nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

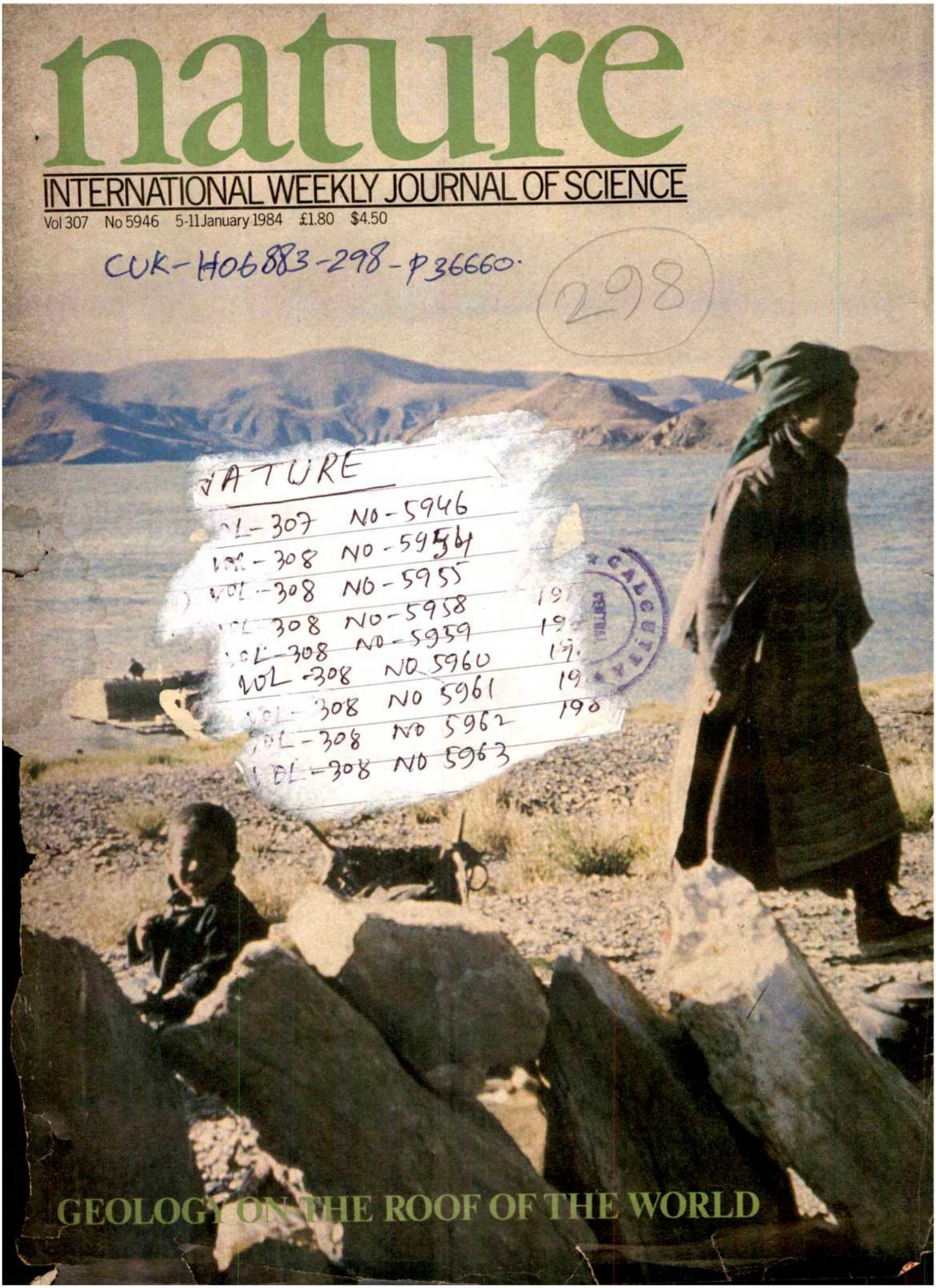
Vol 307 No 5946 5-11 January 1984 £1.80 $4.50

CUK-H06883-298-P36660.

298

NATURE
VOL-307 NO-5946
VOL-308 NO-5954
VOL-308 NO-5955
VOL-308 NO-5958 19
VOL-308 NO-5959 19
VOL-308 NO 5960 19
VOL-308 NO 5961 19
VOL-308 NO 5962 19
VOL-308 NO 5963

CALCUTTA



GEOLOGY ON THE ROOF OF THE WORLD

THE ENZO BIO-PROBE™ SYSTEM

- For non-radioactive DNA labeling.
- For rapid detection of specific DNA & RNA sequences in cells following in situ hybridization.
- For rapid detection of DNA on nitrocellulose membranes.
  — Southerns — Colony hybridizations — Dot blots —

Components of the Enzo Bio-Probe System

Nick Translation Kit for biotinylating DNA Probes.
Biotinylated dUTP, DNA Polymerase I, DNase I, Thymidine Triphosphate, Nucleoside Triphosphate Mix, Lambda DNA, Deoxy(8-^{3}H) Adenosine 5′-triphosphate.

Terminal Labeling Kit for end biotinylating DNA Probes.
Terminal Transferase, Terminal Transferase Dilution Buffer, DNase I, DNase I Dilution Buffer, Terminal Labeling Reaction Buffer, Nucleotide Solution, Biotinylated dUTP, Cobalt Chloride, [methyl-^{3}H] Thymidine-5′-Triphosphate, Control DNA.

Detek™ I-*f* for fluorescent detection of biotinylated DNA probes upon in situ hybridization.
IgG fraction goat anti-biotin, FITC rabbit anti-goat, Evans blue.

Detek™ I-*hrp* for colorimetric detection of biotinylated DNA probes upon in situ hybridization and on nitrocellulose membranes.
Streptavidin-biotinylated horseradish peroxidase complex, 10X buffer, Cobalt Chloride.

The Enzo Bio-Probe System is only available from Enzo Biochem.

Pat. Pend.



How to order the Enzo Bio-Probe System

The Enzo Bio-Probe System allows you to nick translate and choose your means of detection, Detek I-*f* (fluorescent) or Detek I-*hrp* (colorimetric). To find out more about the Enzo Bio-Probe System, write-or call us toll-free.

PRODUCT	CATALOGUE NO.	PRICE
Nick Translation Kit		
for labeling 15μg DNA	**EBP-803-15**	$225.00
for labeling 8μg DNA	**EBP-803-8**	130.00
Terminal Labeling Kit		
for end labeling 15μg DNA	**EBP-809-15**	$275.00
for end labeling 8μg DNA	**EBP-809-8**	155.00
Detek I-*f*		
for detecting 200 slides	**EBP-804-2**	$205.00
for detecting 100 slides	**EBP-804-1**	120.00
Detek I-*hrp*		
for detecting 500 slides or 50 Southern transfers	**EBP-820-2** (81 sq cm ea)	$120.00
for detecting 250 slides or 25 Southern transfers	**EBP-820-1** (81 sq cm ea)	75.00
Biotinylated dUTP		
for labeling 15μg DNA	**EBP-806-15**	$175.00
for labeling 8μg DNA	**EBP-806-8**	95.00
Biotinylated dUTP Substrate Mixture		
for end labeling 15μg DNA	**EBP-810-15**	$185.00
for end labeling 8μg DNA	**EBP-810-8**	98.00
IgG Fraction goat-anti-biotin		
for detecting 200 slides	**EBP-807-2**	$150.00
for detecting 100 slides	**EBP-807-1**	90.00
FITC rabbit anti-goat		
for detecting 200 slides	**EBP-808-2**	$ 55.00
for detecting 100 slides	**EBP-808-1**	30.00

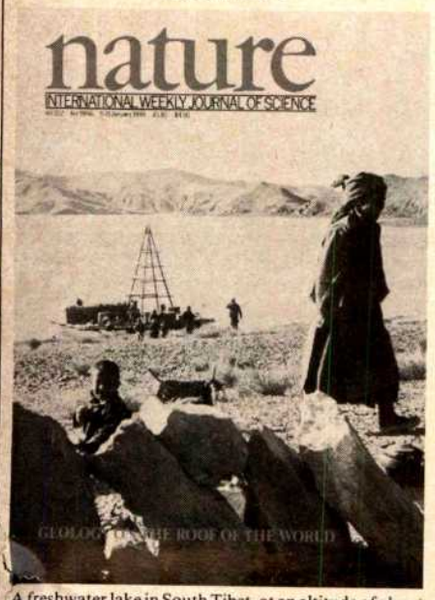
Circle No.19 on Reader Service Card.

[illegible] STREET, NEW YORK, N.Y. 10013 • TEL (212) 741-2828 • TELEX 424683ENZOBIO

nature

NATURE VOL. 307 5 JANUARY 1984

COVER



A freshwater lake in South Tibet, at an altitude of about 5 km. The tripod structure is a floating derrick used for heat-flow measurements. These and other results of a joint French–Chinese study of the North Himalaya–Tibet area are reported on pages 17–36 and in News and Views on page 9.

OPINION

French at odds on new law 1
Ivory tower in space?
The year that never was 2

NEWS

Intellectual property 3
UK research councils
Radiation exposure 4
Indian science
Animal rights 5
Soviet oceanography
US space plans 6
Soviet space programme
France gets hooked
Smallpox eradication 7
US dioxin question is tackled
Richter to head SLAC

CORRESPONDENCE

"Ice-minus" bacteria/Darwin's gradualism/Fly swatting 8

NEWS AND VIEWS

New window on Tibetan tectonics
John Maddox 9

Of Mice and Men
J B Martin 10
Molecular diagnostic medicine
Peter Newmark 11
Antarctic research by satellite link
Dyfrig Jones 12
New light on heavy light
John D Barrow & R R Burman 14
Astronomical angular accuracy
David W Hughes 15
Setting Stonehenge straight
Christopher Chippindale 16

BOOK REVIEWS

Acid Rain: A Review of the Phenomenon in the EEC & Europe *compiled by Environmental Resources Limited*
Kenneth Mellanby 87
Aging: An Exploration *by D P Barash*
Alex Comfort 88
Fast Nuclear Rotation *by Z Szymański*
Neil Rowley
Photoreceptors: Their Role in Vision *by A Fein and E Z Szuts*
Aubrey Knowles 89
Mechanisms of Cell Motility: Molecular Aspects of Contractility *by P Sheterline*
Uno Lindberg
The Philosophy of Popper *by T E Burke*
Donald MacKay 90
Birth Control Technologies: Prospects for the Year 2000 *by M J K Harper*
Warren R Jones

ARTICLES

Structure and evolution of the Himalaya–Tibet orogenic belt
C J Allègre, V Courtillot, P Tapponnier, A Hirn, M Mattauer, C Coulon, J J Jaeger, J Achache, U Schärer, J Marcoux, J P Burg, J Girardeau, R Armijo, C Gariépy, C Göpel, L Tindong, X Xuchang, C Chenfa, L Guangqin, L Baoyu, T Jiwen, W Naiwen, C Guoming, H Tonglin, W Xibin, D Wanming, S Huaibin, C Yougong, Z Ji, Q Hongrong, B Peisheng, W Songchan, W Bixiang, Z Yaoxiu & R Xu 17

Crustal structure and variability of the Himalayan border of Tibet
A Hirn, J-C Lepine, G Jobert, M Sapin, G Wittlinger, X Z Xin, G E Yuan, W X Jing, T J Wen, X S Bai, M R Pandey & J M Tater 23

Lhasa block and bordering sutures — a continuation of a 500-km Moho traverse through Tibet
A Hirn, A Nercessian, M Sapin, G Jobert, X Z Xin, G E Yuan, L D Yuan & T J Wen 25

Tectonic environment and geodynamic significance of the Neo-Cimmerian Donqiao ophiolite, Bangong-Nujiang suture zone, Tibet
J Girardeau, J Marcoux, C J Allègre, J P Bassoullet, T Youking, X Xuchang, Z Yougong & W Xibin 27

High heat flow in southern Tibet
J Francheteau, C Jaupart, S X Jie, K Wen-Hua, L De-Lu, B Jia-Chi, W Hung-Pin & D Hsia-Yeu 32

Evolution of the genes for the β subunits of human chorionic gonadotropin and luteinizing hormone
K Talmadge, N C Vamvakopoulos & J C Fiddes 37

LETTERS TO NATURE

Recurrent bursts in GBS0526–66, the source of the 5 March 1979 γ-ray burst
S V Golenetskii, V N Ilyinskii & E P Mazets 41

The study of metal–oxide interfaces using sputter ion plating
G C Allen & R K Wild 43

Last interglacial reef growth beneath modern reefs in the southern Great Barrier Reef
J F Marshall & P J Davies 44

Late Quaternary pollen records from Easter Island
J R Flenley & S M King 47

A resetting of Phanerozoic community evolution
L M Van Valen 50

Air pollution increases *Aphis fabae* pest potential
G P Dohmen, S McNeill & J N B Bell 52

Direct transfer of carbon between plants connected by vesicular–arbuscular mycorrhizal mycelium
R Francis & D J Read 53

Contents continued overleaf

Nature® (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Annual Index (mailed in February). Annual subscription for USA and Canada US $230 (for subscription prices elsewhere, see next page). USA and Canadian orders only to: *Nature*, Subscription Dept PO Box 1018, Manasquan, New Jersey 08736, USA. All other orders to *Nature*, Brunel Road Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY10010 and additional mailing offices. US Postmaster send form for 3579 to: *Nature*, 15 East 26 Street, New York, NY 10010. Published in Japan by Macmillan Shuppan K.K., 5th FloorEikow Building, 1-10-9 Hongo, Bunkyo-ku, Tokyo, Japan.

© 1984 Macmillan Journals Ltd.

Labelled peptides and proteins

D-**Ala²**-[*tyrosyl*-3,5-³H]**enkephalin (5**-L-**leucine)**	TRK.602
D-**Ala²**-[*tyrosyl*-3,5-³H]**enkephalin (5**-L-**methioninamide)**	TRK.564
[*tyrosyl*-3,5-³H]**Angiotensin I (5**-L-**isoleucine)**	TRK.731
[*tyrosyl*-3,5-³H]**Angiotensin II (5**-L-**isoleucine)**	TRK.733
Arginine⁸[*tyrosyl*-³H]**vasopressin**	TRK.776
[*prolyl* ^{2,3}-3,4(n)-³H]**Bradykinin**	TRK.739
Calmodulin, *N*-[*propionyl*-³H]**propionylated**	TRK.679
Calmodulin, ^{125}I-labelled	IM.126
Cholecystokinin octapeptide (sulphated), [*propionyl*-³H]**propionylated**	TRK.755
Concanavalin A, *N*-[*acetyl*-³H]**acetylated**	TRK.497
[*tyrosyl*-3,5(n)-³H]**Dynorphin(1–9),** opiate ligand	TRK.795
[*tyrosyl*-3,5-³H]**Enkephalin (5**-L-**leucine)**	TRK.516
[3-*iodotyrosyl*-^{125}I]**Enkephalin (5**-L-**leucine)**	IM.155
[*tyrosyl*-3,5-³H]**Enkephalin (5**-L-**methionine)**	TRK.522
[3-*iodotyrosyl*-^{125}I]**Enkephalin (5**-L-**methionine)**	IM.156
[^{125}I]**Epidermal growth factor,** receptor grade	IM.124
N-**Formyl**-L-**methionyl**-L-**leucyl**-L-[4(n)-³H]**phenylalanyl**-L-[4(n)-³H]**phenylalanine**	TRK.763
N-**Formyl**-L-**norleucyl**-L-**leucyl**-L-**phenylalanyl**-L-[3,5-³H]**tyrosine**	TRK.761
[*glycine*-1-¹⁴C]**Hippuryl**-L-**histidyl**-L-**leucine**	CFA.712
[^{125}I]**Iodinated insulin**	IM.38
[¹⁴C]**Methylated avidin**	CFA.701
[¹⁴C]**Methylated fibronectin**	CFA.718
[*tyrosyl*^{3,11}-3,5-³H]**Neurotensin**	TRK.744
[*prolyl*^{2,4}-3,4(n)-³H]**Substance P**	TRK.786
Substance P, ^{125}I-labelled with Bolton and Hunter reagent	IM.157
[*tyrosyl*-³H]**Oxytocin**	TRK.774
[³H]**RX 783006,** agonist at μ-receptors	TRK.681
[3-*iodotyrosyl*-^{125}I]**Vasoactive intestinal polypeptide**	IM.158
Wheat germ agglutinin, *N*-[*propionyl*-³H]**propionylated**	TRK.693

Please contact Amersham or your local representative for further details

Amersham International plc
Amersham England HP7 9LL
telephone Little Chalfont (024 04) 4444

Amersham

Amersham Australia PTY Limited Sydney **Amersham Belgium SA/NV** Brussels **Amersham Buchler GmbH & Co. KG** Braunschweig W Germany
Amersham Corporation Arlington Heights USA **Amersham Denmark ApS** Birkerød **Amersham France SA** Paris **Amersham Japan** Tokyo **Amersham Nederland BV** Utrecht

Circle No.22 on Reader Service Card.

Keep your copies of *Nature* protected in these tough black PVC binders



- Each binder is supplied with a set of wires for the easiest possible loading
- *Nature* logo blocked in gold on spine
- Transparent plastic pocket provided for your own reference
- Each binder takes two volumes of *Nature;* three binders will serve for a year
- Prices and address for orders are set out below

Annual Subscription Prices

UK & Irish Republic		£98
USA & Canada		US$230
Australia & NZ	Airspeed	£150
Continental Europe	Airspeed	£120
India	Airspeed	£115
Japan	Airspeed	¥85000
Rest of World*	Surface	£120
Rest of World*	Airmail	£180

**(not USA, Canada, Europe & Japan)*

Orders (with remittance) to:

USA & Canada
Nature
Subscription Dept
PO Box 1018
Manasquan
NJ 08736
USA

UK & Rest of World
Nature
Circulation Dept
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to:
Japan Publications Trading Company Ltd
2-1 Sarugaku-cho 1-chome
Chiyoda-ku, Tokyo, Japan
Tel: 03-292-3755

Personal subscription rates
These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
15 East 26 Street
New York,
NY 10010, USA
Tel: (212) 689-5900

UK & Europe
Felicity Parker, Nature
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

Credit card orders only (in USA):
Call toll-free: (800) 824-7888 (Operator 246)
In California: (800) 852-7777 (Operator 246)

Back issues
UK, £2.50; USA & Canada, US$6.00 (surface), US$9.00 (air); Rest of World, £3.00 (surface), £4.00 (air)

Binders
Single Binders: UK, £4.50; Rest of World, $10.00
Set(s) of 3 Binders; UK, £12.00; Rest of World, $25.00

Annual indexes (1971–1982)
UK, £5.00 each; Rest of World, $10.00

Nature First Issue Facsimile
UK, 75p; Rest of World (surface), $1.50; (air) $2.00

Nature in microform
For Information:
University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

X-ray diffraction evidence that actin is a 100 Å filament
E H Egelman & R Padrón — 56

Direct observation of motion of single F-actin filaments in the presence of myosin
T Yanagida, M Nakase, K Nishiyama & F Oosawa — 58

Microtubules with more than 13 protofilaments in the dividing nuclei of ciliates
U Eichenlaub-Ritter & J B Tucker — 60

Weaver mutation has differential effects on the dopamine-containing innervation of the limbic and nonlimbic striatum
S Roffler-Tarlov & A M Graybiel — 62

Adrenal chromaffin cells form functional cholinergic synapses in culture
M Ogawa, T Ishikawa & A Irimajiri — 66

Slow transmission of neural activity in hippocampal area CA1 in absence of active chemical synapses
A Konnerth, U Heinemann & Y Yaari — 69

Are baclofen-sensitive $GABA_B$ receptors present on primary afferent terminals of the spinal cord?
G W Price, G P Wilkin, M J Turnbull & N G Bowery — 71

Bonding of molecular oxygen to T state human haemoglobin
A Brzozowski, Z Derewenda, E Dodson, G Dodson, M Grabowski, R Liddington, T Skarżyński & D Vallely — 74

Ferrara β^0 thalassaemia caused by the β^{39} nonsense mutation
M Pirastu, L del Senno, F Conconi, C Vullo & Y W Kan — 76

Most κ immunoglobulin mRNA in human lymphocytes is homologous to a small family of germ-line *V* genes
D L Bentley — 77

A lymphocyte-specific enhancer in the mouse immunoglobulin κ gene
D Picard & W Schaffner — 80

Conservative segregation of nucleosome core histones
I M Leffak — 82

Timing of the steps in transformation of C3H 10T½ cells by X-irradiation
A R Kennedy, J Cairns & J B Little — 85

MISCELLANY

100 years ago — 10
New on the market — 91
Books received — 93
Employment: Key skills and employment growth
R Pearson — 94

NATURE CLASSIFIED

Professional appointments — Research posts — Studentships — Fellowships — Conferences — Courses — Seminars — Symposia: **Back pages**

Next week in *Nature*:

- Successful pregnancy in woman with ovarian failure
- Nucleotide sequence of McClintock's transposable element in maize
- Similarity of human oncogenes and yeast cell division genes
- Human hepatitis B vaccine from yeast
- Maleness in XX individiauls
- Tree rings as volcanic eruption markers
- Radar altimetry of pack-ice reveals sea-swell
- Seasonality of San Francisco earthquake

GUIDE TO AUTHORS

Authors should be aware of the diversity of *Nature*'s readership and should strive to be as widely understood as possible.

Review articles should be accessible to the whole readership. Most are commissioned, but unsolicited reviews are welcome (in which case prior consultation with the office is desirable).

Scientific articles are research reports whose conclusions are of general interest or which represent substantial advances of understanding. The text should not exceed 3,000 words and six displayed items (figures plus tables). The article should include an abstract of about 50 words.

Letters to *Nature* are ordinarily 1,000 words long with no more than four displayed items. The first paragraph (not exceeding 150 words) should say what the letter is about, why the study it reports was undertaken and what the conclusions are.

Matters arising are brief comments (up to 500 words) on articles and letters recently published in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted.

Manuscripts may be submitted either to London or Washington (decisions being made only in London). Manuscripts should be typed (double spacing) on one side of the paper only. Three copies are required, each accompanied by copies of lettered artwork. No title should exceed 80 characters in length. Reference lists, figure legends, etc. should be on separate sheets, all of which should be numbered. Abbreviations, symbols, units, etc. should be identified on one copy of the manuscript at their first appearance.

References should appear sequentially indicated by superscripts in the text and should be abbreviated according to the *World List of Scientific Periodicals*, fourth edition (Butterworth 1963–65). The first and last page numbers of each reference should be cited. References to books should clearly indicate the publisher and the date and place of publication. Unpublished articles should not be formally referred to unless accepted or submitted for publication, but may be mentioned in the text.

Each piece of artwork should be clearly marked with the author's name and the figure number. Original artwork should be unlettered. Suggestions for cover illustrations are welcome. Original artwork (and one copy of the manuscript) will be returned when manuscripts cannot be published.

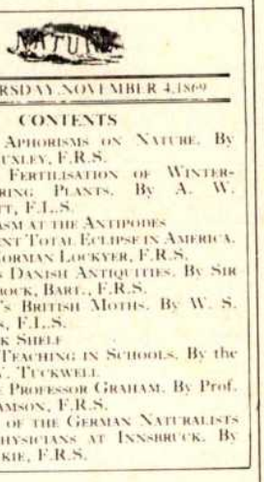
Requests for permission to reproduce material from Nature *should be accompanied by a self-addressed (and, in the case of the United Kingdom and United States, stamped) envelope.*



Fascinating reading and an unusual gift

A high-quality, 40-page facsimile of the first issue of **Nature** is now available at the following prices:

UK	**75p**
Rest of World	**US$1.50** (Surface)
Rest of World	**£2.00** (Air)



THURSDAY, NOVEMBER 4, 1869

CONTENTS

GOETHE: APHORISMS ON NATURE. By Prof. HUXLEY, F.R.S.

ON THE FERTILISATION OF WINTER-FLOWERING PLANTS. By A. W. BENNETT, F.L.S.

PROTOPLASM AT THE ANTIPODES

THE RECENT TOTAL ECLIPSE IN AMERICA. By J. NORMAN LOCKYER, F.R.S.

MADSEN'S DANISH ANTIQUITIES. By SIR J. LUBBOCK, BART., F.R.S.

NEWMAN'S BRITISH MOTHS. By W. S. DALLAS, F.L.S.

OUR BOOK SHELF

SCIENCE-TEACHING IN SCHOOLS. By the REV. W. TUCKWELL

THE LATE PROFESSOR GRAHAM. By Prof. WILLIAMSON, F.R.S.

MEETING OF THE GERMAN NATURALISTS AND PHYSICIANS AT INNSBRUCK. By A. GEIKIE, F.R.S.

Payment may be made in any currency at the prevailing exchange rate. Cheques should be made payable to **Nature**. Prices include postage and packing.

To order please complete the coupon below, or write, enclosing the correct remittance.

Order Form

To: Nature
Macmillan Journals Ltd
Brunel Road, Basingstoke
Hants, RG21 2XS, England

Please send me ..... facsimile(s) of the first issue of NATURE.

I enclose.....

Name .....

Address .....

.....

.....

.....

Reg. No: 785998 England.
Reg. Office: 4 Little Essex St. London WC2R 3LF

Circle No.64 on Reader Service Card.

EDITORIAL OFFICES

London Office
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024

Washington Office
1134 National Press Building, DC 20045
Telephone: (202) 737-2355

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff: Alun Anderson, Tim Beardsley, Philip Campbell, Isobel Collins, Peter Gambles, Melanie Kee, Tim Lincoln, Naomi Molson, Sara Nash, Geoffrey North, Peta Pickering, Miranda Robertson, Robert Walgate, Charles Wenz, Nigel Williams

Washington Editor: Peter David
Washington Correspondent: Stephen Budiansky

Art and Design: Joan Godwin, Joel Chernin, Theresa George, Paul Norris, Marie Xeridat

Administration: Mary Sheehan, Jacqueline Cole, Ruth Cutts, Joanna Czechowska, Ruth Goldenburg, Sharon Isaacs and Penny Walker (Washington)

Publisher: Elizabeth Hughes

Marketing Director: Ray Barker

Promotion Manager: Felicity Parker

International Advertising Manager: Andy Sutherland
Features Advertising Manager: Marion Delaney
Group Classified Advertising Manager: John Barnes — Telephone: (01) 240 1101

Nature: New York Office
15 East 26 Street, New York, NY 10010
Telephone: (212) 689-5900 Telex: 668497

General Manager: Gary Rekstad
American Advertising Manager: Henry Dale
Classified Advertising Manager: Miss Gene Fein
Circulation Manager: Mirta Soto

Canadian Display and Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1—(416) 690-2423

Display Advertising Representatives

New York: Nature Office
Chicago: Didier & Broderick, Inc.—(312) 498-4520
San Francisco: Jobson/Jordan/Harrison/Schulz, Inc.—(415) 392-6794
Los Angeles & Pasadena: Jobson/Jordan/Harrison/Schulz, Inc.—(213) 796-9200
Philadelphia: Daniel Adams Associates, Inc.—(215) 353-6191
Boston: CEL Associates—(617) 848-9306
Fort Lauderdale: Brinker & Brinker—(305) 771-0064
New Jersey: Mary Grunmeier—(201) 572 9018
Dalton, Pennsylvania: Donald E. Northup Associates—(717) 563 2572
West Germany and Austria: Franz Schrecklinger TMW Top Media Werbegesellschaft GmbH Frankfurt—(611) 726046
Holland and Belgium: Adele Struyck G. Arnold Teesing BV Amsterdam—(020) 2636 15
Switzerland: Werner Stähli Agentur Iff AG Schaffhausen—(053) 45821
Scandinavia: Andrew Karnig Andrew Karnig & Associates AB Stockholm—(08) 51 68 70
Japan: Mashy Yoshikawa Orient Echo Inc Tokyo—541-4923
Australia: Gus Bartel & Associates PO Box 1755, Southport, Queensland 4215 (075) 466 577

Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office

©1984 Macmillan Journals Ltd
Vol.307 No.5946 1984

nature

French at odds on new law

The law to regulate French universities has been signed — and opposing interests are already battling for influence over the decrees that will follow

EXCEPT for a little trouble with the French constitution, which should be resolved next month, France now has a new university law, the first major revision of university law since the student revolution of May 1968. But is the reform too little? Has a great opportunity been lost? The vociferous critics of the new law, notable among them Fields Medallist Laurent Schwartz of the Ecole Polytechnique, say loudly that it has been. Strangely enough, Schwartz's opponent at the ministry of education is another mathematician of similar intellectual temperament: Jean-Jacques Payan, ex-director-general of the principal French research council (CNRS) and ex-president of the University of Grenoble I, one of the foremost scientific universities of France. If Schwartz says the government has fumbled its chance, the man responsible is Payan. But Payan does not think he fumbled the ball but, instead, that he has made a pretty clever pass.

The differences between the two men, which epitomize the arguments over the future of the French university, are based less on differences of outlook than on differences of responsibility. Schwartz is a free spirit, outside the universities and with no power to exercise nor political pressure to which to adjust. Payan is in the hottest seat of all, in charge of the reform and acutely aware of what can and cannot be achieved. The new law is pragmatic, he admits, "but it proscribes little and permits much". Payan urges France to wait for the detailed, non-parliamentary "decree of application" which follows every law. Here may be some of the teeth, some of the support for research that Schwartz demands. "I have the same passion [as Schwartz]: the greatness of the French university", wrote Payan recently. So well do these conflicting passions set each other off than one must wonder whether Schwartz and Payan are in league, one creating the political climate that the other needs.

In reality, there are fundamental differences between the two men and the bodies of opinion they represent. Schwartz and those he speaks for wish to reintroduce selection in the first-year entry to university, the absence of which creates a "catastrophe" for both students and teachers. Payan and the law he represents avoid pre-entry selection and speak of post-entry "evaluation". Entry to the second and subsequent years will, in effect, be selective (as at present) but the word "evaluation" implies more: the universities should not evaluate the students according to some predetermined scheme but according to their students' needs, says Payan. Thus by implication in the new laws, the universities are to turn their resources towards their students, rather than the other way about. This is a revolution: it implies that the universities must become, at least in part, more like the existing technical institutes (IUT) or engineering schools (*grandes écoles*), for the students of the 1980s will certainly demand training that will lead to employment.

What of the dominance of French universities by trade unions and "corporatism"? Elections to various powerful committees of administration and promotion play a great — some say overwhelming — role in the post-1968 French university, and often these elections have been dominated by factional politics, with one union or body of staff against another. Schwartz says the new law worsens the situation. Payan, with some force, argues the reverse and, since he is no syndicalist, the benefit of the doubt must rest with him. According to Payan, the fact that under the new law elections will be by proportional representation, with freedom for non-ascribed candidates, rather than the earlier winner-takes-all system, means that corporatism will be much diminished.

And what of research? Is it ignored by the law, as Schwartz says? Wait for the decree that will define the duties of university lecturers and professors, says Payan. Meanwhile, the text of the law introduces a sabbatical year — a welcome innovation for French scientists. It also establishes periodic evaluation of staff to keep them up to scratch, new recruitment procedures that "will leave little room for the establishment of local cliques" and imposes an obligation of mobility on professors. All this will improve the quality of French university research, says Payan. Moreover, the ministry is turning to a system of research support by multi-annual contracts, rather than the annual support related directly to student numbers presently in force. This should give the universities, traditionally dominated by central government, more academic independence.

As for the decree defining duties, that has been through many drafts and tense negotiations with the unions, and is now again being rewritten. The ministry says that it will take research "much more into account" than previous versions, meeting criticism from the presidents of the universities and others. Payan's early insistence on increased teaching duties and a longer working year will be somewhat softened and the duties are now being seen to be an overall requirement on a university, to be distributed according to some as yet undefined rules. The heavier teaching load will not be a direct requirement on individuals. The result of this, however, in conjunction with the law's insistence on free entry and on the evaluation of student need, may be to create a two-tier university structure in France. Some will teach, others will do research.

The pattern of research will also be transformed by the proposed change in the structure of higher degrees. The old *troisième cycle*, and the *thèse d'état*, respectively lesser versions of the PhD and DSc (the latter taking eight or nine years) are to be abandoned in favour of something approaching a PhD. This, most French scientists consider, will bring French science into line with the United States and Britain. But the new law has shrunk from out-and-out advocacy of meritocracy. As things are, would-be researchers can edge their way into laboratories on private means and eventually obtain a permanent post by waiting long enough, rather as in Italy. This system will continue even under the new law unless a comprehensive system of competitive grants is introduced. That step, still neglected, would do more than anything else to better the condition of science in France. □

Ivory tower in space?

NASA should be restrained from mounting a crash programme to build a US space station

THE National Aeronautics and Space Administration (NASA), set up in the heady days of the early 1960s, has always thrived on large engineering projects, undertaken on a crash basis with billions of dollars to throw about. Now that NASA has declared the shuttle operational (which, given recurring problems, including an explosion and fire after the shuttle's most recent

0028-0836/84/010001-02$01.00

landing, is not the case), it is once again at a loose end — whence the plan to build a space station. Although nobody — not scientists, not the military, not even NASA itself — has been able to figure out just why the United States needs a space station, President Reagan is expected to approve the project when he releases his budget for fiscal year 1985 later this month.

Perhaps it was just a coincidence, but just before Christmas, Congress's Office of Technology Assessment released a report that seemed to answer NASA's prayers. The report, a sober description of the Soviet Salyut programme, has been seized upon to provide the ultimate justification for an American space station; the Soviets have one. Unfortunately, an American crash programme to build a space station will fail to correct the real problem in the US manned space programme that the report identifies, and in the process will also do considerable damage to American space science efforts — an area in which the United States really does lead the Soviets.

All the media hoopla over the red menace has ignored the more serious underlying failing of the US space programme compared with that of the Soviet Union: while the Soviets have plodded steadily forward towards a directed goal — a continuous manned presence in space, built up through mastery of automatic docking and long stays in space — the United States has gone in for space spectaculars, virtuoso efforts that reflect a dazzling technical ability and lead nowhere. The Apollo project (which, as a Tom Lehrer song has it, spent billions of dollars of taxpayers' money to put some clown on the moon) was not part of a larger plan.

The shuttle, also a crash programme that took the lion's share of NASA's resources, has suffered from a lack of a sense of direction. NASA, in a political decision to sell the shuttle to Congress, tied everything it could to the shuttle — if space science was needed, the shuttle would do space science; if it was military satellites, the shuttle would do military satellites. To make sure, NASA ditched its reliable expendable launch vehicles. The result was a disaster for space science, though not for the reasons usually given. The shuttle project itself probably did not steal funds away from science; what it did, however, was to delay by four years the Galileo mission to Jupiter (Galileo, of course, was designed to use the shuttle), and the funds needed to keep Galileo going for four years (it will finally be launched in 1986) eliminated the possibility of doing anything else in space science in the meanwhile. Also, the early promises of 28-day missions have given way to the reality that it does not make economic sense to park a billion-dollar launch vehicle in orbit for a month. The presence of men on a scientific platform can also be drawback, particularly for sensitive pointing apparatus. The shuttle was not needed for science; in many ways, it has hurt science.

The space station seems to be following the same path. And it will be a tragedy if science is once again constrained to suit NASA's political expediencies. If the space station goes ahead as another NASA crash programme, there will be another two decades of original research on why astronauts vomit and whether you can make the perfect ball-bearing in space.

If the Administration is truly concerned about staying ahead of the Soviet Union, it would do well to concentrate on the existing strengths in the US programme. The series of inexpensive near-Earth space probes being developed by the Jet Propulsion Laboratory (see p.6) is one obvious avenue to pursue. Another is being studied by the Space Science Board and would make the shuttle into a truly useful scientific vehicle; it is to build a module, called Spartan, that could be dumped overboard from the shuttle, allowed to co-orbit with the shuttle (unmolested by men bumping about) and then picked up just before returning to Earth.

At the same time, more care and effort should be put into the shuttle and the space station, but in terms of an engineering development project — not a crash programme to build the space vehicle for the next decade. It is of course only a matter of time before there will be some real scientific (and perhaps military) needs for a permanent space station, and plans should be made for that. Meanwhile, the United States should learn something from the Soviet Union, and not waste energy on bursts of light and heat that hurt only itself in the long run. □

The year that never was

If 1984 is not as fearsome as in Orwell's novel, there are still important battles to fight.

AT the beginning of George Orwell's year, the first thing to acknowledge is that *1984* never came. The idea that governments might become so preoccupied with their own survival, and so adept at the exploitation of technology for bending the inclinations of their people to that simple goal, that personal liberty would cease to be, remains a nightmare — one, nevertheless, that will not go away. Moreover, this does not imply that governments are consistently more enlightened now than, say, a year ago, or that the suppression of liberty has by magic been banished from the face of the Earth. On the contrary, new pockets of despotism are forever cropping up. The mercy is that the practitioners of wayward offences against people are but crudely practised. Bullets, in their books, are more convenient than mind-control. And, as illiberal governments repeatedly discover, their wish to make their people toe some predetermined line is repeatedly undermined by the personal courage of those they would control. Precisely that obdurate unwillingness of people to forsake human dealings with each other is, after all, what changed Orwell's book from a political tract into a novel. Although the profession of science is superficially indifferent to the ways in which governments choose to treat their citizens, the health of science rests on the freedom with which new ideas flow from one place to another, from any person to any other. That, of course, is why professional scientists are concerned with the way in which their colleagues elsewhere are dealt with and why the fortunate among them have an obligation to assist those less fortunate by all possible legal means.

Against Orwell's predictions, the more serious anxiety during 1984 is not that governments will find it prudent to deprive their people of liberty but that they will choose to blow each other's people to smithereens. Outwardly, this again is an issue separate from the practice of science as such, or in which it might be held that professional people have a right to intervene only in their capacity as citizens. In many circumstances, that would be true. But if it is the case that the means of waging nuclear war, and the means by which the risks of nuclear warfare might be avoided, depend to a large extent on technical considerations, the technical community may be held to have an obligation to throw what light it can on the questions that arise. That is why journals such as this have always taken the position that comment on the steps which are being taken (or not being taken) to avoid the dangers of nuclear and other kinds of wars is not merely permissible but obligatory. And 1984 promises to be a busy year in this respect.

The year ahead also bristles with threats to the well-being of the scientific enterprise. The long recession is far from over (and will not be completed until the present period of rapid technological change has worked its way through the world's advanced societies). So even the most prosperous and far-sighted governments are in their different ways looking for ways of cutting costs wherever possible. The ambition is legitimate and laudable; the practice, unfortunately, is often ill-considered. This is why 1984 is likely to see a running battle between governments anxious to trim budgets by making science a pensioner of industry and those who see that the exploration and understanding of the world we live in cannot be trusted to such chance. Orwell's great book was written in 1948, when the first rudimentary electronic computers had just come into service, when the distinction between the pi and the mu mesons had just been discovered, when neither transistors nor lasers had been invented and when the mechanisms of inheritance were still unknown. That industrial needs have been the engines of many of these developments is not in question, nor is the prosperity that industrial developments have brought. The issue that will be fought in the months ahead is the extent to which free inquiry should depend on such adventitious interests. That battle too, looks like being a long and loud one. □

Intellectual property

UK patents shake-up causes a stir

A PROPOSAL to hive off the British Patent Office from the civil service and establish it as a separate statutory body is sending shock waves through the patent community. That suggestion is just one of several that are likely to prove controversial in a report on intellectual property rights recently published as a green paper*.

The report was produced at the request of the Prime Minister by Dr Robin Nicholson, Chief Scientific Adviser at the Cabinet Office, who holds that "cumbersome and slow" patent procedures are deterring would-be innovators in Britain. Smaller companies and individual inventors, in particular, tend to find the "arcane" world of the patent system a disincentive to stake a claim for patent protection. A new-look Patent Office freed from civil service constraints should, according to the green paper, be given broader terms of reference and encouraged to advertise its services and expertise, thus making fuller use of the database on patents that it maintains.

Nicholson has recently described the patent system in Britain as "dominated by a few very expert individuals" — a view apparently shared by a senior Patent Office figure who declines (for reasons of civil service etiquette) to be named but who feels the workings of the Patent Office have been partly misunderstood. And Mr Bernard Fisher, president of the Chartered Institute of Patent Agents, says that the green paper's main aim of getting the Patent Office to be more responsive to inventors' needs might be achieved more simply and more cheaply by simply allowing it to keep more of the profits it usually makes each year which are now returned to the Treasury. Pension rights and career structures would be greatly affected if the proposed changes were made, however, and allowance would have to be made for compensation payments.

The green paper urges a government policy statement on intellectual property, to include a number of changes. Some, such as the suggestion that service industries should be able to claim exclusive use of registered mark, have already been accepted by the government. Others are more contentious: the proposal to replace design copyright laws by a registered design scheme, in particular, will be unpopular with manufacturers of mass produced items, who are at present very well protected against competing manufacturers of spare parts, for example in the automobile industry. One critic of the Nicholson report said it might be impractical for manufacturers to have to register components individually, whereas as things stand ownership of engineering drawings confers copyright protection.

Nicholson comes down in favour of a system of "petty patents" — a form of short-term patent conferring only limited protection which can be upgraded into a patent proper if the putative invention justifies the cost and effort involved. The government has previously rejected this idea but changes in practice since the introduction of the 1977 Patent Act might now have swung the balance in its favour. The complaint from some industries is likely to be that their research and development programmes would be stultified if the number of potential monopolies were increased by petty patents.

Nicholson identifies several abuses of the patent system that are not adequately checked by existing safeguards. The aim is to prevent potentially valuable innovations from being "sat upon" by patent holders unwilling to risk exploitation. The 1977 act included provisions for compulsory licences to be issued to would-be innovators in such cases, but since the act was introduced only one has been granted. Nicholson says the existing provisions should be used more often and proposes new rights for employee inventors that would allow them to exploit an invention that is not taken up by their employer.

One proposal that is unlikely to be accepted without some resistance is that people other than registered patent agents should be allowed to represent inventors. The aim is to allow wider access to property rights and to promote price flexibility, but it is pointed out that inventors could lose all their rights if an inadequately prepared patent is published. The Chartered Institute of Patent Agents is apparently untroubled however, believing that serious inventors will always prefer the security of a registered agent.

One view of Nicholson's proposals is that they fail to take account of the constraints on Britain stemming from its participation in the European Patent Convention. There are certainly international issues at stake, particularly in seeking a balance between the legitimate interests of manufacturers seeking patent protection throughout the European Community, for example, and the risk of sanctioning anti-competitive practices. The document suggests that Britain should take the initiative in requesting the European Commission to devise proposals to straddle the gap.

Tim Beardsley

Intellectual Property Rights and Innovation. (Cmnd 9117, HMSO, £4.65).

UK research councils

Robbing Peter to pay Paul

THE Advisory Board for the Research Councils (ABRC) has recommended that funds for restructuring the British research councils during 1985–87 should come from within the science budget, according to Mr Peter Brooke, the Under-Secretary of State for Education and Science in a parliamentary reply. This tame-sounding statement has a sting to it. It means that the Medical Research Council (MRC) and the Science and Engineering Research Council (SERC) will, over that period, forgo 0.75 per cent and 1.5 per cent respectively of their budgets to pay for the forthcoming reorganization of the Natural Environment Research Council (NERC) and the Agricultural and Food Research Council (AFRC).

AFRC has been under pressure to reorganize partly as a result of earlier ABRC recommendations to cut back on financial support for the council (see *Nature* 22 December 1983, p.723). More recently, a study carried out by Sir Ronald Mason for ABRC suggested in unspecific terms that the amount of in-house research within AFRC and NERC was excessive. Both research councils are planning reorganization in the long term which is expected to involve expenditure on "early retirements, transfer costs and capital work", according to Mr Brooke. The science budget will be increased by £750,000 in 1984–85 and by £900,000 in the two succeeding years to offset some of this cost, while the total contributions from SERC's and MRC's forward planning budgets will amount to £3.1 million in 1984–85 and £6.3 million in 1986–87.

Mr Brooke also announced that an extra £1 million has been found partially to compensate SERC for increased subscriptions to international research organizations such as the European Organization for Nuclear Research (CERN), on top of the £6 million already secured from the Treasury. This still leaves SERC with about £3 million to find from its current budget next year.

In the circumstances, SERC and MRC are hardly likely to welcome the future loss of funds for research. Indeed, at a press conference in November, Professor John Kingman, chairman of SERC, emphasized the unfairness of a policy that penalized "efficient" councils. This reaction is apparently contradicted by Mr Brooke's statement that ABRC's policy is unanimously supported by the advisory board's members — who include the chairmen of all the research councils.

Philip Campbell

Radiation exposure

US academy study attacked

Washington

THE National Academy of Sciences has stumbled into an uncomfortable controversy about the health of American soldiers exposed to radiation when they were stationed near Hiroshima and Nagasaki after the cities were destroyed by atomic bombs in 1945. Veterans have for years been locked in dispute with the Department of Defense about the long-term health effects of their service in the area.

Last July, in a study requested by the Pentagon's Defense Nuclear Agency, the academy's research arm, the National Research Council (NRC), published a report concluding that there was no evidence to support claims that the veterans had suffered from an abnormally high incidence of the bone cancer called multiple myeloma. The fact that the report was immediately criticized as perfunctory by the National Association of Atomic Veterans (NAAV) caused little surprise.

But now, Congress's Office of Technology Assessment (OTA) has joined the fray. In a rare instance of Congress demanding a review of an NRC study, OTA says the report did not have enough evidence for its conclusions.

The academy's involvement goes back several years. In 1981, the Defense Nuclear Agency asked whether a large-scale epidemiological study of multiple myeloma was warranted. An NRC panel under Harvard's Brian MacMahon concluded that the low level of radiation to which US troops were exposed — about a tenth of a rad — could not have been responsible for the incidence of cancer. But the MacMahon panel also said that an epidemiological study might be necessary if estimates of the level of radiation encountered by American troops were revised upward or if there appeared to be *prima facie* evidence that veterans were suffering in disproportionate numbers from the disease.

In the wake of the MacMahon report, the Pentagon asked NRC to find out what it could about the incidence of multiple myeloma. A panel set up to review all known cases of myeloma among the veterans identified only nine — at the bottom of a range from 9 to 29 expected statistically in the normal population. It concluded in July (see *Nature* **304**, 200; 1983) that there was therefore no evidence to support claims of an abnormally high incidence among veterans.

OTA claims the NRC study has two basic flaws. The methods used to identify only nine cases of myeloma among the veterans were likely to result in an underestimate of the number who had actually contracted the disease. At the same time, NRC probably overestimated the number of cases that could have been expected if the rate of incidence was the same for veterans as for the general population.

Veterans who had contracted multiple myeloma were tracked down through a nuclear veterans' "hot line" established by the Pentagon and through a newsletter and magazine article organized by NAAV. The hot line had received calls from nearly 50,000 people by the time of NRC's study, of whom 678 reported service at Hiroshima and Nagasaki, and seven of those reported having multiple myeloma. NAAV found 21 names of people who had served in the two cities and had the disease.

NRC whittled down the 28 possible cases to nine. Eleven were eliminated because they appeared not to have served in Nagasaki or Hiroshima and six because they, their families or doctor failed to respond to follow-up enquiries. In another two cases, review of the clinical evidence found that they had not, in fact, suffered from the disease.

This procedure, claims OTA, was most likely to produce an underestimate of the number of cases. For one thing, the Pentagon hot line had produced only 4 per cent of the estimated 20,000 veterans who had served in Hiroshima and Nagasaki. For another, nearly half of those who contract multiple myeloma die within the first year, and at least some bereaved relatives may have failed to respond to the hot line, particularly as the advertising did not refer to multiple myeloma. Moreover of the 11 cases eliminated because they appeared not to have been at Hiroshima or Nagasaki, only seven were definitely known to have been elsewhere. And the loss of six cases because of failure to respond to a follow-up letter and telephone call could have had a dramatic impact on the overall result.

Finally, says OTA, the report compared the number of cases expected over a 35-year period with the nine cases it had tracked down — but all nine had been reported within a five-year period. OTA claims the comparison was inappropriate; its own calculation estimated the number of expected cases to be much smaller. Taken overall, OTA concludes, NRC's approach was unlikely to have detected a disproportionate incidence of multiple myeloma even if it existed.

NRC has not yet responded to OTA's criticisms, but in the view of at least one congressman the council has some explaining to do. Paul Simon, the Illinois Democrat sponsoring a bill to compensate "atomic veterans", said NRC's report had "tainted" debate about the complex issues involved. The methods and boldness of the report were bad enough, he added; more troubling was that its conclusions agreed so neatly with the views of the Defense Nuclear Agency, which had long denied government responsibility for the health problems of atomic veterans. **Peter David**

Indian science

Research council in trouble

New Delhi

THERE are skeletons in the cupboard of India's Council of Scientific and Industrial Research (CSIR). A scrutiny by a parliamentary committee has drawn attention to several shortcomings in the functioning of India's leading industrial research organization. The committee complains in particular that very few processes developed by the CSIR laboratories have been found useful by industry.

Only recently, Prime Minister Indira Gandhi was forthright in her criticism of the laboratories on the grounds that their research projects incur substantial losses. At a meeting of directors of national laboratories, she said that these are becoming an "ever-increasing burden on the nation" and asked that if there is no hope of the situation improving, "is it not time just to close them down?"

Now the parliamentary Public Accounts Committee (PAC) has revealed what ails these laboratories. Research projects have often been taken on without adequate planning in advance. The lack of machinery, equipment and other necessary facilities has later led to the abandonment of many such projects. In the past two years, about 300 projects have been dropped, reducing the number of schemes in hand to 1,543. A further hundred are to be terminated next year.

The officials concerned in evaluating these projects display a "superficial and perfunctory" attitude, according to PAC, which says that while unproductive and unfeasible projects should normally be abandoned after one year, some of them were allowed to continue for as long as five years. A glaring example is that of a household pump research project which had to be discarded after two years of work when it was found that the same project had been undertaken by another laboratory of CSIR three years earlier.

PAC has remarked that either the processes developed by CSIR were not selected with the needs of Indian industry in mind or CSIR has been unable to inspire the confidence of potential users in the usefulness of its processes. The council referred 295 of its technical development projects, less than half of those on its books, to the National Research Development Corporation (NRDC) for industrial exploitation, of which only 15 per cent had been taken up by industry by June 1981 — and some of these were withdrawn by CSIR.

Even public sector undertakings are not making full use of the council's research output. CSIR has been complaining of an "unsympathetic attitude towards development and utilization of indigenous know-how", saying that many of its

schemes are exposed to unfair competition "by the import of technology from abroad". It has been demanding a role in the selection of technology imports. It rankles that last year, CSIR technology for colour television was not exploited but that, instead, the government allowed the import of colour television sets.

The director general of CSIR agrees with the criticism that the council's laboratories concentrate on projects which serve the elite sections of society and pay little attention to technologies that could benefit people in economically weaker sections of the community. It has accordingly compiled a list of technologies to be developed for poor people.

Younger scientists have found support from PAC for their complaint that their views are ignored in planning the research work of laboratories. The committee says that funds allotted to their work are often arbitrarily transferred to other projects sponsored by more senior scientists, while younger people tend not to be included in the high-level committees of different laboratories.

CSIR is concerned that it can no longer attract scientists of high calibre for senior posts, partly because its laboratories are poorly equipped. One of its difficulties is the rapid inflation in the prices of chemical apparatus, equipment, books and journals, so that even a 10 per cent increase in its budget is not enough to catch up.

Sunil Saraf

Animal rights

Massachusetts bans strays

Boston

THE giving or selling of dogs and cats from city pounds and animal shelters to laboratories has been outlawed by the Massachusetts legislature. Responding to overwhelming public support for the measure, the bill has now been signed by Governor Michael S. Dukakis.

Animal rights advocates are claiming a major victory, as Massachusetts becomes the eighth state to prohibit pound seizure — the right of licensed laboratories to purchase unclaimed animals after 10 days. The law will end pound seizure from next October and the purchase of dogs and cats imported from out-of-state pounds from two years after that. Massachusetts is the first state to enact this provision.

Researchers who depend on so-called "random source" dogs and cats will now have to buy more costly "purpose-bred" animals from commercial breeders, or switch to other experimental subjects such as sheep, pigs, calves and goats. Ralph Charlwood, assistant director of the Harvard Animal Resource Center, says that the law will dramatically increase costs for heart, kidney and toxicology research that now depends on pound animals. Boston area medical schools are considering solving the problem of supply by establishing a jointly operated animal breeding facility, but even this approach would be more costly than buying cats and dogs from pounds.

Walter Kilroy, vice-president of the Massachusetts Society for the Prevention of Cruelty to Animals (MSPCA), claims that the law will improve the quality of research. "Random source animals are not good research models", he said. "Their genetic background and physical condition are much more variable than bred animals." Opponents of pound seizure believe that domesticated dogs from pounds suffer more stress than those used to kennel conditions and maintenance. "Laboratories taking dogs from pounds accept only those animals with personalities that allow them to be handled and restrained", said Kilroy. These are pets, accustomed to "human companionship and affection, and liberty".

Research institutions in Massachusetts — Harvard University and the University of Massachusetts in particular — have for several years been fighting highly organized efforts to repeal the pound laws; state legislators receive more mail on this issue than any other. ProPets, an alliance between the New England Anti-Vivisectionist Society (NEAVS) and MSPCA, collected more than twice the required number of signatures on a petition to put on this year's ballot a referendum far more



restrictive than the law now to be enacted. Unwilling to face the huge costs of fighting a state-wide referendum, the research institutions agreed to the new legislation in a compromise with ProPets hammered out by State Representative Ray Jordan.

Legislative efforts to prohibit pound seizure appear to be gaining momentum in other states. Aaron Madlock, director of the New England Anti-Vivisectionist Society, says his group will now campaign for similar legislation in Vermont, and points out that animal rights groups in California are engaged in a major battle to ban both pound seizure and importation from other states. Although universities, medical schools and pharmaceutical companies are now attempting to persuade the public of the value of animal research, they have plenty of catching up to do.

Christopher Earl

Soviet oceanology

Institute director in deep water

DR Andrei Sergeevich Monin, director of the Institute of Oceanology of the Soviet Academy of Sciences, has recently come under sharp attack in the Soviet media and from Communist Party activists within the academy. Charges against Dr Monin range from "the embezzlement of socialist property" (having his dacha repaired by institute employees and out of institute funds) to despatching research ships "round the world and back again" without a research programme for the whole journey, and from failing to ensure a "proper moral and political climate" in the institute's oceanological equipment design bureau (which is said to be run by a "close relative" of Dr Monin) to applying for the right to search for sunken treasure. Moreover, it is claimed, he has consistently ignored the criticisms of local and academy party officials, and has attempted to dismiss from the institute anyone who complained too openly of his policy.

As far as the corruption charges are concerned, the institute appears to have been made an example in Mr Andropov's much-publicized campaign for "work discipline". Unauthorized disbursements from the bonus fund and discreet "rectification" of over-demanding plans have become, alas, all too common a feature of Soviet management — including the management of scientific institutions. According to *Sovetskaya Rossiya,* several employees of the Institute of Oceanology's Leningrad branch have already been brought to trial on charges of "fraud and currency machinations". Nevertheless, it is alleged, the deputy director, L.A. Tzymbal, who was discovered to have sanctioned and received illegal salary increments, is still working at the institute under Dr Monin's patronage.

Dr Monin has, apparently, so far managed to quash all complaints against him, including those made to the academy. Letters of complaint get bogged down in the bureaucratic process or else receive smooth and non-committal answers. Several leading oceanologists have allegedly left the institute in disgust at the director's seeming inviolability. Meanwhile, Dr Monin himself has a standard answer to every criticism: it is all due, he says, to the "growing pains" of the relatively young science of oceanology.

Vera Rich

Correction

The accidental sea discharge at Sellafield reported in *Nature* (**306**, 721; 1983) was made through the normal sea pipeline, and not as stated. The 2-inch pipe was used in an attempt to return waste from the sea tank to the plant. □

US space plans

Economy flights in prospect

Pasadena, California

CUT-rate planetary missions have been included in the 1985 budget request of the National Aeronautics and Space Administration (NASA). The series of probes, known as "Observer", will use modified Earth-orbital satellites to explore the nearby planets. The first flight could take place in 1982, with subsequent missions at one to two year intervals.

The new probes would add significantly to the modest resurgence in planetary exploration of the past year. Congress this fall approved funds for the Venus Radar Mapper mission, the first new planetary mission since Galileo. Galileo, which was to have been launched on its journey to Jupiter in 1982, was repeatedly delayed by setbacks in the shuttle programme, and is now scheduled for 1986.

Costs for the Observer probes will be kept down by designing instruments to fit available vehicles, rather than the other way around as in past practice. The Jet Propulsion Laboratory (JPL) here, which has primary responsibility for planetary missions, is ready to issue a request for proposals from satellite manufacturers for supplying the vehicles. Possible candidates include the RCA weather and communications satellites, TRW's satcoms, and Hughes communication satellites. By limiting the Observer missions to the nearby planets, Mars and Venus, requirements for power, temperature control and communications systems will be modest and can easily be adapted to fit the adapted Earth-orbit satellites. One of the first Observers is expected to be a Mars orbiter that will probe the geochemistry and climate of that planet.

The renewed hopes for planetary science come just two years after JPL, finding it hard to keep its staff employed, sought and received unprecedented permission from California Institute of Technology (which operates JPL for NASA) to take on defence contract work in an amount up to one-third of the laboratory's total operating budget. But with things looking up (besides the Venus Radar Mapper and the hoped-for Observers, JPL is busy with Galileo and the forthcoming rendezvous of Voyager with Uranus and Neptune), the laboratory expects to hold the defence work to its present level of 20 per cent.

JPL also has plans for more ambitious planetary missions. So-called Mariner Mark II probes, which similarly would draw on existing hardware designs, are being planned for exploration of more distant planets and perhaps comets; one much discussed idea would be a Saturn orbiter that would carry an entry probe to be dropped into Titan, Saturn's volcanic moon. Both the Observer and Mariner Mark II flights could be carried out under a $300 million annual budget, according to JPL officials. The Mariner Mark II probes would be launched every two to three years.

Much less is being heard about grand, novel missions, such as a sample-return mission to Mars. JPL officials see those as "augmentation" missions that will only be contemplated if extra money becomes available. **Stephen Budiansky**

Soviet space programme

Not without hitches

AFTER more than three months of Western speculation, Soviet cosmonauts Vladimir Lyakhov and Aleksandr Aleksandrov have at last admitted that during their 150-day mission aboard the Salyut-7/Soyuz-T9 complex, there had indeed been a "slight" leak of propellant, although, they said, this "had not affected either the flight or the research programme".

Ironically, on the same day that the cosmonauts talked about their flight at a press conference in Moscow, *Aviation Week and Space Technology* published further claims about the Lyakhov-Aleksandrov mission. During their final weeks aboard the station, it was claimed, the cosmonauts had had to endure "cold, damp and uncomfortable conditions" due to a "solar array problem", that had "reduced electrical power and seriously affected" the environmental control system. The crew's two space walks on 1 and 3 November, *Aviation Week* said, had been necessary to install additional solar panels, in order to save the station from becoming uninhabitable in the near future. When the loss of environmental control became serious, *Aviation Week* claims, the Soviet planners decided to "train and launch" a repair crew — cosmonauts Vladimir Titov and Gennadii Strekalov. When the launch had to be aborted because of a fire in the booster-rocket, however, Lyakhov and Aleksandrov had to carry out emergency repairs themselves.

According to the Soviet flight controllers and the cosmonauts themselves, however, the work on the solar panels had always been "one of the most responsible parts" of the original flight programme. The new panels were needed, they explained, to increase the power supply of Salyut-7 so that more experiments could be performed simultaneously. Deployment of the panels, moreover, had confirmed that the techniques and procedures proposed for large-scale assembly work in space, are, indeed, practicable. It also provided data for evaluating and improving the ground-level training programme for such assembly work in space as well as useful feedback for the designers of space-suits.

The Soviet claim that the deployment of the panels was from the beginning an integral part of the flight programme accords with recent hints that the construction of a permanently-staffed orbital station (always a long-term aim of the Soviet space programme) is now a definite project. Moreover, if it were simply an emergency repair job, carried out in the absence of the trained "repair team", it is remarkable that Lyakhov and Aleksandrov could carry it out without, apparently, undue difficulty. According to their own account, however, the cosmonauts had carefully practised the whole procedure (in the simulation tank) and the flight-plan had been worked out so that the main operations were carried out within the radio-visibility zone and in the illuminated part of the orbit. The special equipment, moreover, used in deploying the panels included not only tools and manipulators, but also non-slip safety handrails — once again, substantiating the Soviet claim that this was a pre-planned "experiment", not an emergency repair job. **Vera Rich**

French get hooked

JUST before Christmas the French post office, PTT, inaugurated "Minitel", an electronic telephone directory designed to lead the French market into the Elysian Fields of the "hooked-up home".

Minitel has been on trial in Brittany and Versailles, but now the experiment has been extended into part of Paris. By March, there should be 50,000 sets operational in the centre of France, and 3 million should be in action by 1986.

Basically, Minitel is designed to replace paper directories: a user dials 11 to link up to a local computer, and then — using a keyboard — answers a few questions to tell the computer the name (and/or address) whose number is sought. The answer is displayed on a small television screen. But of course, with such technology in place many other uses spring to mind — one option is screen-to-screen communication between two Minitels, so the deaf can use the telephone.

In Velizy-Versailles PTT has offered other services: banking, cinema booking, rail timetables, stock exchange prices, even a newspaper; and the take-up has been moderate. One-third of the Minitel owners (who like the others in the experiments have been given them free, though those outside experimental areas can rent them at £6 a month) do not use the machines at all; but the other two thirds use them an average of 40 minutes a week. The biggest users are those with children between 12 and 15; the smallest, retired people.

Where people have been offered the choice of a Minitel or a conventional directory, 46 per cent have chosen Minitel. It is nearly the figure forecast by PTT (50 per cent), but not enough yet to encourage PTT to abandon its printing machines.

Robert Walgate

Smallpox eradication

Military use of vaccine criticized

Washington

NEARLY four years after smallpox was universally eradicated, the US military is still administering the vaccine as a "defensive and deterrent measure" against the use of smallpox virus as a biological weapon. This policy has put the US Army indirectly at odds with the World Health Organization and the US Centers for Disease Control (CDC), both of which have been working to eliminate all use of the vaccine. 'We've had several deaths and significant morbidity from its use for non-indicated reasons", says Dr Stanley Foster, assistant director of CDC's international health programme office. The main civilian users have been physicians who either did not know that smallpox had been wiped out or who believed that smallpox vaccine was effective against genital herpes. CDC last spring persuaded Wyeth Laboratories, the only remaining manufacturer of the vaccine in the United States, to take the product off the commercial market. Wyeth had at last count been selling some 50,000 doses per year.

Wyeth is, however, continuing to supply the military. All active duty personnel and reserves are vaccinated on a five-year schedule — which amounts to several hundred thousand doses per year. The military has also requested Wyeth to supply a stockpile of the vaccine sufficient to dose a classified number of personnel in the case of a general call-up.

Adverse reactions to the vaccine, which contains live vaccinia virus, are rare, but not insignificant. According to CDC, one death can be expected for every million persons vaccinated. Generally less severe reactions (although sometimes fatal themselves) occur more frequently, mostly in certain susceptible groups — immunocompromised subjects (about 7 reactions per million vaccinations) and those with eczema (38 per million).

Dr James Kirkpatrick, an army lieutenant colonel who advises the Army Surgeon General's office on the smallpox vaccination programme, says that by exempting personnel with eczema (or who have a family member with eczema), the military has kept the rate of adverse reactions quite low. All vaccinations (including measles, rubella, tetanus, smallpox and a half dozen others) resulted in 60 hospital admissions a year, he said, with an average hospital stay of two days. Although no numbers are available for the rate of adverse reactions specifically from smallpox vaccinations, CDC says it receives about one request per month for vaccinia immune globin, which is used to treat reactions to the vaccine. All such requests since last spring have been from the military.

The military's reason for vaccinating against smallpox appears to be intelligence information that the Warsaw Pact nations and the People's Republic of China vaccinate their troops. "Nobody can rule out the possibility" that smallpox would be used as a weapon, Kirkpatrick says, and it "would not be prudent" to stop vaccinating. According to CDC, only the United States and the Soviet Union continue to maintain stocks of the smallpox virus. South Africa recently and with much fanfare announced the destruction of its stocks.

Although CDC has been careful not to criticize the military programme directly, CDC officials are known to be uneasy. And a number of smallpox researchers have questioned whether the supposed threat of smallpox as a biological weapon outweighs the known risks of vaccination. Smallpox has an 8–10 day incubation period and is not very infectious. Epidemiologic evidence shows that 30–40 per cent of susceptible members of a household will catch the disease from an infected member, compared with 80–90 per cent for measles, for example. They also point out that smallpox virus would live only a matter of hours in an aerosol form, the presumed method of delivery as a weapon.

The use of smallpox vaccine by civilian physicians as an unorthodox treatment for genital herpes has been a bizarre footnote to the story. Controlled studies have demonstrated no benefit in preventing or healing herpes lesions; CDC has nonetheless received many calls from herpes patients protesting against CDC's role in removing the vaccine from the market and claiming that the vaccine was the only thing that had helped them. CDC officials say that some physicians had also apparently been using the vaccine as a general "tonic", supposedly to boost a patient's immune defences.

Stephen Budiansky

US dioxin question is tackled

Washington

A MAJOR new programme to deal with dioxin has been unveiled by the Environmental Protection Agency (EPA), fulfilling a promise made by William Ruckelshaus when he became its administrator last spring. The programme will include investigations at hundreds of chemical plants and waste disposal sites at a cost of up to $250 million over four years. Alvin Alm, EPA's deputy administrator, says the agency regards dioxin as one of the most perplexing and potentially dangerous chemicals ever to pollute the environment. The programme would concentrate on what was considered the most toxic of the 75 dioxin isomers — 2,3,7,8-TCDD, which is known to cause chloracne in humans. In laboratory animals, it has been known to cause cancer, reproductive failure, reduced effectiveness of the immune system and significant changes in enzyme systems. EPA's Cancer Assessment Group says it should be regarded as both an initiator and a promoter of cancer.

The agency's top priority will be an investigation of sites at which the herbicide and feedstock chemical 2,4,5-TCP was manufactured or disposed of. The chemical was used in the manufacture of 2,4,5-T and silvex, well-known herbicides widely used until recently to control weeds and as defoliants. 2,4,5-T was an ingredient of Agent Orange, the defoliant used in Vietnam. In a background document, EPA admits that little is known about the effect of dioxin on humans. A single dose of less than a millionth of a gram can kill a guinea pig, but its potency varies enormously between species. Information on the effects of human exposure is scarce. It is agreed that dioxin can cause chloracne but it has not been proved that it can also cause soft tissue sarcoma.

The uncertainty has resulted in a chequered history of regulation, and in October last year the Dow Chemical Company voluntarily cancelled its registration of the products.

Peter David

Richter to head SLAC

Washington

BURTON Richter has been named to succeed Wolfgang Panofsky as director of the Stanford Linear Accelerator Center (SLAC). Richter, who won the Nobel Prize for Physics in 1976 (with C.C. Ting) for the discovery of the psi particle, will take up the post next September. Richter is the designer and prime mover of SLAC's linear collider, on which construction began two months ago. The $113 million machine consists of a one-and-a-half mile loop attached to the main linear accelerator; electrons and positrons accelerated in the linear stretch will collide in the loop at centre-of-mass energies of up to 100 GeV. The linear collider, like the much larger LEP machine under construction at the European Organisation for Nuclear Research (CERN) at Geneva, will find its greatest application in detailed studies of the Z^0 particle. The electron-positron collisions are much cleaner and easier to interpret than the proton-antiproton collision used at CERN last year to detect the heavy bosons (Z^0 and W^+/W-). Unlike LEP, however, the linear collider will be breaking new ground in accelerator design — a design that Richter says will yield considerable cost savings over LEP's traditional ring structure.

Panofsky, who has directed SLAC since 1961, decided to follow his own policy of retirement at the age of 65 for the laboratory's administrators but will act as a technical adviser. Stephen Budiansky

Releasing "ice-minus" bacteria

SIR — Two strategies for the biological control of those bacteria that act as seeds for ice-crystal formation (and the consequent crop damage) have recently been presented. Researchers at the University of Colorado propose spraying fields with bacterial viruses (bacteriophage)[1], while researchers at the University of California propose spraying fields with genetically engineered bacteria whose "ice-nucleation gene" has been deleted[1,2]. Most of the media attention has gone to the latter stratagem, largely because of the controversy surrounding the release of recombinant DNA organisms into the environment[2,3]. However, successful implementation of these strategies may be limited by ecological and evolutionary factors subsequent to the deployment of predators (bacteriophage) or competitors (recombinant bacteria). The application of bacteriophage to populations of sensitive bacteria engenders intense selection for phage-resistant mutants[4] whose increase could defeat future, if not present, control. The application of recombinant "ice-minus" bacteria requires that they outcompete naturally occurring ice-nucleating forms[2], whereas the arbitrary deletion of an evolved gene would likely be disadvantageous, or at best neutral.

An integrated strategy might overcome these difficulties, and might even begin to counter some of the fears associated with releasing recombinant bacteria. Bacteriophage infect bacterial cells by first adsorbing to some surface protein or other component of the cell wall; phage are generally very specific in their site of adsorption[5]. By comparing the sensitivities of "ice-minus" and "ice-plus" isogeneic bacteria to a diverse set of bacteriophage, one might identify a phage whose site of adsorption is the ice-nucleating surface protein. By applying this bacteriophage in concert with the "ice-minus" recombinant bacteria, one would provide the "ice-minus" phage-resistant bacterium with a strong selective advantage over the "ice-plus" phage-sensitive natural counterpart. Moreover, as phage-resistant mutants appeared among the naturally occurring bacteria, they too would probably be "ice-minus" since resistance to phage typically arises via the alteration or loss of function of the gene encoding the specific surface protein to which the phage adsorbs[5]; even if such phage-resistant mutants were not "ice-minus", selection favouring their increase would be ameliorated by high densities of the phage-resistant recombinant competitor. Finally, with this strategy, it should be possible to release "disarmed" strains of "ice-minus" recombinant bacteria — recombinant strains bearing genes (or deletions of genes) which ensure their competitive inferiority outside the range of simultaneous application of bacteriophage, yet whose phage-resistance renders them selectively favoured within the range of simultaneous phage application. Thus, the spread of recombinant bacteria would be prevented by virtue of their competitive inferiority coupled with their inability to support the very phage whose presence is necessary to permit their establishment.

Is there a precedent for this strategy? Williams Smith and Huggins[6] successfully treated experimental bacterial infections in mice using bacteriophage, precluding the rise of phage-resistant bacterial mutants in a manner similar to that proposed above. First, pathogenicity of the target bacterium was shown to depend on the presence of a particular surface antigen[7].

Second, a bacteriophage was isolated whose site of adsorption is that surface antigen. Third, it was shown that the bacteria became resistant to the phage through the loss of that surface antigen. In essence, Williams Smith and Huggins chose phage for which resistance was incompatible with pathogenicity of the target bacterium. Whether phage can be isolated for which resistance is incompatible with ice-nucleation by the target bacteria remains to be seen.

RICHARD E. LENSKI

Department of Zoology,
University of Massachusetts,
Amhurst, Massachusetts 01003, USA

1. Miller, J.A. *Science News* **124**, 132 (1983).
2. David, P. *Nature* **305**, 262 (1983).
3. Budiansky, S. *Nature* **305**, 564 (1983).
4. Levin, B.R. & Lenski, R.E. in *Coevolution* (eds Futuyma, D.J. & Slatkin, M.) 99–127 (Sinauer, Sunderland, Massachusetts, 1983).
5. Schwartz, M. in *Virus Receptors* Part 1, *Bacterial Viruses* (eds Randall, L.L. & Philipson, L.) 59–94 (Chapman and Hall, London, 1980).
6. Williams Smith, H. & Huggins, M.B. *J. gen. Microbiol.* **128**, 307 (1982).
7. Williams Smith, H. & Huggins, M.B. *J. gen. Microbiol.* **121**, 387 (1980).

How "gradual"?

SIR — The recent article by Rhodes[1] on "Gradualism, punctuated equilibrium and the *Origin of Species*" is a major event in clarifying Darwin's views on both rates of evolution and "gradualism". The claim that "gradualism" refers specifically to long-term regular changes in the geological record[2] has led to much confusion. It is now clear[1] that Darwin's views on gradualism should be understood on a shorter biological or ecological time scale[3], and that they do not require (nor could they explain) slow, long-term, orthogenetic changes in the fossil record.

There is one point that has not been covered and which helps to establish how (apart from misquotation[3]) the misunderstanding arose. It may also help to prevent such errors in future. This is that terms such as "fast", "slow" and particularly "gradual" have no scientific relevance unless they are used with respect to a specified time scale.

The term "temporal solipsism" has been used by Kamen[4] to describe the confusion that arises when conclusions are applied to the wrong time scale. His example is photosynthesis and he pointed out that it can be studied over many different time scales. Radiation physicists study quantum processes that occur within 10^{-15} – 10^{-9} seconds, or even longer. This "era" extends over at least six orders of magnitude, and overlaps with the time frame of photochemistry which studies events from 10^{-9} to about 10^{-4} or 10^{-3}s. Biochemists study events occurring from about 10^{-3} up to 10 s, and physiologists extend the range up to 10^{4}s. Crop physiologists, farmers and ecologists extend the domain to at least 10^{9}s. But long-term evolutionary changes such as the relative sensitivity of carboxylating enzymes to CO_2 and O_2 (ref. 5), development of C_4 photosynthesis and crassulacean acid metabolism, extend into the domain of more than 10^{16}s.

Given this extraordinary range of 10^{-15}–10^{16}s, it is clear that "fast", "slow" and "gradual" are meaningless unless a temporal frame of reference is given. The interpretation suggested is that some palaeontologists have shown "temporal solipsism" by assuming that gradualism referred specifically to a geological time scale. It is clear[1,3] that Darwin, in writing a book for a general readership, was referring to a biological or ecological time scale[1,3] in discussing small, gradual changes between generations. Such a process does not predict orthogenetic trends in the fossil record.

DAVID PENNY

Department of Botany and Zoology,
Massey University,
Palmerston North, New Zealand

1. Rhodes, F.H.T. *Nature* **305**, 269 (1983).
2. Eldredge, N. & Gould, S.J. in *Models on Palaeontology* (ed.Schopf, T.J.M.) 82–115 (Freeman, Cooper, San Francisco, 1972).
3. Penny, D. *Syst. Zool.* **32**, 72 (1983).
4. Kamen, M.D. *Primary Processes in Photosynthesis* (Academic, New York, 1963).
5. Miziorko, H.M. & Lorimer, G.H. *A. Rev. Biochem.* **52**, 507 (1983).

Be kind to flies

SIR — E.G. Gray's method for swatting flies[1] may work[2] but must always result in the death of the fly which cannot be justified unless the insect in question really is a health hazard. Last Christmas I bought my arachniphobe family a simple device called a "Spider Scoop". This consists of a transparent plastic dome mounted scissor-like on a broad plastic base. The dome is put over the offending arthropod and the base gently brought into place trapping the animal inside it. The principle resembles that of the card and beaker technique[3]. I recommend this method of fly trapping as efficient, clean and kind.

F.E.G. COX

Department of Zoology,
King's College London,
London WC2R 2LS, UK

1. Gray, E.G. *Nature* **304**, 678 (1983).
2. Miller, A. *Nature* **305**, 664 (1983).
3. Kruythoff, P.N. *Nature* **305**, 570 (1983).

New window on Tibetan tectonics

The latest reports from the Sino-French expedition in Tibet show a rich and surprising harvest of discovery. Has not the time come for more open access to this important region?

TIBET has always been a mysterious place. In the nineteenth century, wide-eyed explorers wrote of the region with an innocence unclouded by the circumstance that Marco Polo had passed that way some centuries earlier. Physical isolation has all along been enhanced by human geography, and in particular by the self-imposed isolation of the Tibetan people, broken chiefly by expeditions bent on climbing Mount Everest. The consequence is that very little has been learned at first hand of the most rudimentary geological characteristics of this remarkable region and that many of the attempts in the past few years to relate the tectonic structure Tibet (and much of the rest of central Asia) to the tectonic collision of the Indian subcontinent with the Asian land-mass have had to rely to an unhealthy degree on inferences from topographical features.

Now, at last, there is a good chance that some of these uncertainties will be removed. The group of articles appearing on pages 17–36 is the second instalment of the fruits of the Sino-French collaboration on the geology of Tibet (see also *Nature* **294**, 405-417; 1981). In two successive seasons (1980 and 1981), modern techniques have for the first time been applied to the study of the gross tectonic structure of Tibet. By means of seismic records from artificial explosions, it has in particular been possible to map beneath Tibet the Mohorovicic discontinuity which is probably the best indicator of the continental basement. The result is both important and surprising.

That the collision of the Indian subcontinent with the Asian underbelly must have had a dominating influence on the structure of central Asia has been evident since it was first recognized that the Indian subcontinent traversed the whole length of the Indian Ocean from about 50 million years ago until its collision with Asia rather more than 20 million years ago. The join between the two continents is recognizable in the long east-west suture along part of which the Brahmaputra flows eastwards, north of the Himalayas. The Himalayas themselves are explicable as tectonic consequences of the collision between two land-masses — formed from material uplifted from the northern leading edge of the Indian subcontinent and partly metamorphosed in the process.

In recent years, the bigger puzzle has been that of understanding what may have happened north of the suture, beneath Tibet itself. The great elevation of central Asia suggests that an ancient continental crust has been thickened, while the predominantly east-west alignment of major features between the Caspian Sea in the west and mainland China in the east suggests crustal shortening in a north-South direction and extension at right angles. Whether the elevation of central Asia might be explained by the addition of Indian material to the underlying basement, almost as if the southern continent were sliding beneath Asia, has been a lively issue amongst geophysicists.

What now emerges from the Sino-French study of Tibet along two north-south traverses is that the simple picture of a once-and-for-all collision is too simple. The most striking result of the data gathered by the 1981 expedition is that there is a second suture in the tectonic structure of Tibet which, on the face of things, is in every way comparable with the southern suture but some hundreds of kilometres further north. The inference is that the collision of India with the underside of Asia was preceded some tens of millions of years earlier by the collision of another continental fragment with the same region. And that layer of material has been thickened, and elongated from the east to west, between the anvil of the main body of Asia to the north and the still-moving hammer of India, now shown to be moving relative to the Tibetan blocks at about 5 centimetres a year.

The evidence for this new view of the recent history of south-central Asia is impressive. Palaeomagnetic measurements show that the Lhasa block, the first tectonic structure north of the Himalayas, was sited at or south of the Equator in Cretaceous times. In the region covered by the Sino-French surveys, the continental basement is a patchwork suggesting that the high plateau of central Asia is made up not simply of two fragments (the Indian subcontinent and its predecessor) but of several. And there is evidence to suggest just how relics of past continental fragments underline each other, contributing to the thickening of the continental crust throughout this region.

For those with an interest in the evolution of Gondwanaland, these developments must be portentous. On the simplest reconstructions, Gondwanaland is an amalgam of those continents lying on or south of the Equator. Both the manner of their assembly into a larger continent and the timing of their dispersal are reasonably well catalogued. But the reconstruction of the supposedly earlier super-continent in which the northern land-masses are also combined has always been more speculative and less certain.

If now it is clear that the shapes of many of these structures have been distorted by later tectonic events, the awkwardness of more ambitious reconstructions will be better understood. But naturally, the question will be sharpened why a whole series of continental fragments should have been sent scurrying north across the Indian Ocean in the past few hundred million years. Why in that direction and not some other? Or was the assembly of southern Asia an even more complicated process than it now appears to have been?

For others, the work now published is still not enough to satisfy legitimate curiosity about this geologically important region. Moreover, it is clear that what has been accomplished in a few short seasons of work with modern equipment is merely a scratching of the surface. In an ideal world, there would now follow a systematic programme of exploration designed to amass the details on which accurate interpretations can alone be based. (Why, in any case, should there have been a single land mass in the early Cambrian?)

The obvious snag is that Tibet is almost as inaccessible now as in the days before its annexation by the People's Republic of China. The collaboration with the French is the only significant opening to the outside world so far. In everybody's interests, those of the people of China especially, there need to be many more. And readers of the articles now published will be struck by the irony that the 1981 Sino-French expedition was prevented for administrative reasons from continuing north beyond Tibet into China proper. The time has surely come when such restrictions should be swept away.

John Maddox

Sir Charles Frank

IN an article on snowflakes in *Nature* on 3 November (**306**, 13; 1983), it was erroneously stated that Professor F.C. Frank (whose name was mis-spelled) of the University of Bristol had died in 1982. *Nature* is glad to report that this is not the case and wishes to apologize to Sir Charles, his friends and colleagues and its other readers for this embarrassing mistake. □

0028-0836/84/010009-01$01.00

Neurological disease

Of Mice and Men

from J. B. Martin

SPONTANEOUS autosomal recessive mutations in mice are providing novel neurobiological models for studies of disorders of the central and peripheral nervous systems[1]. The clinical syndromes observed in such mice are given graphic anthropomorphic names: weaver, shiverer, wobbler, totterer, reeler, twitcher, and so on. Such mutants are being carefully investigated in search of clues to the pathogenesis of their disorders, both to gain an understanding of normal development of the brain and to seek hints to the nature of genetic disorders that affect the human central nervous system. Anatomic and biochemical studies of each mutant type have revealed some interesting abnormalities in brain, spinal cord or peripheral nerve. In general, homozygotes are severely affected; heterozygotes less so. Two such mutants have recently been the subject of new biochemical and anatomical investigations. In the shiverer mouse, which fails to synthesize myelin basic protein (MBP) — the most important constituent of the sheath of central nervous system axons — the defect has now been identified as a mutation in the structural gene for the protein[2]. And weaver mice, best known for cerebellar abnormalities, are, in a paper published in this issue of *Nature*, shown for the first time to have a highly selective degenerative disorder of subcortical dopaminergic systems somewhat reminiscent of certain human neurological disorders[4].

The brains of homozygous shiverer mice contain less than one per cent of normal levels of MBP; heterozygotes contain about 50 per cent, indicating a gene dose effect. Demyelination is prominent throughout the CNS and is believed to be a contributory cause of the clinical symptoms.

The recent work by Roach and co-workers[2] is the first successful attempt to relate a structural protein defect to an abnormal gene in mutant mice with neurological syndromes. Working from the known structures of mice and rat MBP, which share close homology[3], the investigators synthesized DNA probes based on the reverse translation of the amino acid sequence of rat MBP. Two cDNA clones encoding MBP were selected and a restriction enzyme fragment from one was completely sequenced. When translated, a portion of this sequence was identical at 126 of 127 positions with the known amino acid sequence for small MBP in the rat. Brains of shiverer mice homozygous for the MBP defect had greatly reduced concentrations of MBP mRNA compared to wild type. With the use of Southern blots for genomic DNA, it was shown that MBP sequences are deleted in shiverer mice. These studies have considerable implications for the investigation of demyelinating diseases in man. Multiple sclerosis, though usually a sporadic not an inherited disorder, has an increased incidence in individuals with certain tissue types (HLA-A3, B7, Dw2) suggesting a genetic propensity for the disorder, at least in some individuals. More important, the fact that rodent (rat and mice) MBP is closely similar in structure to human MBP points to the potential advantage of using such animal models to investigate human afflictions. It is likely that an understanding of the gene transcription and post-translational processing of MBP will increase our understanding of the aberrations of this important protein in the human.

In this issue of *Nature*, Roffler-Tarlov and Graybiel[4] describe an intriguing biochemical and anatomic study of the dopamine abnormality known to occur in the brain of the weaver mutant. Previous studies of this mouse focused on the cerebellum where abnormal migration and death of granule cells were noted[5]. The weaver strain also has a partial deficiency of dopamine in the forebrain[6]. The important contribution of the new report is the demonstration that one dopamine system, the nigrostriatal, is affected, whereas the other, the mesolimbic, is normal. The biochemical deficiency of dopamine in the putamen and caudate is mirrored by an alteration in the specific histofluorescence of the amine-containing neuronal elements. The dorsal striatum, which has a rich innervation from the nigrostriatal tract, shows dopamine depletion, whereas the ventromedial striatum and the nucleus accumbens, innervated by the mesolimbic system, are normal. The authors point to a lack, at the moment, of a similar disorder in humans but make analogies between this genetic disorder and the pattern of cell degeneration in Huntington's disease, an autosomal dominant human disease, recently shown to be associated with a DNA polymorphism on chromosome 4 (see refs 6 and 7). The regional distribution of the cell death that occurs in Huntington's disease is similar. Specifically, the area of the basal ganglia most affected in Huntington's disease is the dorsal lateral region of the caudate and putamen (neostriatum). In contrast, the substriatum, in particular the nucleus accumbens, is relatively spared[8]. The possibility of discovering genetic models of disease with topographic features similar to those present in man is raised by these observations.

The defect in the weaver mouse more closely approximates that of Parkinson's disease, which is usually a sporadic illness, but occasionally occurs as a hereditary or familial disease. The exact nature of the cellular pathology responsible for the dopamine deficiency in the weaver mouse has yet to be defined, and its genetic basis is unknown.

Mutants may also be useful models for exploring new therapeutic approaches. When grafted nerves of twitcher mice, which are defective in the enzyme galactosylceramidase (a model of Krabbe's disease), are transplanted into normal mice, the enzyme defect is corrected after several weeks[9]. Enzyme replacement occurs from adjacent normal tissues pointing to a possible strategy for correcting enzymatic defects. It is likely that future studies of mice mutants will provide additional examples of nature's errors which are applicable to an understanding of human diseases. In search of these, it is evident that defined biochemical changes rather than descriptive anatomic studies will provide the important leads to follow. □

J. B. Martin is Bullard Professor of Neurology in the Massachusetts General Hospital, Harvard Medical School, Fruit Street, Boston 02114.

1. Baumann, N. *Neurological Mutations Affecting Myelination*. (Elsevier/North-Holland Biomedical, Amsterdam, 1980).
2. Roach, A., Boylan, K., Horvath, S., Prusiner, S.B. & Hood, L.E. *Cell* **34**, 799 (1983).
3. Martenson, R.E. In *Biochemistry of Brain* (ed. Kumar, E.) 49–74 (Pergamon, New York, 1980).
4. Roffler-Tarlov, S. & Graybiel, A.M. *Nature* **307**, 63 (1984).
5. Sidman, R.L. *in Physiological and Biochemical Aspects of Nervous Integration* (ed. Carlson, E.D.) 164–193 (Prentice-Hall Inc., Englewood Cliffs, 1968).
6. Lane, J.D., Nade, N.S., McBride, W.J., Aprison, M.N. & Kusano, K. *J. Neurochem* **29**, 349 (1977).
7. Gusella, J. *et al., Nature*, **306**, 234–238 (1983).
8. Von Sattel, J-P, Ferrante, R.J. & Richardson, E.P. *J. Neuropath. and Exp. Neurol.* (in preparation).
9. Scaravilli, F. & Suzuki, K. *Nature* **305**, 713 (1983).

100 years ago

PROF. J.P. Licherdopol writes from Bucharest, Roumania, that on January 1, at 6.13 a.m., two horizontal shocks of earthquake, from north to south and *vice versâ*, were felt there, and were preceded by a loud noise, as of a distant train coming from the north. The furniture was slightly shaken and crackings were heard. The atmosphere was calm, but charged with a very thick and persistent fog.

A series of ornithological observatories has been established throughout Austria-Hungary at the instance of Crown Prince Rudolf, with a view of paying special attention to the migrations of birds, as well as to their breeding habits. The work done by these stations is satisfactory enough; yet it has been found that a complete insight into the periodical movement of birds cannot be obtained so long as similar stations are not spread over the whole globe. The subject is to form one of the principal topics for discussion at the approaching Ornithological Congress, which will be held under the auspices of the Crown Prince at Vienna on April 16 next and the following days.

From *Nature* **29**, 244; January 10th, 1884.

Medical genetics

Molecular diagnostic medicine

from Peter Newmark

FOR too many years those who felt obliged to respond to the taunt that molecular biology, for all the medical research money it had soaked up, had never done anything to help diagnostic medicine, could, at best, just about fall back on the case of thalassaemias. The extent to which the situation has changed in the last few years was well illustrated at a recent Titisee conference* and has since been spectacularly demonstrated in the case of Huntington's disease.

A key to much of the recent progress, including that with Huntington's disease, has been the use of restriction fragment length polymorphisms as genetic markers of disease. Remarkably, they provide a means of detecting the presence of a hereditary genetic lesion, even when the lesion itself is completely elusive. The theoretical basis of that approach was described in great detail three years ago (Botstein, D., White, R.L., Scolnick, M. & Davis, R.W. *Am. J. hum. Genet.* **32**, 314; 1980).

Restriction fragment length polymorphisms are the fortuitous result of variations in DNA sequence which are detectable because they generate (or eradicate) cutting sites (short, specific sequences of base pairs) for one or another restriction enzyme. Therefore the restriction fragments produced by the enzyme from the two alleles will differ in length. Since fragments of different length can be separated (by agarose gel electrophoresis) and detected with specific DNA probes (Southern hybridization) it is possible to determine (prenatally, if necessary) which form of a polymorphic sequence is carried by any individual and through any family.

To make use of that information for diagnosis it is necessary to find at least one and preferably several polymorphisms that are close enough to the chromosomal locus of the disease to minimize the chances of becoming separated from it by genetic recombination during formation of gametes.

It is important to remember that the polymorphisms are not the result of the lesion that caused the disease but simply a pre-existing variation in the population at large. Therefore which of the polymorphic restriction fragment lengths will be linked to the disease in any one family will depend upon which one existed on the ancestral allele in which the lesion arose and from whose bearer the family is descended: the case of Huntington's disease illustrates how two families have a different restriction fragment marker linked to the disease. Moreover, there is always a chance, sometimes high, that the same restriction fragment that characterizes the ancestral lesioned allele will be 'imported' into the family on the same chromosome as an unlesioned allele — hence the necessity for family studies.

In Titisee, Duchenne muscular dystrophy and the Fragile X mental retardation syndrome were discussed as examples of diseases for which restriction fragment length polymorphisms should aid both prenatal diagnosis and, eventually, the ability to identify the mutant gene. Both diseases are known to be located on the X chromosome and so it has been immediately possible to narrow down the hunt for restriction fragment length polymorphisms that lie close enough to the mutant gene usually to remain attached to it. K. Davies (St. Mary's Hospital Medical School, London) briefly described a polymorphism that is loosely linked to the 'fragile site' near the tip of the X chromosome whose breakage is associated with mental retardation; a more closely linked polymorphism is one that is known by its association with the gene for the blood coagulation factor IX (G. Camerino *et al. Nature* **306**, 761; 1983).

For Duchenne muscular dystrophy, Davies offered two polymorphisms, one on either side of the mutant gene. Neither is linked closely enough for confident prenatal diagnosis in affected families but they have nonetheless proved useful for carrier detection, particularly in families where both occur. Unfortunately, between one-third and one-half of muscular dystrophy cases are the result of new mutations and so will never be detectable without prenatal population screening, which is unthinkable.

The distance of each polymorphism from the mutant gene — around 15×10^6 base pairs — will also make difficult the other goal, that of identifying the mutant gene by working in from the polymorphisms. Davies makes the reasonable assumption that the mutation is in a gene that is normally expressed and therefore should be identifiable by an RNA transcript that corresponds to the gene. She estimates that the search is for one transcript in about a hundred that will be specified by the sequences lying between the two polymorphisms.

An alternative approach was described by E. Southern (MRC Mammalian Genome Unit, Edinburgh). It begins with some very rare female sufferers from muscular dystrophy (it being a recessive disease, females are usually only carriers). The female sufferers are characterized by the presence of an X chromosome translocation, always involving the same band of the X chromosome but with different autosomes partaking in the reciprocal cross. Southern's assumption is that the X chromosome breaks at the muscular dystrophy gene on the active member of the pair of X chromosomes, and that the sequence across the breakpoint should therefore lead directly to the relevant gene. (If the gene is not at the breakpoint it must be close enough to it to be inactivated as a consequence of the translocation.) To help overcome the difficulties of the direct approach, his group is also trying to produce a probe for human ribosomal genes that should help them in a specific case where it is the ribosomal genes from chromosome 21 that abut the broken end of the X chromosome as a result of the reciprocal translocation.

Lesh-Nyan is also an X-linked recessive disease but one in which the genetic lesion is known. It is the result of a deficiency of hypoxanthine-guanine phosphoribosyl transferase. However, according to C.T. Caskey (Baylor School of Medicine, Houston), there is seldom a lack of messenger RNA, presumably because the genetic lesion is usually a point mutation. Defects at the level of DNA have been detected in about half of the 28 families investigated but in no two cases are they the same. The optimal means of prenatal diagnosis of Lesh-Nyan disease is not yet certain but the use of restriction fragment

*The International Titisee Conference of the Boehringer Ingelheim Foundations on Impact of Modern Molecular Genetics on Human Biology and Medicine, was held on October 5–9, 1983.

THE International Titisee Conferences, held in Titisee in the Black Forest, have always been sponsored by the German company of Boehringer Ingelheim. Sponsorship used to come through their subsidiary, Dr Karl Thomae GmbH, but for the past year the new Boehringer Ingelheim Foundation, under its director Dr Hasso Schroeder, has taken over the job.

In addition to continuing to hold two or three Titisee conferences each year, the foundation has announced two other ways in which it will support basic research in medical sciences in the Federal Republic of Germany. First, it will sponsor an annual scientific lecture series in which the lecturer not only speaks at several academic centres but also spends a day or two at each as a consultant. Second, it will provide three types of grant. There are to be grants of one to three years for postgraduates working on basic clinical research projects; grants to industrial researchers, not necessarily in the pharmaceutical industry; and grants to cover trips of not more than sixty days to learn a technique or attend a conference.

The foundation has a budget of about DM 1.5 million (£0.4 million) this year and expects to have half as much again next year. Decisions are made by a board of trustees. □

length polymorphisms may prove to be best. Already this seems to be the case for phenylketonuria in which there may also be no consistent or readily detectable lesion accounting for the deficiency of phenylalanine hydroxylase (S.L.C. Woo *et al. Nature* **306**, 151; 1983).

Another, very elegant, use of restriction fragment length polymorphisms (R. White, University of Utah School of Medicine, Salt Lake City) showed that retinoblastoma is the result of development by at least two different mechanisms, of homozygosity for the mutant allele on chromosome 13 (see *News and Views* **305**, 761 and Cavanee *et al. Nature* **305**, 779).

It was left to D. Weatherall (John Radcliffe Hospital, Oxford) to address some of the practicalities of prenatal detection of genetic diseases based on the accumulated evidence of thalassaemias. For example, will the new technique of sampling chorionic villi in pursuit of first trimester diagnosis have an acceptable risk rate? It is too soon to know, said Weatherall. Why is clinical phenotype not always similar for one class of genetic lesion, making genetic counselling that much harder? Co-existent α-thalassaemia can sometimes dampen down the effects of homozygous β-thalassaemia, explained Weatherall, by way of example. Will prenatal population screening for genetic lesions ever be justified? Very rarely. But in the case of β-thalassaemia in Cyprus where 20–30 per cent of the population are carriers, 1–2 per cent of the children are homozygous and will die before reaching adulthood. In 95 per cent of the homozygous children the β-globin mutation is directly detectable and the co-existence of β-thalassaemia that reduces the risk of death from the disease can also be diagnosed. Here, there is a good case for prenatal screening of the population. □

Peter Newmark is Deputy Editor of Nature.

Space plasma physics

Antarctic research by satellite link

from Dyfrig Jones

WHAT do long term experiments in Antarctica have in common with experiments on scientific satellites? One obvious answer is their remoteness. And in what way do they differ? Data from satellites are available in real time and can even be analysed directly by dedicated computers on the ground, allowing scientists, or even the computers, rapidly to change how the experiment is operating. But data from experiments in Antarctica are usually stored on magnetic tape and collected once a year by ship — so producing a long delay between data acquisition and analysis, and an even greater delay before the experiment can be reconfigured. The delay is particularly frustrating in the increasing number of studies where satellite and Antarctic data are correlated in an effort to understand highly complex magnetospheric processes, because the spacecraft data have to be shelved until the Antarctic data arrive. Now, steps are being taken at the British Antarctic Survey, to overcome the problem, by transmitting scientific data from Antarctica to Cambridge via an INMARSAT geostationary communications satellite.

The space plasma physics section of the British Antarctic Survey (BAS) with the physics department, University of Sheffield, has operated VLF radio receiving equipment at Halley Station, Antarctica for a number of years. The main thrust of the research lies in the analysis of natural and man-made radio waves in the frequency range up to 10 kHz, the aim being to understand the generation and propagation of the natural waves and to determine what influence man-made waves may have on the magnetosphere. NASA's satellite Dynamics Explorer 1 is in an orbit admirably suited for correlative measurements with Halley, and a close collaboration exists between BAS and the Universities of Stanford and Iowa, and NASA. It greatly helps the project if the Antarctic data are readily available.

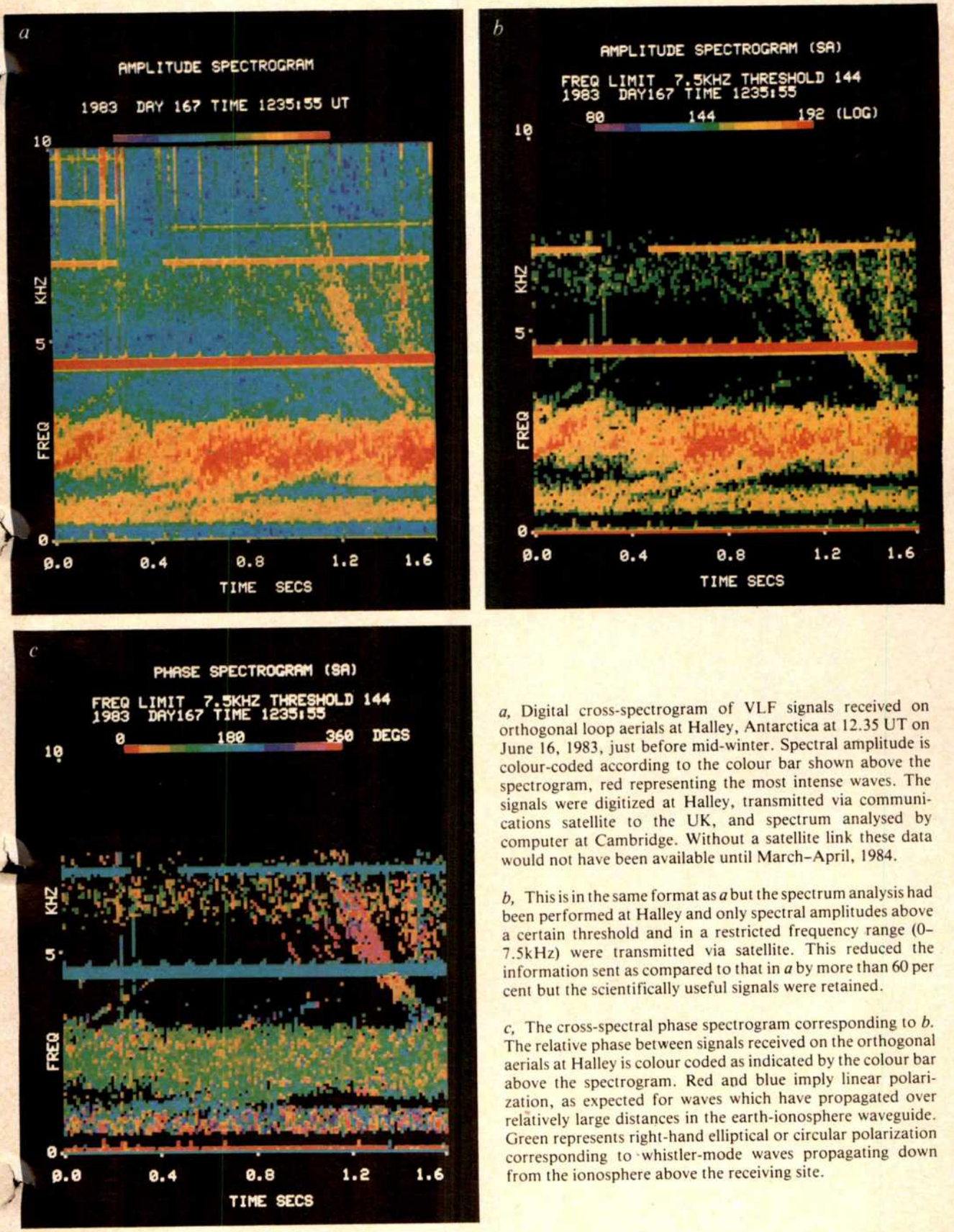
A satellite terminal was installed at Halley in January 1983. The first VLF data were transmitted to Cambridge in July. VLF wave experiments are highly demanding in terms of the amount of information that has to be acquired and telemetered. This is because natural waves, which occur randomly, may vary rapidly in frequency with time. To ensure that most significant events are captured, a wide band, typically 10 kHz, needs to be monitored continuously. Further, studies often require that the waves be recorded on more than one sensor. At Halley, two orthogonal vertical loop antennae are used, the minimum required for obtaining information on the waves' direction of arrival. If signals with a 10-kHz bandwidth are sent in digital form via satellite, 350,000 bits of information need to be transmitted for each second of data. For a bit-rate of 2.4 kbits s^{-1}, each second of data takes 2.5 minutes to transmit. Obviously, there is a strong financial incentive to decrease transmission time, and investigations are under way to see how this can be done without losing scientifically significant data.

On the ESA satellite GEOS, which carried a sophisticated wave experiment, a similar problem was resolved by incorporating a digital correlator on the spacecraft. This first computer in space calculated the auto- and cross co-variance functions of signals which, after reception on the ground, were Fourier transformed to produce dynamic spectra. The technique achieved a data compression factor of 20; this could have been increased with more powerful on-board processing. One technique being investigated using GEOS data by BAS, collaborating with the Laboratoire de Physique et Chimie de l'Environnement, Orleans, is the extraction of signals using cross-spectral phase information. The method is now being applied to Halley data, the preliminary results being shown in the figure. Signals from the orthogonal loops were digitized at Halley and despatched, without pre-processing, to Cambridge via satellite. Cross-spectral analysis was performed on the Cambridge IBM 3081 computer, the resulting spectrogram being shown in *a*. The constant frequency line near 5 kHz propagated from a VLF transmitter at the US station Siple. Intermittent lines at higher frequencies are due to navigation transmitters. The signals sweeping down from 10 kHz to 3 kHz are whistlers, that is, radio waves from lightning in the Northern Hemisphere which have propagated along the magnetic field line to Halley. The analysis of these gives invaluable information on plasma conditions out to 30,000 km from Earth.

The emissions below 3 kHz are natural waves produced in the Earth's magnetosphere. All the blue area is background noise. To remove this, the same data were pre-processed at Halley and only the spectral amplitude above a certain threshold was transmitted via satellite. The result is shown in *b*. Virtually all the signals visible in *a* are retained but the noise has been rejected; the maximum frequency was limited to 7.5 kHz. Figure *c* shows the corresponding cross-spectral phase spectrogram, the phase between signals on the orthogonal antennae being colour-coded. The relative phase of the waves from 1 to 3 kHz is as expected for a right-hand polarized wave propagating down from the ionosphere above the receiving site. The phases of the whistlers and the emissions below 1 kHz are different, implying that they have propagated to the receiver from a considerable distance in the Earth-ionosphere waveguide. Further computations will determine their direction of arrival.

These examples serve to illustrate not only the potential of satellite links for scientific research at remote stations, but also the importance of data pre-processing if the link is to be efficiently utilized. It should be added that the satellite link provides two-way communications and it is envisaged that experiments at Halley will be reprogrammed from Cambridge in much the same way as a satellite experiment is reconfigured by ground command.

Dyfrig Jones is Head of Space Plasma Physics, British Antarctic Survey, Natural Environment Research Council, Cambridge, CB3 OET.



a, Digital cross-spectrogram of VLF signals received on orthogonal loop aerials at Halley, Antarctica at 12.35 UT on June 16, 1983, just before mid-winter. Spectral amplitude is colour-coded according to the colour bar shown above the spectrogram, red representing the most intense waves. The signals were digitized at Halley, transmitted via communications satellite to the UK, and spectrum analysed by computer at Cambridge. Without a satellite link these data would not have been available until March–April, 1984.

b, This is in the same format as *a* but the spectrum analysis had been performed at Halley and only spectral amplitudes above a certain threshold and in a restricted frequency range (0–7.5kHz) were transmitted via satellite. This reduced the information sent as compared to that in *a* by more than 60 per cent but the scientifically useful signals were retained.

c, The cross-spectral phase spectrogram corresponding to *b*. The relative phase between signals received on the orthogonal aerials at Halley is colour coded as indicated by the colour bar above the spectrogram. Red and blue imply linear polarization, as expected for waves which have propagated over relatively large distances in the earth-ionosphere waveguide. Green represents right-hand elliptical or circular polarization corresponding to whistler-mode waves propagating down from the ionosphere above the receiving site.

Particle physics and cosmology

New light on heavy light

from John D. Barrow and R. R. Burman

DURING the past three years it has been extremely fashionable to consider the results of endowing the neutrino with a non-zero rest mass, and there now exists experimental evidence to suggest that this idea may one day pass from the realm of the fashionable to that of the factual[1]. However, while a small neutrino mass has dramatic consequences for astrophysics[2] it has a surprisingly small effect upon the structure of microphysical theories. This is because the neutrino does not mediate the weak interaction and hence the reciprocal of its mass does not determine the range of the weak force. If we choose to endow the photon with a non-zero mass the consequences will be more far-reaching: the masslessness of the photon is responsible for the infinite range and gauge invariance of the electromagnetic interaction.

Classical electrodynamics with a non-zero rest mass is described by the Proca equations[3,4] which reduce to Maxwell's when the photon mass (m_γ) is zero. These equations contain a characteristic length scale, $\lambda = \hbar/cm_\gamma$, and a small non-zero m_γ will lead to significant physical effects on the scale of the photon Compton wavelength λ. The length λ is an effective 'range' of the electromagnetic interaction here and static electric and magnetic fields will exhibit an exponential, $\exp(-r/\lambda)$, fall-off at distances $r > \lambda$ from sources. Other new features include a systematic variation of the velocity of light with frequency and a violation of Amperes law.

In an intriguing letter to *Nature*, Georgi, Ginsparg and Glashow[5] have resurrected the idea of a non-zero photon mass, but with a new twist. They suggest that in addition to the usual low energy symmetries $SU(3) \times SU(2) \times U(1)$ governing the strong, weak and electromagnetic interactions respectively, there exists an additional $U'(1)$ symmetry in nature, and oscillations occur between the different 'photons' which mediate the U(1) and $U'(1)$ interactions. This structure allows the 'ordinary' massless photon to obtain a mass, (see also refs. 6 and 7). The consequent classical electrodynamics would obey Proca rather than Maxwell field equations. This photon oscillation picture provides a natural scheme wherein a photon mass can enter, and is entirely analogous to the more familiar idea of neutrino oscillations. Georgi *et al.* point out some observational consequences that arise even if m_γ is so small as $\sim 5.10^{-18}$ eV/c^2. The oscillation of photons between cosmological relic distributions of the two kinds at 2.9 K (the measured temperature of the cosmic microwave background radiation) leads to small distortions from the precise Planckian spectral form that would arise if m_γ were zero. These distortions happen to be in the same sense and of the same magnitude as the purported spectral distortions measured by Woody and Richards[8] balloon-borne experiment in 1979. It is only fair to say that cosmologists have been wary of drawing any conclusions from the existence of these distortions because of their ambiguous statistical significance and the real possibility of systematic error. More exciting is the prediction of Georgi *et al.* that a definite sinusoidal variation of flux with wavelength should be observed shortward of 2 cm if the above spectral distortions arise from photon oscillations.

The photon mass level of 5.10^{-18} eV/c^2 required for measurable effects to exist is an interesting one. It is consistent with the observational[4,9] limits on m_γ obtained by direct experiments and all other limits obtained until the early 1970s which require only that $m_\gamma \lesssim 6.10^{-16}$ eV/c^2. However, there exist a variety of astrophysical limits on m_γ, which Georgi *et al.* do not consider, that appear to rule out a value so high as 5.10^{-18} eV/c^2 by a very wide margin. They all arise from detailed consideration of astrophysical objects in which magnetic fields, and hence the Maxwellian form of electrodynamics, play a key role in maintaining equilibrium or creating long-lived stable structures. Following a suggestion of Goldhaber and Nieto[4], Byrne and Burman[9] derived an upper limit of $\sim 3.10^{-20}$ eV/c^2 on m_γ by considering the electric current density and concomitant Joule heating in the interstellar medium. Barnes and Scargle[10] produced a similar limit from a study of what appear to be magneto-acoustic waves ('wisps') in the Crab Nebula. The existence of moderately dense, magnetized (1–2.10^{-5} gauss) interstellar gas clouds which appear to have contracted across the Galactic magnetic field, compressing flux, leads to an upper limit on m_γ of about 10^{-25} eV/c^2 in order that the repulsive Proca stresses do not overwhelm the effects of gravity and prevent collapse[9,11]. A similar physical effect would prevent the equilibrium of interstellar gas in the disc of our Galaxy unless $m_\gamma \lesssim 10^{-25}$ eV/c^2. Finally, it has been argued by Chibisov[12] that the magnetically-dominated interstellar plasma observed in the Small Magellanic Cloud would not exist in magneto-gravitational equilibrium unless the photon Compton wavelength, λ, which sets the effective range of the interaction, exceeds the characteristic field scale ~ 3 Kpc. This leads to a very restrictive upper limit on m_γ of about 10^{-27} eV/c^2. All these limits should be regarded as uncertain by about an order of magnitude numerically and each has associated experimental and theoretical uncertainties. We have

Table 1 Summary of limits on m_γ

m_γ limit	Site	Uncertainties
10^{-10} eV/c^2	Variation of light speed with frequency (terrestrial)[4,19]	Irregularity of medium in which light propagates; possible measurement error
5×10^{-12}	Dispersion of radiation from Crab pulsar[4,26]	Dispersion in interstellar plasma mimics the effect of photon mass
10^{-14}	Deviations from Coulomb's law (laboratory)[4,18]	
3×10^{-14}	Terrestrial Schumann resonances[20]	Cavity geometry
2×10^{-15}	Geomagnetic field variation on and near Earth[4,21,22]	Regularity of field; incomplete mapping of field; externally generated fields
4×10^{-16}	Pioneer-10 measurement of Jovian magnetic field[23]	Regularity of field; perturbation by external fields
2×10^{-16}	No low-frequency cut-off for hydromagnetic waves in the Earth's magnetosphere and solar wind[24]	Limited data; interpretation of a positive results could be non-unique
2×10^{-20}	Magneto-acoustic 'wisps' in Crab Nebula[10]	'Wisps' may not be magneto-acoustic waves
2×10^{-20}	Galactic magnetic field[4,9]	Properties and uniformity of interstellar medium
10^{-25}	Interstellar gas clouds[4,9]	Initial conditions in plasma that forms the gas clouds
10^{-25}	Interstellar gas in Galactic disc[4,9]	Regularity of field lines and plasma
10^{-27}	Interstellar gas in Small Magellanic Cloud[12,25]	Regularity of field lines and plasma; theory not presented in detail
10^{-33}	Smallest mass measurable in age of the Universe	

BOX 1: PROCA EQUATIONS

$$\nabla \times \mathbf{E} = -\frac{1}{c}\frac{d\mathbf{B}}{dt}, \qquad \nabla \cdot \mathbf{B} = 0$$

$$\nabla \times \mathbf{B} = \frac{4\pi}{c}\mathbf{j} + \frac{1}{c}\frac{d\mathbf{E}}{dt} - \frac{m_\gamma^2 c^2}{\hbar^2}\mathbf{A}$$

$$\nabla \cdot \mathbf{E} = 4\pi\rho - \frac{m_\gamma^2 c^2}{\hbar^2}\phi$$

A and ϕ are the space-time components of the 4-vector potential $A\mu$, **E** and **B** the electric and magnetic 3-vectors; **j** the current density; ϱ the charge density. The equations are not gauge invariant if $m_\gamma = 0$, and the Lorentz gauge ($d_\gamma A^\alpha = 0$) must be employed for consistency.

mentioned some of these uncertainties in a tabular summary of the experimental situation. It remains to be seen whether the huge superficial conflict between the astrophysical upper limits on m_γ and the value of about 5.10^{-18} eV/c^2 required for observable effects on the 2.9 K background can be side-stepped by a more rigorous scrutiny of the data and a wider examination of the theoretical alternatives.

One of the most interesting consequences of photon oscillations would be, as Georgi *et al.* remark, the striking fact that observations of what might be the lowest energy scale in nature, $m_\gamma \lesssim 10^{-18}$ eV/c^2, would tell us definite things about physics at ultra-high energies $\sim 10^{15}$-10^{19} GeV/c^2. In the last decade the most fruitful approach to the problem of elementary particles has been to follow the unified gauge theory idea to its logical and most elegant conclusion, namely the existence of a 'grand unification' of the SU(3) × SU(2) × U(1) symmetries of the strong and electro-weak interactions within a simple gauge group like SU(5), as first suggested by Georgi and Glashow[13] in 1974. However, if there exists a non-zero photon mass and an SU(3) × SU(2) × U(1) × U′(1) symmetry at low energy, then it is not possible for this low-energy behaviour to have resulted from the breaking of a single grand unified symmetry, like SU(5), at high energy. A proof of this result first appeared in a paper by Calogeracos, Dombey and Kennedy[14] and its truth is realized independently by Georgi *et al.* in their *Nature* letter. This theorem comes about on account of a fundamental difference in behaviour between a theory such as quantum electrodynamics (QED), which is invariant under the Abelian gauge group U(1) (the group under multiplication by complex numbers of unit modulus), and a theory such as quantum chromodynamics (QCD), which has a non-Abelian gauge group (SU(3) in the case of QCD).

Many years ago Stueckelberg[15] showed for QED that, even if the U(1) gauge invariance is broken by the addition of a small photon mass, the theory still exists and the limit $m_\gamma \to 0$ is smooth. Van Dam and Veltman[16], however, showed in 1970 that this was not the case for Yang-Mills theory (or SU(2)), which is the simplest non-Abelian gauge theory. Here, if the gauge particle is given a mass m, the limit $m \to 0$ does not produce the original massless theory (it is, in effect, a 'singular perturbation' of the massless theory). Dombey and Vayonakis[17] then showed that this peculiar behaviour arose from the existence of a longitudinal polarization state when the gauge particle possesses mass. In an Abelian theory gauge invariance causes this longitudinal mode to decouple when $m_\gamma \to 0$ (as it does in classical electrodynamics), whereas in a non-Abelian theory the gauge invariance is more complicated and, as a result, the longitudinal mode fails to vanish in the zero-mass limit.

In a hybrid theory, such as electro-weak SU(2) × U(1) or the SU(3) × SU(2) × U(1) × U′(1) proposed by Georgi *et al.*, the QED situation persists (because of the U(1) subgroup) and there can be an arbitrarily small photon mass. On the other hand, if electrodynamics were contained in a Grand Unified Theory such as SU(5) which, being a simple group, has no U(1) subgroup, then there would be no way of adding a small photon mass term within normal theories without violating charge conservation.

The discovery, therefore, of evidence for photon oscillations in the way suggested by Georgi *et al.* would be dramatic: not only would it show Grand Unification to be a false route towards understanding elementary particles but it would also compromise physicists' central article of faith — gauge invariance — as the most reliable guide to nature's ultimate structure.

Finally, it is worth pondering that a photon mass could well exist at a level below the reach of all direct experimental techniques, however accurate. The Heisenberg Uncertainty Principle gives the lowest mass m that can be measured in the age of the universe $T \sim 5.10^{17}$ sec as $m \sim \hbar T^{-1} c^{-2} \sim 10^{-33}$ eV/c^2 — surprisingly close to the strongest astrophysical limit on m_γ. Photons with a mass less than 10^{-33} eV would have a Compton wavelength greater than the size of the observable Universe, $cT \sim 1.5.10^{28}$ cm. Such incoherent quantum wave functions would, at the very least, shed new light on determinism. □

John D. Barrow and R.R. Burman are in the School of Mathematics and Physics, University of Sussex, Brighton BN1 9QHJ.

1. Lyubimnov, V.A., *et al. Sov. Phys. JETP* **54**, 616 (1980).
2. Barrow, J.D., *Fund. Cosmic Phys.* **8**, 83 (1983).
3. Proca, A. *J. Phys. Radium Ser. VII* **8**, 23 (1936).
4. Goldhaber, A.S. & Nieto, M.M. *Rev. Mod. Phys.* **43**, 277 (1983).
5. Georgi, H., Ginsparg, P. & Glashow, S.L. *Nature* **305**, 765 (1983).
6. Okun, L.B. *Sov. Phys. JETP* **56**, 502 (1982).
7. Barr, S.M., Reiss, D.B. & Zee, A. *Phys. Rev. Lett.* **50**, 317 (1983).
8. Woody, D.P. & Richards, P.L. *Phys. Rev. Lett.* **42**, 925 (1979).
9. Byrne, J.C. *Astrophys. Sp. Sci.* **46**, 115 (1977).
10. Barnes, A. & Scargle, J.D. *Phys. Rev. Lett.* **35**, 117 (1975).
11. Murphy, G.L. & Burman, R.R. *Astrophys. Sp. Sci.* **56**, 363 (1978).
12. Chibisov, G.V. *Sov. Phys. Usp.* **19**, 624 (1976).
13. Georgi, H. & Glashow, S.L. *Phys. Rev. Lett.* **33**, 451 (1974).
14. Calogeracos, A., Dombey, N. & Kennedy, A.D. *Phys. Lett.* **104B**, 444 (1981).
15. Stueckelberg, E.C.G. *Helv. Phys. Acta.* **14**, 51 (1941).
16. van Dam, H. & Veltman, M. *Nucl. Phys.* **B22**, 397 (1970).
17. Dombey, N. & Vayonakis, *J. Phys.* A **9**, 1381 (1976).
18. Williams, E.R., Faller, J.E. & Hill, H., *Phys. Rev. Lett.* **26**, 721 (1971).
19. Al'pert, Y.L., Migulin, V.V. & Ryazin, P.A., *ZH. Tech. Fiz.* **11**, 29 (1941).
20. Jackson, J.D. *Classical Electrodynamics* (Wiley, New York, 1962).
21. Schrödinger, E. *Proc. Roy. Irish Acad* **A49**, 135 (1943).
22. Frank, I.A. *J. geophys. Res.* **72**, 3753 (1967).
23. Davis, L., Goldhaber, A.S. & Nieto, M.M. *Phys. Rev. Lett.* **35**, 1402 (1975).
24. Gintsburg, M.A. *Sov. Astron.* **7**, 536 (1964).
25. Dolgov, A.D. & Zeldovich, Y.B. *Rev. Mod. Phys.* **53**, 1 (1981).
26. Feinberg, G. *Science* **166**, 879 (1969).

Astronomy

Astronomical angular accuracy

from David W. Hughes

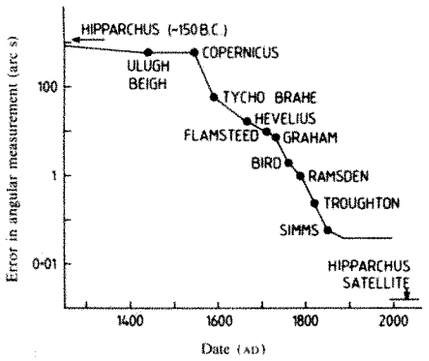
PRACTICAL astronomy before the middle of the nineteenth century was mainly concerned with the measurement and cataloguing of the positions of stars and planets. Large-radius sextants, quadrants and circles were used. Accuracy was paramount, and depended on three main factors: accurate graduation of scales, careful alignment and skilful technique.

In many cases the quest for accuracy was fuelled by the demands of the theoretician. A typical example concerns stellar parallax. The Copernican insistence that the Earth orbited the Sun had the concomitant consequence that close-by stars should execute annual displacements as seen against the more distant 'fixed' stars. Proxima Centauri moves about 1.5 arc s because of this process. Three centuries elapsed between the prediction of this effect and its observation, when the craftsmen and astronomers and their instruments and techniques caught up in the 1830s.

The advance in angular accuracy is summarized in the figure, and the subject has been reviewed by Allan Chapman of Wadham College, Oxford in a recent edition of the *Journal for the History of Astronomy* (**14**, 133; 1983).

Three important developments in instrument-making were responsible for the changes shown in the figure. The first was introduced by Tycho Brahe. He augmented the normal radial scale divisions on the limbs of instruments by interspersing a series of diagonal lines. This enabled him to read the scale to a fraction of a degree whilst still retaining the small compact stable features of the instrument. Brahe also improved the sights of sextants and quadrants, thus eliminating most of the alignment errors.

Before 1660 angular measurement was



The observational error in the astronomical measurement of stellar and planetary positions plotted logarithmically against the year. The data have come from H. Mineur (see H.T. Pledge, *Science since 1500*, HMSO; 1939), A. Chapman (*J. hist. Astr.* **14**, 133; 1983) and *European Space Agency DP/PS(78)13*.

also limited by the 1-arc-min resolution power of the human eye. The adoption of the telescopic sight and the micrometer overcame this barrier and the angular error fell to 15 arc s by 1700 and 8 arc s by 1725. This made it possible to detect stellar aberration (small positional wanderings due to the vectorial addition of the velocity of light to the Earth's orbital velocity) and nutation (an 18.6-year wobble in the Earth's spin axis produced by the gravitational influence of the Moon and planets).

The third breakthrough came in the late eighteenth century. Instrument-makers Jesse Ramsden and Edward Troughton replaced the quadrant with the full circle. Circles could be rotated thus enabling the graduations to be cross-checked with micrometer microscopes. By 1800 the error had been reduced to 0.5 arc s. 1850 saw the installation of G.B. Airy's transit circle at Greenwich. Troughton and Simms were responsible for the optics and instrumentation. Six cross-checking micrometer-microscopes placed at 60° intervals around the circle gave a final accuracy of 0.06 arc s.

Unfortunately the accuracy of astronomical measurements of stellar position has only improved very slightly since then. The accuracy of the main applications of astrometry — the motions of the Moon and planets, the kinematics and dynamics of the galaxy, stellar mass determination and the rotation of the Earth and polar wandering — have also remained almost stationary. The next leap forwards will come from the ESA Hipparchus satellite. This is designed to measure stellar position to an accuracy of ± 0.0015 arcs and stellar parallax to ± 0.002 arcs. □

David W. Hughes is in the Department of Physics, University of Sheffield, Sheffield S3 7RH.

Archaeology

Setting Stonehenge straight

from Christopher Chippindale

FOR years the dismal state of Stonehenge, Europe's premier prehistoric monument, has been a disgrace — and an embarrassment to British archaeology. Always the most famous English ancient monument, it has become even more popular since archaeo-astronomers tried to identify it as a neolithic observatory. Yet many of its visitors, up to 800,000 annually, and two-thirds of them from abroad, find the place a sad let-down — hemmed in by busy main roads, approached through a nasty grey concrete tunnel, and each June the unwilling focus of an immense drug and rock music festival.

Partly it is a simple question of numbers. Stonehenge is rather a small monument — the central ruin with its three standing trilithons is only thirty-five paces across — and the grass there cannot survive the tramp of a million feet. Since 1978 the Department of the Environment (DoE), the government agency responsible, has closed the centre. Instead visitors are shepherded along a tarmac path, camouflaged a chemical shade of green, well away from the stones. In consequence it is impossible properly to see those features, the mortise-and-tenon joints of the lintels, the bluestone settings, the solar alignment of the axis, that make Stonehenge extraordinary and unique. And since aids for visitors are minimal, it is hard to know just what you should be looking out for.

The romantic ideal is to see Stonehenge alone, preferably by moonlight and with a storm brewing (it is still strongly recommended but is now very hard to manage). Over 200 years ago, William Stukeley, the first great student of Stonehenge, grumbled about its 'infinite number of daily visitants'. It was a famous shambles at Victorian bank holidays, with 'vanloads of uproarious humanity, dressed in all the colours of the rainbow, and in many others of aniline origin', and the dominant sound not lark-song but the crash of souvenir-hunters' hammers on the stones[1].

So great was the threat, during the 1920s, of a suburban sprawl of shapeless development — bungalows, cafés, an intensive pig-farm — crowding in on Stonehenge, that a public appeal bought 700 hectares of surrounding land and gave it to the National Trust, which would preserve Stonehenge for ever in a sweep of empty chalk downland. There were schemes to suppress the main A344 road which ran a couple of metres from the Heel Stone, so that the "circle itself seems almost submerged by the congestion, vulgarity, speed, and noise". But the road stayed open and soon spawned a car-park hard by the stones. (Calls for lavatories were at first resisted: "after all, the whole Plain is available for the convenience of the public".) Over the years, more facilities have grown on that site — extra space for cars several times, lavatories (both underground and up in the air), a café, souvenir shop, and bunker for the DoE's guardians — as well as a tunnel access-way to save the dangerous crossing of the road[2].

After four decades of such piecemeal growth, Stonehenge presents a classic case of how a cultural resource should not be managed. The main road across its edge is still open. The facilities are too close and too obtrusive, but because the site is cramped they are also inadequate. Management is bureaucratic and remotely directed from London, while the DoE shows no sign of imagination or initiative, or even of noticing the enormous advances being made elsewhere in managing and displaying historic monuments. It has suppressed the report of an independent working party[3] which recommended new facilities at a sensible distance and on a sensible scale. Instead it wants to enlarge on the present site, which is on the land bought by public subscription expressly to prevent tasteless development. The National Trust, recently bruised by a very public row over letting the air force develop a military bunker on another of its holdings, is, with every reason, declining to cooperate. And since the National Trust is the actual landowner, it has an effective veto.

In April 1984, the DoE will lose control over Stonehenge, which will pass to a new, autonomous Historic Buildings and Monuments Commission. Certainly, the new commission will know what a disaster it inherits — one of its commissioners calls Stonehenge today a 'squalid scandal' — and there is every hope it will abandon the fossil attitudes of the old regime.

The downs around Stonehenge contain, within a few square kilometres, an astonishing concentration of prehistoric sites[4], currently the subject of remarkable fieldwork by Julian Richards of the Trust for Wessex Archaeology. That ancient landscape, with its barrows, henges, cursuses and boundary earthworks, is the environment into which its builders put Stonehenge. A new policy for Stonehenge gives the chance of an imaginative scheme to show the place in that context — to set it once again, as William Stukely explained in 1740, "in clean and distinct areas, distant from profane buildings and traffic"[5]. □

Christopher Chippindale is at the Department of Archaeology, University of Cambridge CB2 3DZ.

1. C. Chippindale, *Stonehenge Complete* (London 1983).
2. C. Chippindale, *Antiquity* **57**, 172 (1983).
3. Stonehenge Working Party *Report to the Department of the Environment* (1979, unpublished).
4. RCHM(E), *The Environs of Stonehenge* (Edinburgh 1979).
5. W. Stukeley, *Stonehenge* (London, 1740).

Structure and evolution of the Himalaya–Tibet orogenic belt

C. J. Allègre*, V. Courtillot*, P. Tapponnier*, A. Hirn*, M. Mattauer†, C. Coulon‡, J. J. Jaeger§, J. Achache*, U. Schärer*, J. Marcoux‖, J. P. Burg†, J. Girardeau*, R. Armijo*, C. Gariépy*, C. Göpel*, Li Tindong¶, Xiao Xuchang¶, Chang Chenfa¶, Li Guangqin¶, Lin Baoyu¶, Teng Jiwen¶, Wang Naiwen¶, Chen Guoming¶, Han Tonglin¶, Wang Xibin¶, Den Wanming¶, Sheng Huaibin¶, Cao Yougong¶, Zhou Ji¶, Qiu Hongrong¶, Bao Peisheng¶, Wang Songchan¶, Wang Bixiang¶, Zhou Yaoxiu¶ & Ronghua Xu¶

* Institut de Physique du Globe, Université Paris 6, UER Sciences Physiques de la Terre, Université Paris 7, 75230 Paris Cedex 05, France
† Laboratoire de Géologie Structurale, USTL, 3400 Montpellier, France
‡ Laboratoire de Pétrologie, St Jérôme, 13397 Marseille, France
§ Laboratoire de Paléontologie, UER Sciences de la Terre, Université Paris 6, 75230 Paris Cedex 05, France
‖ Laboratoire de Géologie, Université de Reims, 51000 Reims, France
¶ Academy of Geological Sciences, Ministry of Geology, Baiwanzhuang Street and Academia Sinica, Beijing, China

The 1981 French–Chinese expedition to Tibet focused on the Lhasa block, extending earlier coverage 400 km north of the Tsangpo suture. The Lhasa block stood between 10 and 15° N latitude over most of the Upper Cretaceous and Eocene and, if Gondwanian in origin, had detached from Gondwana by early Permian. Seismic profiles reveal a complex Moho topography resulting both from multiple continental thrusting and large-scale strike-slip faulting. Subduction related granitoids representing mixtures of mantle and crustal components and anatectic granitoids have been analysed and dated. This study emphasizes the role of smaller blocks in the accretion of the continental mosaic.

A 3-year programme of French–Chinese cooperation for the study of Tibet and the northern Himalayas began in 1980. Preliminary results of the first campaign have already been published[1–4], and a second campaign, started in 1981, has yielded much additional data and has clarified several significant problems. The 1981 results have been discussed at a French–Chinese conference[5] and here we present a synthesis of the major results. Details of observations, laboratory measurements or modelling in each specialized field will be published separately. Some preliminary results from the 1982 campaign have been used when available.

We will summarize our results on a north–south cross-section, supposed to run from 28° to 32° N lat. not far from the longitude of Lhasa (Fig. 1). A geographical map of the area is shown in Fig. 2. Information gathered along trails which are perpendicular to the main trend of the section will be included whenever necessary. Observations start at the main central thrust (MCT) in the vicinity of Katmandu (Nepal) and extend northwards to the small town of Anduo, close to the administrative boundary between Xizang (Tibet) and Qinghai provinces.

Blocks and sutures

From a palaeogeographic standpoint, three blocks separated by two major suture zones have been recognized (Fig. 1). The Indus Tsangpo suture (ITS) marks the boundary between the Indian continent to the south and the Lhasa block to the north. The Lhasa block is in turn bounded to the north by the Bangong–Nujiang suture (BNS), with the Qangtang (or Tanggula) block lying to the north. On the basis of earlier work[6], at least two other sutures, the Kokoxili (KS) and Chilien (CS) sutures, can be recognized farther north, before reaching the Tarim basin (Fig. 1). The four sutures will be labelled ITS, BNS, KS and CS with increasing age from south to north. The identification of three blocks and two sutures in southern Tibet has been proposed by Chang and Cheng[6] and has been corroborated by further work, including French–Chinese work in 1980 and 1981[1,2,5]. The key observations for such a distinction are stratigraphical and are summarized in Fig. 1. We present here additional constraints provided by new data.

Earlier palaeomagnetic results for the Lhasa block come from the Middle to Upper Cretaceous Takena formation (red beds and Orbitolina limestones) and the Lingzizong volcanic formation of Palaeocene to Eocene age, in the vicinity of Lhasa[4,7]. The authors concluded that the Lhasa block stood at a latitude of 20° N 90-Myr ago and at 10° N 65-Myr ago. Our results confirm only (and partly) the second of these results. During the 1981 mission, sampling has been extended north to Anduo. Laboratory measurements on 18 sites in Takena red beds revealed a multicomponent magnetization. A pre-folding stable component can be confidently isolated, with positive fold tests at a confidence level higher than 99%. Palaeomagnetic data do not indicate any significant latitudinal movement within the Lhasa block, suggesting that it formed a single unit before folding of the Takena formation (around 70 Myr ago?). Although it cannot as yet be demonstrated whether this magnetization is primary or a remagnetization acquired just before folding, the fact that all sites show a normal polarity suggests that magnetization was acquired before 80 Myr, the end of the Cretaceous Long Normal Interval. The corresponding palaeolatitude of Lhasa, possibly as late as 80 Myr, is 12° N. At the same time, the northern margin of India may have been between 20 and 30° S. Results from nine sites in the Palaeocene–Eocene Lingzizong formation indicate a palaeolatitude close to 15° N. Very few precise isotopic ages are available for the Lingzizong formation[7,8]: they are a $^{40}Ar/^{39}Ar$ age of 60 Myr on an andesite and a K–Ar age of 48 Myr on an ignimbrite. Thus it appears that the Lhasa block remained approximately stationary in latitude for a period that extended at least from 80 to 60 Myr and may have been as long as from 100 to 48 Myr. Since the suturing of India to Asia, the block has moved about 20° north.

The most significant piece of new palaeontological information is given by fossil plant remains from the Takena formation. Immediately north of Lhasa, but also further north near Lake Peng-Co, close to Barda, new species of silicified woods

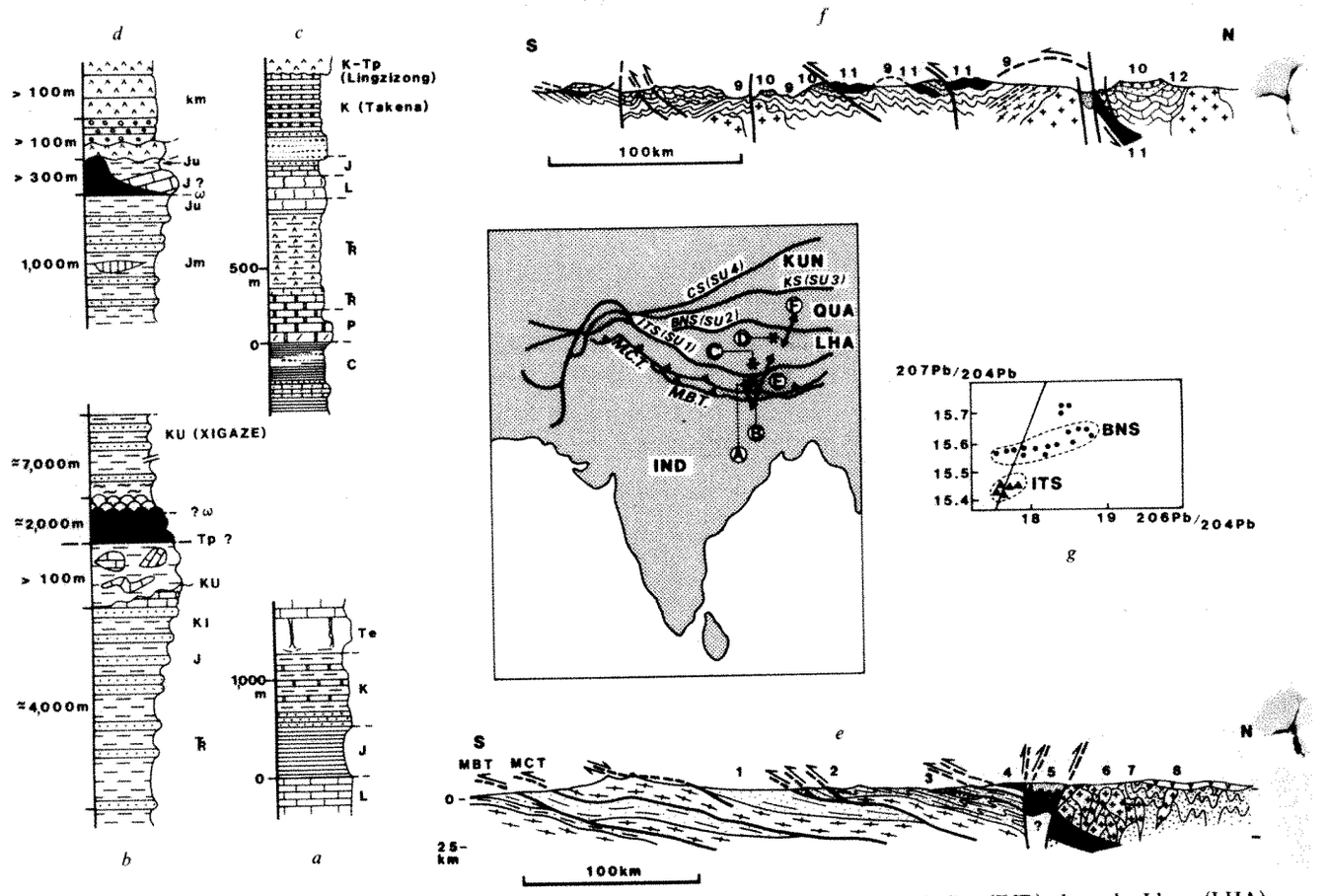


Fig. 1 Major tectonic blocks and boundaries in the Tibet–Himalaya. From south to north are the Indian (IND) plate, the Lhasa (LHA), Quantang (QUA) and Kunlun (KUN) blocks and the Asian (ASI) plate and the main boundary thrust (MBT), main central thrust (MCT) and Indus Tsangpo (ITS or SU1), Bangong Nujiang (BNS or SU2), Kokoxili (KS or SU3) and Chilien (CS or SU4) sutures. Stratigraphical sections from the southern (*a*) and northern (*b*) parts of South Tibet and from the southern (*c*) and northern (*d*) parts of the Lhasa block (see ref. 1 for details of sections *a* and *c*). *e*, Schematic cross-section through the ITS (see also ref. 37). 1, South Tethyan series (Indian shelf sediments); 2, North Tethyan series (Triassic and Jurassic of the Indian continental rise); 3, Upper Cretaceous or Palaeocene (?) wildflysh; 4, ophiolite series; 5, Xigaze group flysh; 6, Gangdise plutonic belt; 7, folded Mesozoic series of the Lhasa block (including Takena red beds); 8, Lingzizong volcanic formation; *f*, schematic cross-section through the BNS; 9, Middle to Upper Jurassic flysh and slates; 10, Middle Cretaceous volcanics and red series; 11, ophiolite series; 12, Jurassic limestone and slates. *g*, Lead isotope diagram for the ITS magmatic section (triangles) and BNS (circles) ophiolites. The isotopic composition of the three points above the main field of the Donqiao ophiolite is due to alteration processes. The reference line is the geochron.

belonging to the genus *Protopodocarpoxylon* (gymnosperms, Cheirolepidiaceae) have been discovered that are always associated with Albian orbitolinids. This genus, which is widely represented in the Lower Cretaceous of western Europe, northern Africa and south-east Asia, has never been recorded in India or other south-Gondwana areas. Its distribution seems to fit palaeolatitudinal boundaries between 0 and 35° N. All the numerous fossil woods sampled from the Lhasa block show the presence of growth rings suggesting a palaeolatitude not too close to the Equator during Albian and Aptian times, in agreement with palaeomagnetic results. Our observations confirm previous work[9].

The discovery of abundant palynomorphs in a level from the latest Triassic (Rhaetian) north of Lhasa suggests that an equatorial or tropical latitude was reached by the Lhasa block as early as late Triassic. The flora indicates very close relationships with the equatorial (and north equatorial) province and lacks any significant Indian taxa. Most of the taxa are commonly found in Europe and the Caucasus and more recently in Szechuan province[10]. The latter find suggests that not only the Lhasa block, but also the south Chinese block were situated near the Equator or not far from the tropical area as early as late Triassic[11,12]. Somewhat surprisingly, there seems to be no unambiguous palaeontological proof of the Gondwanian origin of the Lhasa block as doubts can be cast on the significance of the Damxung–Linzhu tillites. Examination of these 'tillites' is not sufficient to demonstrate that they are a glacial sediment or alternatively rift infill. If they are indeed late Carboniferous glacial tillites, they imply a climate and latitude in striking contrast with the early Permian climate and latitude implied by the Cathaysian flora which has been discovered in the Lhasa block, and which does not contain a single Gondwanian element[9]. This indicates that if the Lhasa block indeed originated as a Gondwanian microcontinent it had detached from Gondwana as early as early Permian.

The tectonic environments of the ITS and BNS sutures are quite distinct. To the north of the ITS, the southern part of the Lhasa block can itself be divided into three zones[1,13]. North of the Gangdise (Transhimalayan) plutonic belt, a Precambrian continental basement and its Mesozoic cover have been tightly folded in the Cretaceous with east–west trends and vertical cleavage[1,14,15]. Within the southern part of the Gangdise belt, amphibolites, meta-pillow lavas and meta-cherts may be associated with a meta-ophiolitic suite. A thin Triassic to Lower Cretaceous sequence with low metamorphic grade occurs to the south of Lhasa. This sequence displays at least three phases of deformation: small scale (<10 m) isoclinal folds (north–south stretching lineation, slaty cleavage (S1) with southward vergence, increasing in intensity towards the ITS) are followed by a second phase, with larger (>100 m) folds (fanlike cleavage (S2) and vertical axial planes with undecided vergence). However, no unconformity which would allow precise separation of these two phases has been observed and they may be pulses of a single event. The second phase was over when the

uncomformable Lingzizong volcanics were deposited. Tectonic and stratigraphical observations suggest that the Gangdise belt intruded a continent–ocean margin, leaving remnants of continental crust to the north and of (Triassic?) oceanic crust to the south. The associated Lingzizong volcanics, which span possibly from the Palaeocene to recent times[7,8], have themselves undergone a third phase of crustal shortening, with long wavelength folds, and both high and low angle thrust faults with small (less than a few kilometres in size) displacements.

South of the crystalline Gangdise belt, the Xigaze series may be both transgressive on the plutons to the north, and conformably deposited on the Tsangpo ophiolites to the south. The bulk of the Xigaze group consists of a thick turbidite sequence earlier thought to represent a fore-arc basin (see refs 1, 14). It has suffered east–west folding with clear southward vergence and exhibits steeply dipping cleavage and shortening of at least 30%. The ophiolite sequence on which the Xigaze formation rests has been described by Nicolas *et al.*[3]. Using radiolaria, Marcoux *et al.*[16] have determined an age of 110 Myr for the cherts overlying the pillow lavas. Although a complete sequence from the pillows and radiolarian cherts to the Xigaze series has been observed near Tiding[16], ophiolitic massifs are generally bounded by thrust or strike slip contacts and are intensely deformed. New (1982) observations have revealed important tectonic zones within the Xigaze formation and throw doubt on the previously suggested continuity of this formation between the batholith and the ITS ophiolites[1]. It has already been pointed out that a very late compressive phase, postdating the Oligo-Miocene(?) Liuxu conglomerate, has in some places led to a northward backthrusting of the ophiolites. The tectonics of the Upper Cretaceous (?) mélange, Triassic flysch and autochtonous Indian series south of the ITS involve four shortening phases, with intense isoclinal folding and southward thrusting and symmetrical folding with steeply dipping cleavage and varying vergence between the Upper Cretaceous and the Eocene[1]. This complex history of superimposed deformations may be consistent with an Upper Cretaceous to Palaeocene southward obduction of the ophiolites on top of India (see ref. 37). Alternatively, it could be related to progressive deformation within an accretionary prism at different depths. Post-Eocene deformation is mostly related to strike-slip faulting.

The tectonic style of the BNS is rather different. The suture zone has been studied in particular near Anduo and between Gyanco and Donqiao. There, an ophiolitic nappe is thrust over a thick series of Middle to Upper Jurassic sandstones and black slates. This series is locally strongly folded, slightly metamorphosed and displays a steep cleavage with variable dips both to the south and to the north. The ophiolites are in turn locally tectonically covered by a metasedimentary nappe of Palaeozoic (?) rocks. All these units are uncomformably overlain by a Middle Cretaceous volcanic and red detrital formation. These formations have undergone isopaque (constant thickness) folding, with N140° axes, show a cleavage with undecided vergence, and thrust faulting accompanying late emplacement of the ophiolites on the red beds. Although the ophiolites have been severely deformed, they exhibit all the traditional components of the suite (pillow-lavas), a sheeted dolerite complex overlying garnet-bearing amphibolites, isotropic to layered gabbro, troctolites, wehrlites to dunites and serpentinized residual harzburgites containing chromite rich bodies. The ophiolites have been reworked in a grey neritic limestone of Upper Jurassic age. Moreover, in Donqiao, the ophiolites seem to be covered in turn by a transgressive marine detrital Upper Jurassic to lowermost Cretaceous series.

The Quangtang block, north of Anduo, is less well known, as observation there was far less extensive. It seems to involve rather simple, broad, folding of dominantly neritic Jurassic sediments. Rapid surveys and inspection of the geological map of China seem to indicate that plutonic and volcanic formations are far less abundant to the north of BNS, which, of course, raises questions as regards the polarity of the former subduction zone. A southward dipping subduction would be favoured by the scanty presently available data, opposite to what was previously thought (see ref. 6).

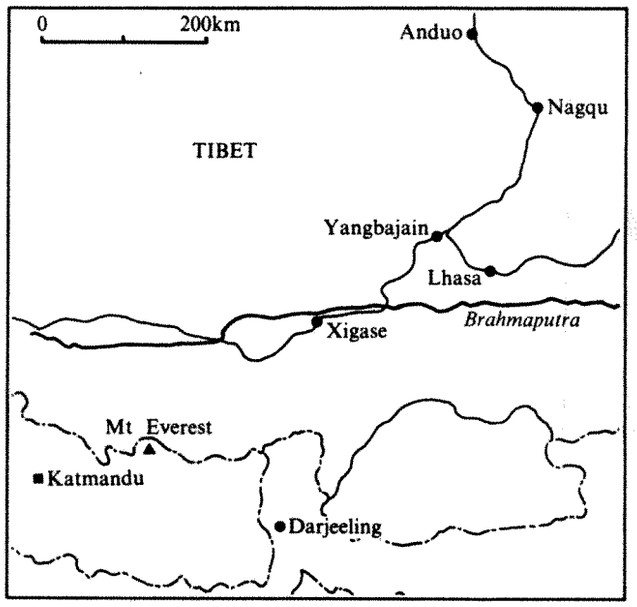


Fig. 2 Map showing the relationship of southern Tibet to the Himalaya. The place names given are those used by the French–Chinese three-year geoscience cooperation programme.

The analysis of lead isotopes in samples of ophiolites from the ITS and BNS sutures reveals striking differences (Fig. 1). The Pb isotopic composition of the magmatic sequence from ITS falls in the field of dredged samples from the Indian Ocean Ridge[18]. This may indicate a genetic relationship between the sub-Indian Ocean mantle and the ophiolites. A U–Pb total rock age of 119 ± 25 Myr has been obtained from the magmatic section of the ophiolite near Xigaze. In contrast, rocks from BNS show higher 207/204 ratios and resemble ophiolites from the eastern Mediterranean[19]. This is compatible with the Bangong-Nujiang ophiolites have formed in a small back arc or interarc basin, as indicated by the palaeolatitudinal studies reported above.

The ultramafic part of the Tsangpo ophiolite has an isotopic signature that is similar to that of the Donqiao pillow-lavas with higher 207/204 ratios than in the magmatic section. This suggests that the two parts of the Tsangpo ophiolite may not be cogenetic.

The volcanic environment of the two sutures is also contrasted. Volcanic rocks are very abundant in the south of the Lhasa block. They occur as ignimbritic tuffs in the Middle Cretaceous Takena formation and form most of the 1,500-m thick Lingzizong formation, with piles of andesite flows and rhyolitic ignimbrite flows. This thick calc-alkaline sequence contrasts with the less abundant mostly basic volcanic rocks of BNS. Only a few ignimbrite flows are observed there. In Nagqu, volcanic rocks overly the black Jurassic slates and seem to postdate the Cretaceous red beds in Anduo. These red beds are sometimes thrust over the basalts and andesites. Thus, the volcanic environments of ITS and BNS appear to be suggestive of Andean and island-arc subduction respectively, although the age of the northern volcanics remains to be determined.

Plutonic rock and basement gneisses have been sampled in a fairly systematic way to obtain geochronological data (U–Pb: accessory minerals) and to identify the source regions of the granitoid magmas (Nd, Sr and Pb initial isotopic compositions). The ages at which the basement material was transformed into gneisses, although poorly defined because of the heterogeneity of the inherited Pb components, appear to be synchronous with the plutonic activity. Inherited Pb-components in zircons yield

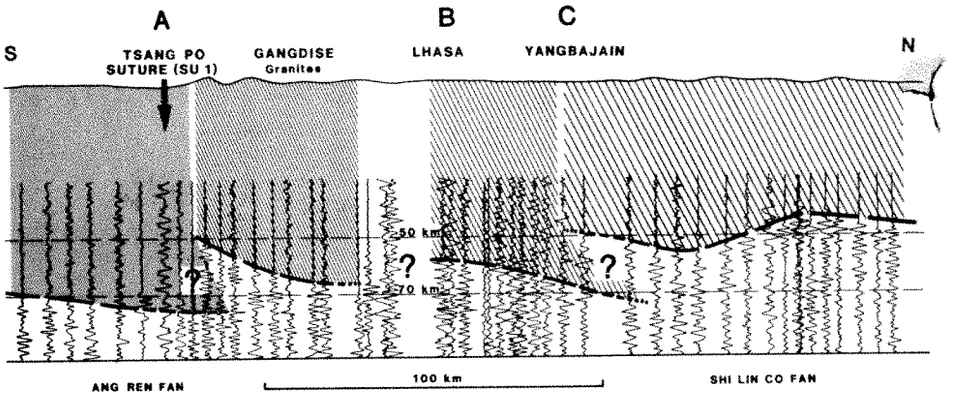


Fig. 3 Wide-angle seismic refractions from the Moho along the section shown in Fig. 2 reveal a complex Moho topography (after refs 20–22). First arrivals are picked at the dashed lines, providing the Moho topography. Particularly noteworthy are the 20-km step in the Moho near ITS and the presence of two superimposed Mohos at the latitude of Yangbajain. The profiles result from two shots in Angren and the Shi Lin Co (about 100 km outside of the figure to the west) recorded in fans along the main road from Quxu to Nagqu. No vertical exaggeration.

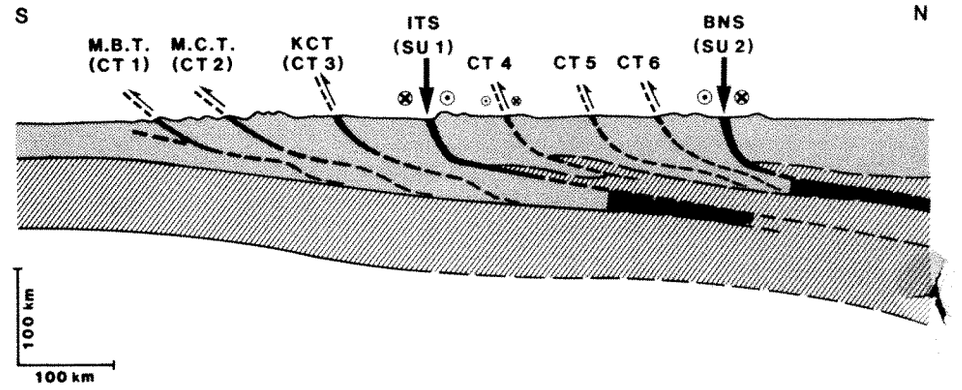


Fig. 4 A model for the structure of the lithosphere in the Tibet–Himalaya. The role of obduction related sutures (Indus Tsangpo suture or ITS, Bangong-Nujiang Suture or BNS) and of continental thrusts on the Indian plate (main boundary thrust, main central thrust, Kangmar thrust labelled CT1 to CT3) and in the Lhasa block (CT4 to CT6 corresponding to possible thrusts near Yangbajain, Gulu and South of Anduo) is emphasized. Continental and oceanic crusts are shaded in light and dark grey respectively. The subcrustal lithosphere is ruled. Older sutures and continental thrusts are rotated into the vertical and reactivated as strike-slip faults.

minimum ages up to 2,200 Myr in the Everest area and also Precambrian ages in the Yangbajain and Anduo areas. Clearly, all crustal blocks are made of old basement materials. The leucogranites of the High Himalayas, and those intruding the Tethyan sediments further north, yield ages of 15–30 Myr and seem to be purely anatectic. The Gangdise plutonic belt comprises numerous bodies of gabbro, diorite, granodiorite and granite which have been intruded from 95 to 40 Myr ago. Initial Nd, Sr and Pb isotopic compositions show large heterogeneities and plot along trends interpreted as a mixture of mantle-derived component(s) with continental crust materials. The volcanic rocks of the Lingzizong formation have the same isotopic characteristics implying a similar origin. In both cases, the crustal components appear to be dominant. Farther north, the Yangbajain, Gulu and Anduo granitoid belts yield ages around 50, 100 and 100 Myr respectively. The Pb-isotopic composition of these rocks is compatible with an origin by partial melting of the basement materials. From the point of view of geochemistry, the Gangdise belt looks like a subduction related complex built on continental crust, similar to the Sierra Nevada batholith, whilst other intrusives farther north in Tibet are anatectic granitoids.

Thus, the available information suggests that the ITS corresponds to an Andean subduction environment, which has been terminated between the Upper Cretaceous and the Eocene by the collision of India with the Lhasa block, acting as the southern margin of Asia. On the other hand, the BNS is different and a likely interpretation at this stage involves an island-arc subduction environment, which has been terminated in the Upper Jurassic to Lower Cretaceous by a collision between the Lhasa block and the Quangtang block, then the southern margin of Asia.

Crustal repetition

A 300-km long north–south seismic section has been obtained by Hirn *et al.*[20–22], using critical reflection profiling on the Mohorovicic discontinuity, complemented by fan profiles. The detailed data and interpretation are given elsewhere[20–22] and only the major features are summarized here (Fig. 3). The section reveals a thick crust (between 50 and 70 km) with a complex structure. An outstanding feature is a 20-km step in the Moho precisely below the surface trace of the ITS which is consistent with strike-slip reactivation of a major thrust contact. The data do not allow resolution of whether or not the Lhasa block is actually underlain by Indian crust. Crustal repetition occurs along parts of the section, particularly north of Yangbajain: there two superimposed Mohos are observed with the lower crustal member being ~20 km thick. Another 20-km step in the Moho, very similar to that observed below the ITS has been found near Anduo below the BNS (outside of the range of Fig. 4, see refs 20–22). Thus, the Tibetan Moho displays a complex topography with slopes and faults and in places implies crustal repetition (although the two crustal members are not necessarily complete) by continental thrusting.

It is now widely accepted that the structure of the High Himalayas is associated with a major intracrustal thrust, the MCT, with the northern Himalayan crust thrust southwards. The throw on the MCT is not yet known but may be very large. It seems that the MCT is no longer the active thrust but that tectonic activity has jumped southward, with a new continental (crustal) thrust, the MBT, presently active[23–25]. Intense shear along the MCT within the lower structural levels of the Tibetan slab is linked with the genesis of the 20-Myr old anatectic Himalayan leucogranites. A detailed field study of the Lhozag–Kangmar–Lhagoi–Kangri belt of granite plutons, about 100 km to the north of the High Himalayas[26], also implies that a major continental thrusting episode occurred there, possibly leading to crustal thickening, and associated with a high grade metamorphic episode contemporaneous with thrusting on the MCT (around 20 Myr).

The gneisses around Yangbajain, about 100 km north of the ITS, have a Precambrian primary age but have undergone a young metamorphic episode at around 50 Myr. This is also the age of the Yangbajain anatectic granites. The U–Pb systematics of zircons imply that these gneisses have been formed at a depth of ~15 km. They may have been thrust over the Cenozoic Lingzizong volcanic formation and thus be related to another intracrustal fault with 15 km of vertical offset. It may be sug-

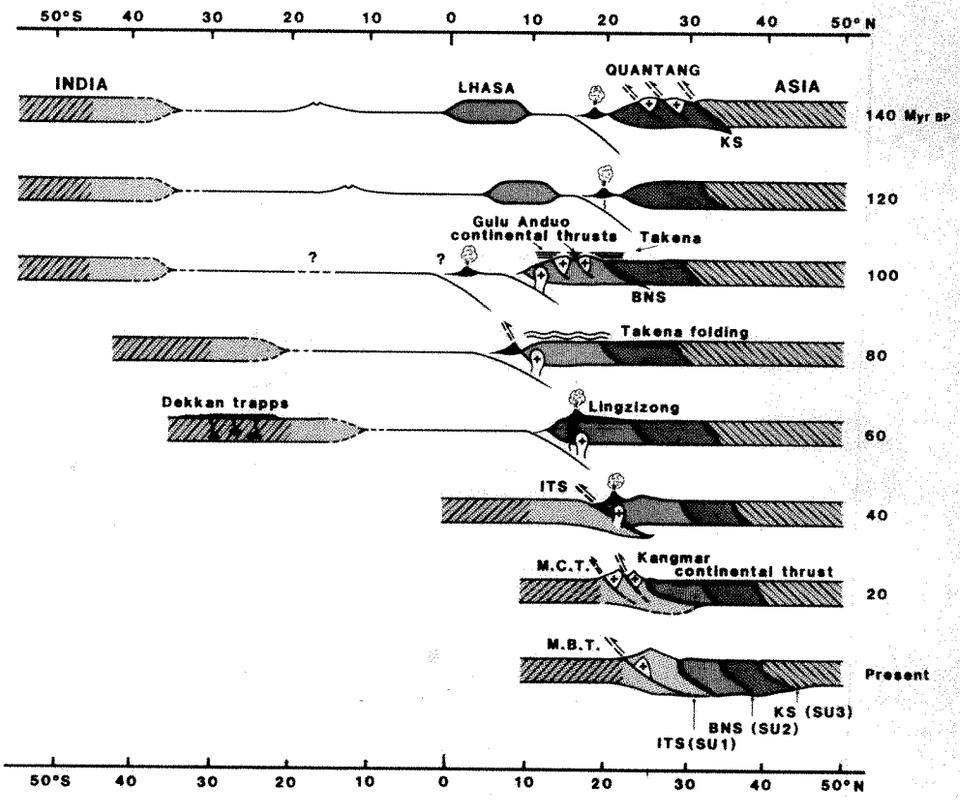


Fig. 5 An attempt to reconstruct the evolution of the Lhasa block in relation to the Indian and Asian continents since the beginning of the Cretaceous, at 20-Myr intervals. The blocks are located in latitude in agreement with palaeomagnetic (after 100 Myr) and palaeontological (before 100 Myr) data. Shortening of the Lhasa and Quantang blocks by a factor of two and of the northern margin of India by about 1,000 km have been included. Closing of sutures, activation of continental thrusts and related granite formation, Takena deposition and folding and Lingzizong volcanic activity have been emphasized. Controversial aspects are, of course, present such as the origin of Takena folding by collision with an island arc (see text for another possibility).

gested that the genesis of granitoid rocks in Tibet is related to large-scale intracontinental thrusts (leading to crustal doubling or thickening) and that, for instance, the Gulu and Anduo belts are the signature of older (100 Myr old) thrusting events. This would be a major means of crustal thickening in Tibet: a picture emerges where two sutures, both related to major continental collisions, separate thickened continental blocks in which thickening (crustal repetition) is related to as many as six major thrusts (MBT, MCT, Kangmar, Yangbajain, Gulu, Anduo from south to north; see Fig. 4). These continental thrusts might result from post-collisional continuing convergence.

Some of the authors of this article are, however, reluctant to accept Yangbajain as the site of a major southward verging Tertiary intracontinental thrust. Some structural observations show that between Yangbajain and Nagqu deformation of late Mesozoic to Cenozoic age is characterized by steep cleavage dipping mostly south and, locally, horizontal stretching lineations, and thus presumably indicative of large scale strike slip motion, dominantly in an east–west direction. The relative importance of thrusting and strike slip faulting with respect to crustal shortening in Tibet has yet to be analysed in detail.

Kinematic evolution

The stratigraphical and tectonic observations, together with the isotopic age determinations and palaeomagnetic data reported here allow us to propose a geodynamical model for the evolution of blocks which are now part of the Indian–Asian continent over the past 120–140 Myr. The time and space distribution of our data is, of course, still far from complete and the interpretation that we propose is not unique at all periods. The present model (Fig. 5) includes the possibility of lithospheric shortening by continental thrusting and is controlled in latitude by palaeomagnetic and palaeontological data. It begins around 140 Myr ago. The Quangtang block was sutured to Asia along the Kokoxili suture ~200 Myr ago and some crustal shortening may subsequently have occurred on (hypothetical) continental thrusts. Around 140 Myr ago, a small ocean basin between the Quangtang and Lhasa blocks closed along the BNS (its size at 140 and 120 Myr has been exaggerated on Fig. 5). Continental shortening followed along the Gulu and Anduo thrusts and continental red beds were deposited over this southern margin of the Asian continent. Folding of the red series may have occurred around 80 Myr or later. Its origin is still puzzling and it may either be due to interaction with a hypothetical island arc (which would have to have been completely obliterated subsequently), or be linked with the subduction process (Andean folding) or be related to the obduction and collision themselves. At this time, the Lhasa block was at low latitudes. The Lingzizong arc volcanics were erupted between 60 and 45 Myr, at which time the subduction zone was blocked and obduction occurred along the ITS. Continental shortening then occurred in the Yangbajain (40 Myr), Kangmar and MCT areas (20 Myr), recently jumping to the MBT. An unknown length of Indian continental lithosphere may also have been thrust under the MCT. Our schematic representations suggest that it may be up to 1,000 km long as suggested by Argand in 1924[27].

Geodynamical consequences

Our observations have important geodynamical implications: first, they may provide constraints on the mechanism of continental accretion and evolution, second they can tell us something about the driving mechanism of plate tectonics.

We have suggested that there may be as many as two continental collisions and six continental thrusts between 26 and 33° N at the longitude of Lhasa. There are two more collisions (suture zones) and possibly several continental thrusts north of the area that we surveyed, between 33 and 40° N. Each one of these tectonic features leads to the disappearance of some continental surface, to crustal thickening and to high elevations. As time passes, erosion will destroy high mountains and plateaus, thin the crust and bring it back to its 'normal' 35-km continental thickness. In the process, continental crust will have been removed not only along horizontal dimensions (surface area) but also along the vertical dimension (volume loss). The eroded material will, of course, be redeposited elsewhere, although much of it is likely to be returned to the mantle in nearby active subduction zones. A very quick and crude estimate of the surface and volume loss in Tibet over say, the past 150 Myr can be made: over a 2,500 km long front, from the Pamir to the Assam syntaxis, blocks that now extend over 1,500 km from north to south with a double continental crustal thickness may have originally extended over as much as 3,000 km. The total surface

loss is of the order of 4×10^6 km^2 (or between 2 and 3×10^{-2} km^2 yr^{-1} in average surface loss) and the final volume loss will amount to over 100×10^6 km^3, or between 0.5 and 1 km^3 yr^{-1} on average. It is interesting to compare this value with the rate of possible sediment loss in subduction zones, which is of the order of 1–2 km^3 yr^{-1} (taking average sediment thickness, length of trenches and subduction velocity of 0.5 km, 40,000 km and 5 cm yr^{-1} respectively). Thus, continental collision acts as a significant limiting factor for continental growth (which is itself related to volcanic and plutonic activity in subduction zones).

Nevertheless, continental collision has enlarged the Asian continent by adding allochtonous continental fragments to it. The older sutures are the remnants of continental collisions related to previous accretion of allochtonous blocks to Asia since the beginning of the Palaeozoic[6]. The situation encountered in western North America[28] is reminiscent of what we observe in Tibet. The origins of each individual block may be quite diverse, as shown by the analysis of faunas and floras. The Lhasa block for instance is a Cathaysian block and not necessarily of Gondwanian origin. Together with much of Indochina, the Lhasa block may have been part of Pacifica[29]. Thus the Asian continent actually results from the juxtaposition of many blocks of diverse origins and is a typical continental mosaic.

The sutured edges of previously accreted continental blocks will induce large-scale heterogeneity and anisotropy of deformation when a new block is accreted through collision. Those earlier sutures which are favourably oriented with respect to the principal compressive stress due to the new collision will be reactivated, often as large strike-slip faults[30]. Some of the major strike-slip faults described by Tapponnier and Molnar[31] in Tibet are rejuvenated sutures. This is the case for large segments of most sutures described here. It is also true for other parts of China, and in Tibet for several continental thrusts (such as the ones near Yangbajain and Gulu, for instance). However, active faults resulting from a collision need not necessarily all be reactived block boundaries: the most efficient way to join two reactivated strike slips faults may in cases be to create a new fault through some particular block in the mosaic. Still, an active tectonic phase is in some ways guided by tectonic features resulting from previous phases. If the mode of accretion of the Asian continent is to be regarded as the rule rather than the exception for continental accretion, then earlier continental collisions are important in establishing the tectonic grain of a future continental collision.

Following the work of Forsyth and Uyeda[32], many authors have considered the pull of descending slabs at oceanic subduction zones as the main force acting to drive plate motion. Other forces, such as the so-called 'ridge push', gravity sliding and shear coupling to the asthenosphere have been regarded as less important and in any case not resolvable. However, it seems clear that slab pull cannot be the leading force in the case of the relative motion of the Indian plate with respect to Asia. Subduction along the ITS stopped at least 45 Myr ago and since then, there has been no oceanic lithosphere left between India and Asia. Continuing slab pull beneath Java and Sumatra is not likely to be sufficient to counteract alone resistance to convergence in the Himalayas. Yet, intracontinental motion continues on the MCT and Kangmar CT around 20 Myr, and now on the MBT, at a rate of about 2 cm yr^{-1} (ref. 33). The total motion between India and Asia is 5 cm yr^{-1} (ref. 31).

Continental shortening is also taken up along sets of large strike-slip faults[31,34]. Models of continental delamination (see ref. 35) cannot account for the continued penetration of India either, and other boundary forces must be sought. The northward motion of India lasted since the Jurassic and the subcontinent was subjected to considerable tensile stress from maybe as early as 110–60 Myr ago when the Dekkan traps were produced. Recent geochemical analyses suggest that the traps result from the fusion of the continental lithosphere and do not involve deeper components[36]. This observation seems difficult to reconcile with any significant contribution from the ill-named 'ridge push' force. Moreover, before India was rifted away from Gondwana, other continental fragments, rifted earlier either from Gondwana or elsewhere, were already 'attracted' towards Asia in a dominantly northward direction and collided with it[30].

Thus, there is a long history, probably more than 200 Myr long, of polarized accretion of blocks to the southern margin of the Asian continent. A similar long-lived polarization of motion is suggested by the study of allochtonous terranes accreted to North America and sheared along the San Andreas–Denali strike-slip system (see ref. 28). These observations seem to imply the persistence over several hundred million years of large-scale convection currents, more or less stationary with respect to the mantle, and coupled to the lithosphere. A possible mechanism is gravity sliding over the hot ascending limb of such a large convection cell. The initial breakup of Gondwana, the motion of small blocks and then of India, the successive collisions and present continental shortening would thus all be due to northward gravity sliding over a large hot mantle zone lasting at least 200 Myr. This zone may well correspond to the Atlantic–African geoid high, as recently argued by Anderson[17]. The trend of the southeastern part of this geoid high, associated with the Réunion, Kerguelen and Crozet hotspots, is compatible with the observed direction of polarized motion, that is perpendicular to it.

This work was carried out within the Cooperative Research Project of the Chinese Geological Academy, Chinese Academy of Sciences, French Centre National de la Recherche Scientifique and Institut National d'Astronomie et de Géophysique. This programme benefited greatly from the help of the French Ministère des Affaires Etrangères, and the Service des Relations Internationales of the CNRS. Special acknowledgments go to G. Aubert, J. Mirabel, A. Curien and Li Chengda for administrative assistance. This is IPGP contribution no. NS 689.

Received 27 June; accepted 10 October 1983.

1. Tapponnier, P. *et al. Nature* **294,** 405–410 (1981); *Résultats de la coopération franco-chinoise au Tibet (mission 1980)* (eds Mercier, J. L. & Guangqin, Li) (CNRS, Paris, in the press).
2. Tapponnier, P., Mercier, J. L., Armijo, R., Tonglin, H. & Ji, Z. *Nature* **294,** 410–414 (1981).
3. Nicolas, A. *et al. Nature* **294,** 414–417 (1981).
4. Pozzi, J. P. *et al. Nature* **297,** 319–321 (1982).
5. *Colloque Franco–Chinois sur la Géologie de l'Himalaya, Abstr.* Guilin (1982).
6. Chang Chenfa & Cheng Hsilan, *Sci. Sinica,* **16,** 257–265 (1973); in *Proc. Symp. on Qingai-Xizang (Tibet) Plateau,* (Gordon & Breach, New York, 1981).
7. Westphal, M., Pozzi, J. P., Yaoxiu, Zhou, Lisheng, Xing & Xianyao, Chen, *Geophys. J. R. astr. Soc.* **73,** 507–521 (1983).
8. Maluski, H., Proust, F. & Xuchang, Xiao, *Nature* **298,** 152–154 (1982).
9. Hsü, J. *Scient. Geol. Sinica* **10,** 323–331 (1978).
10. Schang, Y. *5th int. palyn. Conf.,* (Cambridge University Press, 1980).
11. Buffetaut, E. *Bull. Soc. géol. Fr.* **23,** 587–593 (1981).
12. Ingavat, R. & Janvier, P. *Geobios* **14,** 725 (1981).
13. Burg, J. P., Proust, F., Tapponnier, P. & Guoming, Cheng. *Eclog. Helv.* (submitted).
14. Shackleton, R. *J. struct. Geol.* **3,** 97–101 (1981).
15. Bally, A. W. *et al. U.S. geol. Surv. Open File Rep. 80-501* (1980).
16. Marcoux, J. *et al. Ofioliti* Suppl. 6, 31–32 (abstr.) (1981).
17. Anderson, D. *Nature* **297,** 391–393 (1982).
18. Dupré, B. & Allègre, C. J. *Nature* **303,** 142–146 (1983).
19. Hamelin, B., Dupré, B. & Allègre, C. J. *Earth planet. Sci. Lett.* (in the press).
20. Hirn, A., Jobert, G., Wittlinger, G., Zhongxin, Xu & Enyuan, Gao, *Ann. Géophys.* (submitted).
21. Hirn, A., Nercessian, A., Zhongxin, Zu, Deyan, Lu & Jiwen, Teng, *Terra Cognita* **3,** 267 (1983).
22. Hirn, A. *et al. Nature* **307,** 23–25 (1984).
23. Barazangi, M. & Ni, J. *Geology* **10,** 179–185 (1982).
24. Mattauer, M. *C. R. hebd. Séanc. Acad. Sci., Paris* **296,** 481–486 (1983).
25. Seeber, L., Armbruster, J. G. & Quittmeyer, R. in *Zagros-Indukush-Himalaya Geodynamic Evolution* (eds Gupta, H. K. & Delany, F. M.) 215–242 (1981).
26. Burg, J. P., Guiraud, M. & Guoming, Cheng, *Earth planet. Sci. Lett.* (submitted).
27. Argand, E. *C. r. Congr. géol. int.* **13,** 171–372 (1924).
28. Ben Avraham, Z., Nur, A., Jones, D. & Cox, A. *Science* **213,** 47–54 (1981).
29. Nur, A. & Ben Avraham, Z. *J. geophys. Res.* **87,** 3644–3661 (1982).
30. Tapponnier, P., Mattauer, M., Proust, F. & Cassaigneau, C. *Earth planet. Sci. Lett.* 52, 355–371 (1981).
31. Tapponnier, P. & Molnar, P. *Nature* **264,** 319–324 (1976); *J. geophys. Res.* **82,** 2905–2930 (1977).
32. Forsyth, D. & Uyeda, S. *Geophys. J. R. astr. Soc.* **40,** 465–474 (1975).
33. Armijo, R., Tapponnier, P., Mercier, J. L. & Tonglin, Han, *EOS* **63,** 1093 (1982).
34. Molnar, P. & Tapponnier, P. *Science* **189,** 419–426 (1975).
35. Bird, P. *J. geophys. Res.* **84,** 7561–7571 (1979).
36. Allègre, C. J., Dupré, B., Richard, P., Rousseau, D. & Brooks, C. *Earth planet. Sci. Lett.* **57,** 25–34 (1982).
37. Burg, J. P. thesis, Montpellier Univ. (1983).
38. *Geological map of the Tibetan Plateau,* 1/1,500,000, Chengdu, China (1981).

Crustal structure and variability of the Himalayan border of Tibet

Alfred Hirn*, Jean-Claude Lepine*, Georges Jobert*, Martine Sapin*, Gérard Wittlinger†, Xu Zhong Xin‡, Gao En Yuan‡, Wang Xiang Jing‡, Teng Ji Wen§, Xiong Shao Bai§, M. R. Pandey‖ & J. M. Tater‖

* Laboratoire d'Etude Géophysique des Structures Profondes, associé au CNRS, Institut de Physique du Globe de Paris, Université Pierre et Marie Curie, 4 place Jussieu, Tour 14, 75230 Paris Cedex 05, France
† Laboratoire de Sismologie, Institut de Physique du Globe, Strasbourg, France
‡ Department of Geophysics, Institute of Geology, Changchun, Ministry of Geology, China
§ Institute of Geophysics, Academia Sinica, Beijing, China
‖ Department of Mines and Geology, Kathmandu, Nepal

Reversed critical wide angle reflection profiles of the crust–mantle boundary south of the Yarlung Zangbo suture in Tibet reveal a deep (70 km) Moho extending north from the High Himalayas, whilst to the south the Moho is 15 km higher. Even further south, reflections from 35 km depth may correspond to the Moho of peninsular India.

In September–October 1981 seven charges ranging from 2 to 10 tons were detonated in two lakes and at one drill-hole shotpoint between the High Himalayas and the Yarlung Zangbo Jiang as part of the French–Chinese geoscientific exploration of southern Tibet. Forty portable tape recording seismic stations were laid out to cover a 500-km long east–west line with a system of reversed and overlapping profiles, together with auxiliary profiles (Fig. 1). Twelve additional stations in Nepal tested the propagation through the High Himalayas. Details of the method, experiment and interpretation are presented elsewhere[1–3] and partly summarized below.

Interpretation of critical reflections

Critical reflections recorded from the crust–mantle boundary (Fig. 2*a*) are exceptionally clear when compared with former experiments on thick crusts of complex origin[4–9], a result partly attributable to propagation along the strike of geological units. The crust–mantle boundary can be modelled (using synthetic seismograms[10]) as a transition zone about 12 km thick at 75 km beneath the present-day surface (4,500 m mean elevation). Such a large crustal thickness was unexpected so near to the High Himalayas. In the upper crust strong variations in seismic response along the Tibetan line indicate significant structural variation, but reflectors at mid-crustal depth can be detected on most profile sections. Crustal mean velocity, derived from reversed observations of the Moho reflection, is of the order of 6.3 km s^{-1}, with upper and lower crust mean values around 6.0 and 6.4 km s^{-1}, respectively. The slope of the spectral ratio and the comparison of overall amplitudes of waves reflected respectively, on a mid-crustal interface and on the Moho provide a significantly high value for P-wave attenuation in the lower half of the crust of the order of 10^{-2}. Another peculiar feature

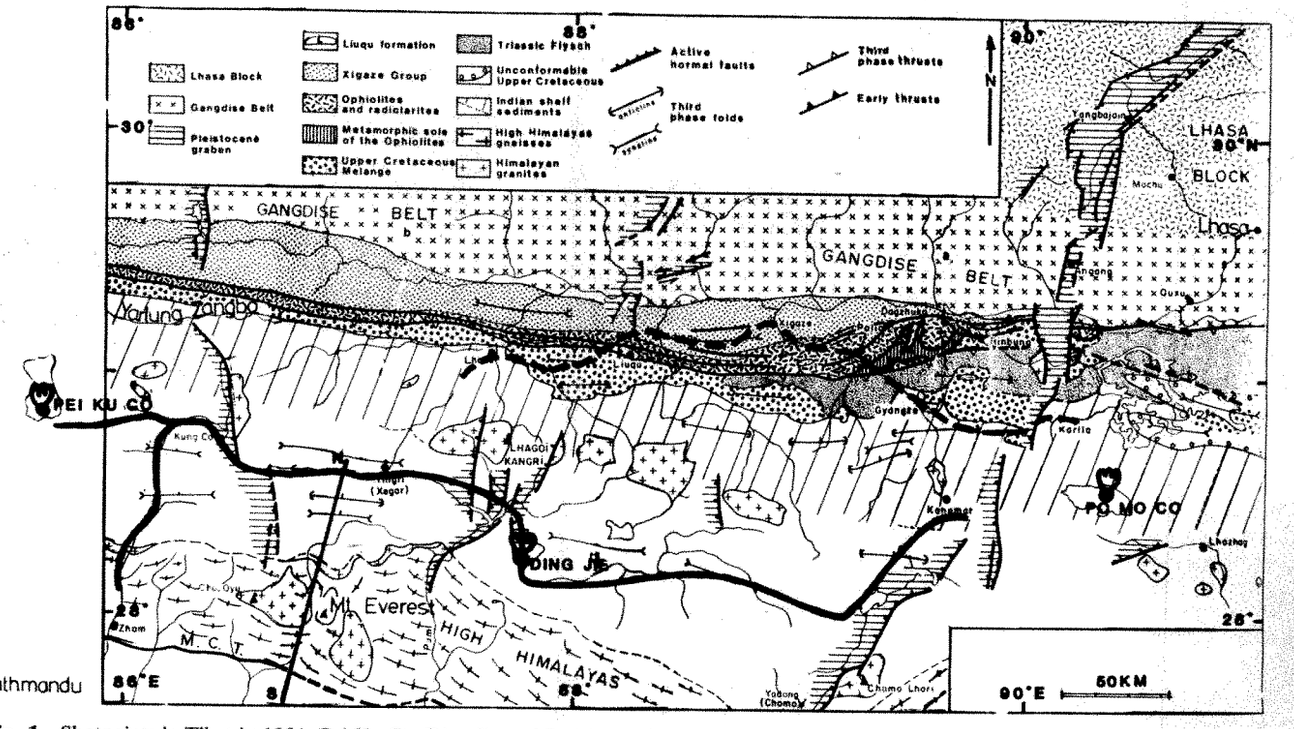


Fig. 1 Shotpoints in Tibet in 1981: Pei Ku Co, Ding Jie and Po Mo Co. Heavy lines are the location of profiles of seismographs with 10 km spacing. Geology is from Tapponnier *et al.*[18]. S–N is the position of the crustal section of Fig. 3.

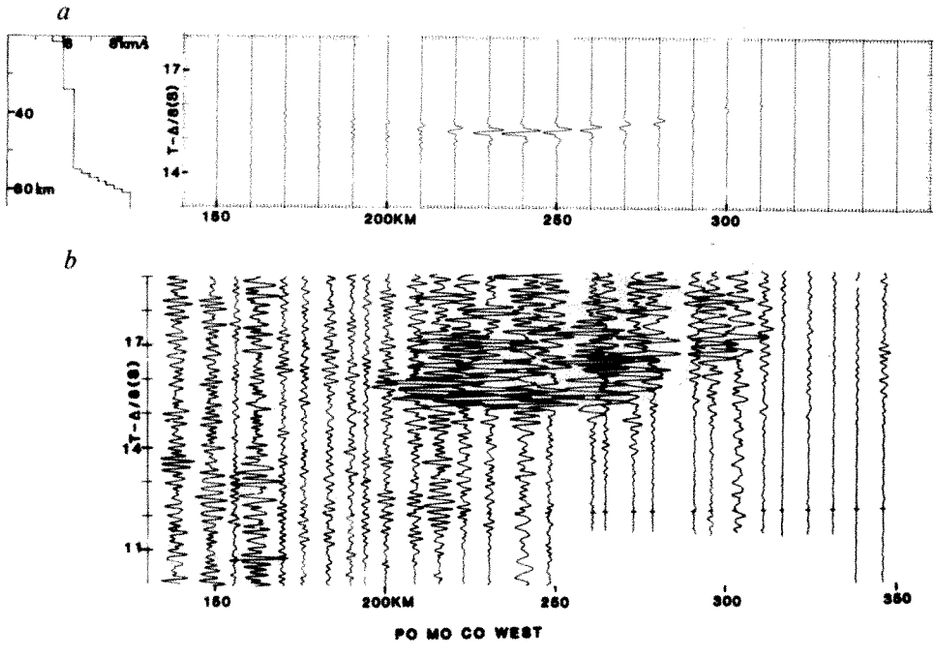


Fig. 2 *a*, Synthetic record section for a 12-km thick transition from crust to mantle velocity around 75 km beneath the surface. *b*, Shots Po Mo Co recorded on the east–west in-line profile. True amplitude record section reduced to 8 km s^{-1} velocity. Note the prominent burst of energy returned between 200 and 300 km distance and 15 and 16 s reduced time due to reflection at the Moho.

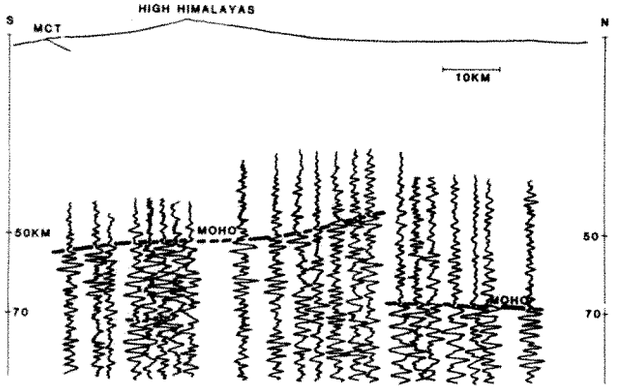


Fig. 3 Fan-profile to the west from shotpoint Dingjie. Correlated phases at these shotpoint distances from 180 to 300 km correspond to reflections at the crust–mantle boundary (see Fig. 2). Reflection times are corrected to a constant offset of 200 km, so that the time-section is directly transformed in a depth-section (S–N on Fig. 1) across the High Himalayas under the assumption of negligible variation of mean crustal velocity along it.

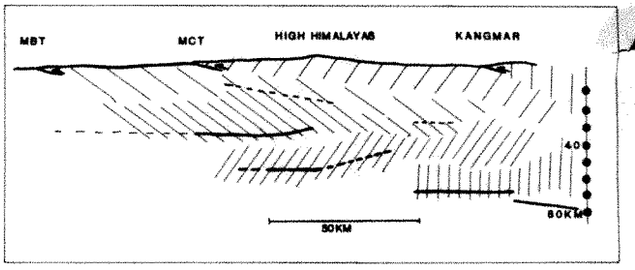


Fig. 4 Sketch of elements of seismic structure across the High Himalayas. Dashed line: interface between upper and lower crustal velocity material. Solid line: interface between crust and mantle velocity material. Loose hatching: upper crust layers. Their superposition is supposed to occur along flat thrusts at the base of the overriding unit. Tight hatching: lower lithospheric units. These are comprised of the lower crust, Moho and a sole of uppermost mantle material and are assumed to be brought to the present position, attested by the position and shape of the Moho marker, as a result of thrusting and superposition along levels of decoupling in the middle or lower crust and at shallow depth in the uppermost mantle. Possible correspondence of upper and lower units is indicated by hatching of same direction. MBT, Main Boundary Thrust; MCT, Main Central Thrust.

derived from the east–west reversed profile is a value of Pn velocity greater than 8.5 km s^{-1}.

North–south crustal profile

North–south crustal variation at the level of the Moho has been investigated using the relative arrival times of the reflections at critical-wide angle, that is, at 200–300 km range, on fan profiles in southern Tibet and across the High Himalayas to Nepal. Figure 3 shows a topographical cross-section of the deep crustal interfaces west of Mt Everest, obtained from a time-section of reflected waves at their mirror position. This section has been corrected for different offset distances and transformed into a depth-section assuming negligible variation of average crustal velocity. The deep Tibetan Moho is seen to extend to southwards to within a few tens of kilometres of Mt Everest. Another crust–mantle interface, probably dipping northwards, is seen at 55 km depth under the High Himalayas and partly on top of the Tibetan Moho. The mantle is deeper than expected beneath and south of the High Himalayas. Nevertheless, the depth to the mantle can be reconciled with the observed values of the gravity field[11,12] by taking into account the abnormal thickness of the low velocity upper half of the crust and the density deduced from the intermediate velocity value at the crust–mantle transition. However, the crustal section derived from explosion seismology no longer supports a continuous, smoothly north-dipping trend for lithosphere features beneath the Himalayan border of Tibet (a model which was suggested by gravity data and was consistent with a simple underplating of the crust causing a doubling of crustal thickness to the North[13]), nor slicing and superposing of crustal units on top of a decoupled, smoothly depressed Moho[14,15].

Evidence for further sharp topographical variations of the Moho to the north is derived from a fan profile between the High Himalayas and the Yarlung Zangbo. Here the crust–mantle transition zone appears to be composed of reflecting elements which may overlap each other at depth intervals of the order of 10 km (ref. 3).

To the south, and possibly on top of the Everest Moho (55 km depth), a reflector which could be another segment of crust–mantle interface is shown by a profile in Nepal through Kathmandu, in line with the western shotpoint in Tibet. Here the depth difference between these interfaces is of the order of

20 km so that the shallower one could be the Moho of peninsular India at 35 km depth[2].

Conclusions

Figure 4 summarizes the explosion seismology data by projection onto a cross-section from the Main Boundary Thrust (the southern extremity of the lower Himalayan domain) to the Yarlung Zangbo. This figure suggests that the observed thickening of the crust from north to south is not due to a smoothly north-dipping Moho. The amplitude of the steps is significantly smaller than normal crustal thickness, suggesting that the crustal doubling is not the result of the superposition of two whole normal crusts on each other, but rather the result of separate doubling (by decompling and thrusting) of the upper and lower crustal layers (plus Moho). The resulting structure would be consistent with the observation that the 70-km thick Tibetan crust can be divided into two layers of different mean velocities, each being twice as thick as the corresponding layers of normal crust.

The decoupling at mid-crustal depth thus implied may have occurred at the lower limit of the brittle layer or at the base of the upper, less basic layer in a compositionally layered crust[16]. Decoupling beneath the Moho might be related to the behaviour of a low velocity layer at a shallow depth in the mantle lithosphere[17].

The Sino–French geoscientific programme in Tibet has been promoted by the Foreign Ministries of both countries and discussed and supported by the French Institut National d'Astronomie et de Géophysique of CNRS and the Chinese Ministry of Geology and Academia Sinica. Additional effort in Nepal came from the Department of Mines and Geology. Contribution IPG 684.

Received 6 July; accepted 14 October 1983.

1. Hirn, A., Jobert, G., Wittlinger, G., Xu, Z. X. & Gao, E. Y. *Ann. Géophys.* (in the press).
2. Lépine, J. C., Hirn, A., Pandey, M. R. & Tater, J. M. *Ann. Géophys.* (in the press).
3. Hirn, A. & Sapin, M. *Ann. Géophys.* (in the press).
4. Ocola, L. C. & Meyer, R. P. *Geophys. J. R. astr. Soc.* **30,** 199–209 (1972).
5. Ocola, L. C., Aldrich, L. T., Gettrust, J. F., Meyer, R. P. & Ramirez, J. E. *Bull. seism. Soc. Am.* **65,** 1681–1695 (1975).
6. Finetti, I., Giorgetti, F. & Poretti, G. *Boll. Geof. teor. appl.* **21,** 159–170 (1979).
7. Beloussov, V. V. *et al. Tectonophysics* **70,** 193–221 (1980).
8. Kaila, K. L., Krishna, V. G., Chowdhury, K. R. & Narain, H. *J. geol. Soc. India* **19,** 1–20 (1978).
9. *Acta geophys. Sinica* **24,** 155 (1981).
10. Fuchs, K. & Mueller, G. *Geophys. J. R. astr. Soc.* **23,** 417–433 (1971).
11. Kono, M. *Geophys. J. R. astr. Soc.* **39,** 288–299 (1974).
12. Tang, B. X., Liu, Y. L., Zhang, L. M., Zhon, W. H. & Wang, Q. S. *Geological Ecological Studies of Qinghai–Xizang Plateau* Vol. 1, 683 (Science Press, Beijing, 1981).
13. Powell, C. & Conaghan, P. J. *Earth planet. Sci. Lett.* **20,** 1–12 (1973).
14. Lyon-Caen, H. & Molnar, P. *J. geophys. Res.* **88,** 8171–8191 (1983).
15. Mattauer, M. *Terra Cognita* **3,** 269 (1983).
16. Meissner, R. *J. Geophys.* **40,** 57–73 (1974).
17. Hirn, A., Steinmetz, L., Kind, R. & Fuchs, K. *Z. Geophys.* **39,** 363–384 (1973).
18. Tapponnier, P. *et al. Nature* **294,** 405–410 (1981).

Lhasa block and bordering sutures— a continuation of a 500-km Moho traverse through Tibet

Alfred Hirn*, Alexandre Nercessian*, Martine Sapin*, Georges Jobert*, Xu Zhong Xin†, Gao En Yuan†, Lu De Yuan‡ & Teng Ji Wen§

* Laboratoire d'Etude Géophysique des Structures Profondes, associé au CNRS, Institut de Physique du Globe de Paris, Université Pierre et Marie Curie, 4 place Jussieu, Tour 14, 75230 Paris Cedex 05, France
† Department of Geophysics, Institute of Geology, Changchun, Ministry of Geology, China
‡ Geophysical Team 562, Ministry of Geology, Beijing, China
§ Institute of Geophysics, Academia Sinica, Beijing, China

A north–south wide-angle fan-profile through the Yarlung Zangbo suture, across the Lhasa block and through the Bangong-Nujiang suture shows several significant and sharp changes in crustal thickness. Both sutures appear as vertical 20-km steps in the Moho and may have been the loci for eastward strike-slip motion of the Tibetan lithosphere.

THE Yarlung Zangbo Jiang suture zone between southernmost Tibet, part of the Indian continental plate, and the Lhasa continental block, part of Asia since before the Cretaceous, was a major target for the seismic exploration of deep structure in the 1981–82 French–Chinese joint programme. The results of the first phase (an east–west profile in the south Tibetan zone between the High Himalayas and Yarlung Zangbo suture) are reported in the accompanying article[1] and elsewhere[2–4]. The second phase in June 1982 was intended to record a parallel line in the Lhasa block, to the north of this suture, to allow a comparison of structures. However, difficulty of access obliged us to displace it 300 km to the north, near the other limit of the Lhasa block. Consequently fan-recording was used to investigate crustal variation between these lines.

From a methodological viewpoint it is worth noting that the width of individual crustal segments between Moho disruptions thus defined is of the order of their thickness (50–100 km). As the critical distance for waves reaching the Moho is much larger (200 km) than this width, these segments could hardly have been resolved by waves propagating across them in a conventional north–south in-line shooting and recording except perhaps by using numerous tightly spaced shotpoints. Although, in principle, vertical seismic reflection profiling of the continental crust[5] offers the possibility of improved resolution of horizontal variations, significant attenuation in the deep Tibetan coast[3] would affect high frequency signals.

Depth-section

The depth section of deep crustal reflectors presented here can be tied in with sections further south discussed in the accompanying article[1] to establish a 500-km long Moho traverse north of Kathmandu (Fig. 2). Parts of it, principally in the northern third, are provisional, as the interpretation now in progress of east–west in-line profiles may add or modify some conclusions. The depth-section is obtained under the assumption that the average crustal velocity is regionally constant. Most seismograms were obtained at constant distance, 200–250 km, from shotpoints, so the plot is that of the time-section; in some parts, however, the data are corrected for larger offset distances. The largest uncertainty in the conversion of time into depth results from lack of control of possible variations in the sharpness of the velocity contrast across the Moho along the section. In contrast to vertical

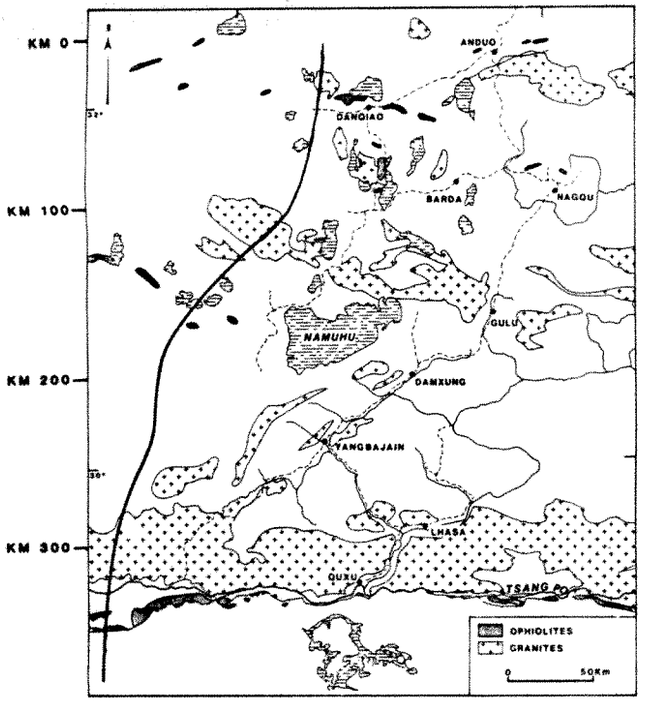


Fig. 1 Map with simplified geology, southern Tibet. The black line is the location of the seismic cross-section of Fig. 2. Data were recorded in a fan-like manner by seismic stations aligned along the main road through Quxu, Yangbajain, Gulu, Nagqu and Anduo at approximately equal distances of 250 km from two shotpoints to the west near latitudes 32° N at Shi Lin Co Lake and 29°5 N in drill-holes at Ang Ren. Distances (in km) from Fig. 2 are taken from the left side scale. South of km 300 the section of Fig. 2 is a composite of data discussed in the accompanying paper[1].

reflection profiling, only interfaces for which the offset of 250 km corresponds to the angle of incidence for critical reflection respond by sending back strong signals. Clear correlated onsets correspond to deep interfaces between crust and mantle material, and cannot originate as simple or multiple reflections from shallow levels or from deep levels with only small velocity contrasts.

North of the Himalayan region discussed previously[1], the 70-km deep Moho can be traced at approximately that depth until it is abruptly interrupted in the region of the Yarlung Zangbo. The Moho rises from a depth of 70 km only about 50 km to the north to a depth of 50 km below the river at km 330, where it is interrupted. What we detect here is a very sharp limit at the Moho level to the south of the Lhasa block. Lithospheric perturbation in this region of former oceanic subduction and suture seemed likely. However, the sharpness of the Moho depth variation was unexpected. We should stress that seismograms on both sides of it were obtained at strictly the same offset from the shotpoint just on the suture, so that there can be no ambiguity of data reduction. If the mantle wedge on the northern side is indicative of the direction of thrusting during subduction, the exact coincidence of the Moho step with the edge of the Gangdise granitic belt, a major structural and mechanical boundary, points to a vertical contact of crustal segments. This is a strong argument for considering this boundary as related to post-suture strike–slip motion. This heterogeneity in the lithosphere may represent one of the major shear zones for the eastward expulsion of lithospheric segments in the continuing motion of India towards Asia[6]. This motion could have preserved and enhanced the sharpness and verticality of the shear zone and taken advantage of the east–west linearity of the original suture.

Three hundred kilometres further north the Lhasa block is thought to be limited by an earlier suture, the Bangong-Nujiang line. The northernmost part of our section crosses a branch of the related ophiolite belt. There is a striking similarity between the northern 70 km of the Moho section and the topography beneath the Yarlung Zangbo suture. Here also we see a very sharp step of slightly less than 20 km in the depth of the Moho and the same polarity. The location of this step is coincident to within about 10 km with the geological boundary at the surface, so that the same aspect of probable verticality of contact between lithospheric segments is documented and the same considerations on post-suture strike–slip activity may hold.

Deep crustal features

For logistical reasons seismic recording stations were situated along the road through Lhasa, Yangbajing, Gulu and Nagqu, which is also a geologically well studied section. Because of the offset, however, seismic data sample the deep structure 100 km to the west, on the other side of the Niainqentangla range which interrupts the east–west structural trends. Thus, deep crustal features cannot be compared with geology in a simple way. In particular the extension to the west of the Damxung accident is not known and conversely the geological significance of the ophiolitic belt and of structural limits which are indicated on general maps and crossed by the seismic section, is not assessed. This central region appears to be the most complicated in the section. From km 330 to km 170 the average depth to the shallowest reflector of crust–mantle type apparently decreases from 70 to 50 km. However, at least four overlapping Moho segments can be identified, three of which are north-dipping, contrary to the general trend. From the Yarlung Zangbo, where it is at 50 km depth, the Moho dips to 70 km at km 300. At this point a further Moho segment is seen on top of it, dipping northwards from 55 km depth at km 300 to more than 75 km at km 230.

Near km 260, data to the south are from the Ang Ren shotpoint whereas to the north they are from Shi Lin Co. When approaching from the south the Moho dips from 60 to 70 km over 30 km distance. When approaching from the north the shallowest reflector is at 50 km, also dipping north. The energy, at this offset, of strong late arrivals suggests these can be attributed to a deeper crust–mantle velocity contrast. The depth and dip of this second level allow it to be tied in continuously through km 260 with the Moho defined from the other side and from another shotpoint. The geometry of these reflector segments could be described as the southward over-thrusting of three north-dipping lower crust layers on each other.

From km 230 to km 170 the depth to the Moho decreases from 50 to 40 km, although with local changes in dip. The succession of overlapping north-dipping reflectors followed in the south of the Lhasa block is interrupted by a new step when the shallowest level, 45 km, is reached at km 160. Contrary to findings in the south, one interpretation here is the northward overthrusting of a south-dipping deep crust segment. Alternatively, as second arrivals after those of the shallow reflector are not followed far to the south, a vertical step in the Moho without overlapping can be postulated.

In the region to the north (from km 120 to km 70), throughout the Bangge–Gulu granitic belt, the base of the crust is apparently about 50 km deep, the shallowest in the whole section. This zone is however tectonically very complicated, with further large ophiolite bodies. Variations in structure are also deduced from an additional east–west seismic profile perpendicular to the section presented here which may thus not be completely representative; furthermore, reflections deeper than this shallow Moho may be present. That such structure exists deeper can also be supposed from the significant signal level in later parts of the seismograms; it is likely that further crustal material is present beneath the shallowest mantle material at 50 km depth.

Tectonic interpretation

The considerable departure of the crust–mantle boundary from the horizontal along the section suggests that crustal thickening did not occur through mere underplating of several hundred kilometres of continuous Indian crust beneath Tibet, unless the

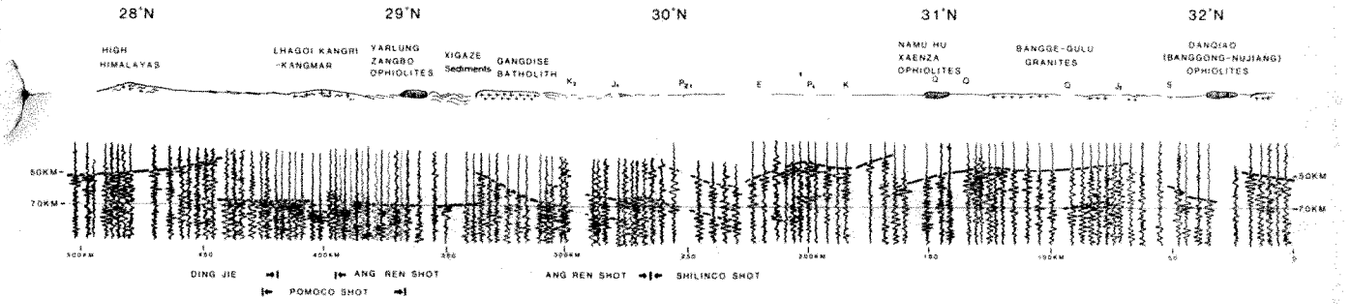


Fig. 2 Time-section of Moho reflections at constant offset of 250 km, that is, critical to the wide angle. This is a depth-section of the topography of the crust–mantle boundary along the line of Fig. 1 assuming negligible variation in average crustal velocity along the section. Approximate distances are counted along the north–south projection. Note the 20 km change in Moho depth across sutures (km 330, Yarlung Zangbo; km 30, Bangong-Nujiang; possibly km 160, Namu Hu ophiolites) thought to indicate later strike–slip motion on vertical block limits. Note also, for example, between km 180 and km 350, successive dipping and overlapping segments of crust–mantle reflectors indicating thickening of deep crustal levels as a result of the superposition of neighbouring pieces of lower crust along deep thrusts.

deep crust topography is due to more recent disruptions. We attribute the occurrence of vertical steps in the Moho, coinciding with major surface lineaments vertically above them (as documented for the two former suture zones bordering the Lhasa block), to such phenomena, mainly to horizontal strike–slip on major east–west lithospheric fractures due to the expulsion of Tibet to the east. The Moho step at km 160 could be interpreted in the same way unless the nearby ophiolite belt (west of Namu Hu) is an allochthonous unit or if prominent strike–slip features can be identified at the surface, as in the case of the Damxung accident on the other side of the Niainqentangla. The overlapping of dipping segments of the Moho noted at several places would suggest intracontinental thrusting of neighbouring crustal slices onto each other as a means of thickening the crust. No data on a possible subdivision of the thick crust of the Lhasa block are available as they were for the southern block towards the Himalayas.[1] It is worth noting however, that, as was the case there, the thickness of crustal units which are seen to interact at depth is less than 20 km. A similar picture of thickening of the lower crust by superposition of neighbouring lower crust units may thus be obtained. The complete thickening of the crust would then also have needed superposition of neighbouring segments of upper crust layers along major thrusts. They could have the same polarity as the lower crust thrusts or the opposite one, as seems to be the case at the Himalayan border. As the part of the Lhasa block north of the Gangdise belt and west of Niainqentangla is not easy to reach, no geological study documenting surface expression of crustal thrusts is available with which to consider this hypothesis.

The Sino-French geoscientific programme in Tibet has been promoted by the foreign ministries of both countries and discussed and supported by the French Institut National d'Astronomie et de Géophysique of CNRS and the Chinese Ministry of Geology and Academia Sinica. Contribution IPG 685.

Received 6 July; accepted 14 October 1983.

1. Hirn, A. *et al. Nature* **307**, 23–25 (1984).
2. Hirn, A., Jobert, G., Wittlinger, G., Xu, Z. X. & Gao, E. Y. *Ann. Géophys.* (in the press).
3. Lépine, J. C., Hirn, A., Pandey, M. R. & Tater, J. M. *Ann. Géophys.* (in the press).
4. Hirn, A. & Sapin, M. *Ann. Géophys.* (in the press).
5. Oliver, J., Cook, F. & Brown, L. *J. geophys. Res.* **88**, 3329–3347 (1983).
6. Tapponnier, P., Peltzer, G., le Dain, A. Y., Armijo, R. & Cobbold, P. *Geology* **10**, 611–616 (1982).

Tectonic environment and geodynamic significance of the Neo-Cimmerian Donqiao ophiolite, Bangong-Nujiang suture zone, Tibet

J. Girardeau*, J. Marcoux†, C. J. Allègre*, J. P. Bassoullet‡, Tang Youking§, Xiao Xuchang§, Zao Yougong§ & Wang Xibin§

* Institut de Physique du Globe, 4 place Jussieu, 75230 Paris Cedex 05, France
† Laboratoire de Géologie, Faculté des Sciences, Moulin de la Housse, 51062 Reims, France
‡ Laboratoire de Géologie Stratigraphique et Structurale, Université de Poitiers, 40 Avenue du recteur Pineau, 86022 Poitiers, France
§ Institute of Geology, Chinese Academy of Geological Sciences, Beijing, China

The Bangong-Nujiang suture zone is underlain by a discontinuous and roughly EW ophiolite belt. Around Donqiao–Gyanco, ophiolitic and metasedimentary Palaeozoic series form nappes thrusted over Jurassic flysch series. All these series have undergone a late symmetrical folding accompanied by generally northward reverse faulting. Although strongly dismembered, a complete ophiolitic sequence comprising harzburgites with large podiform-type chromite deposits, wehrlites, cumulate gabbros, sheeted dolerite dykes, foliated garnet-bearing amphibolites and pillow lavas, has been found.

THE Tibetan plateau, which probably results from successive accretion of several continental blocks to the south of Eurasia, is cross-cut by two major EW suture zones[1,2]. To the south, the Yarlung Zangbo suture zone is underlain by a nearly continuous ophiolite belt over several hundred kilometres. The latter separates the Indian plate to the south from the Lhasa block to the north. In the Xigaze area, the ophiolites form a slice a few kilometres thick thrusted to the south onto the Himalayan series. Although strongly dismembered, it shows a continuous ophiolite sequence, whose most peculiar feature is the occurrence of dolerite sills and dykes throughout the whole sequence from the basal fresh peridotites up to the Upper Albian–Lower

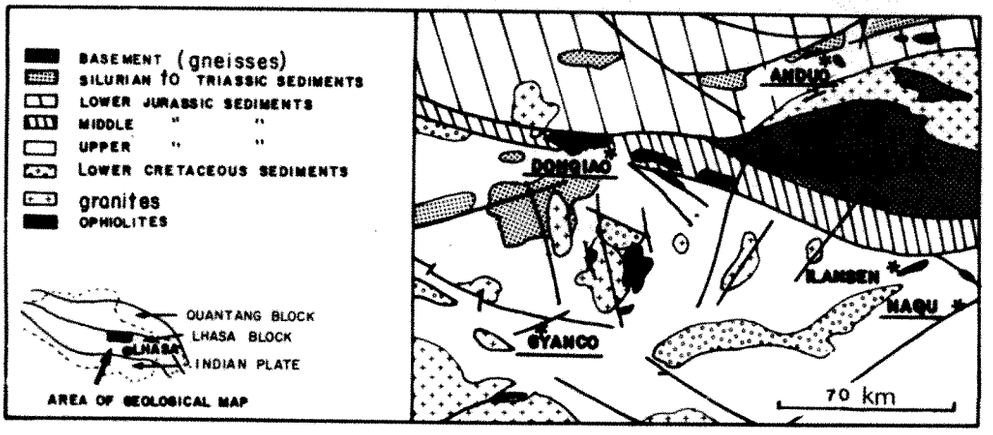
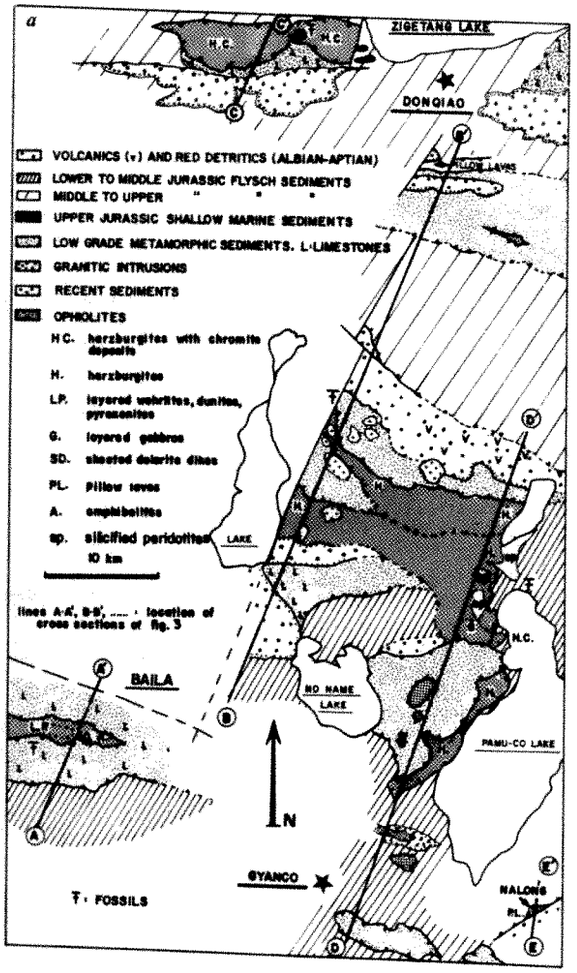


Fig. 1 The Bangong-Nujiang suture zone in the Anduo–Donqiao–Gyanco-Gulu area (from the Chinese 1/1,500,000 scale geological map).



Cenomanian pillow lavas[3–5]. The ophiolite was probably obducted during the Eocene[6,7].

The Bangong-Nujiang suture zone[8–11] separates the Lhasa block to the south from the Quantang block to the north (Fig. 1). It is located approximately 300 km north of the Yarlung Zangbo suture zone and forms poorly defined EW belt several hundred kilometres long running from the north of Laddack to the west, to Nujiang valley (Salouen River) to the east, and is locally underlain by ophiolitic massifs. Very few data have been published on the tectonics of this suture zone[8–11].

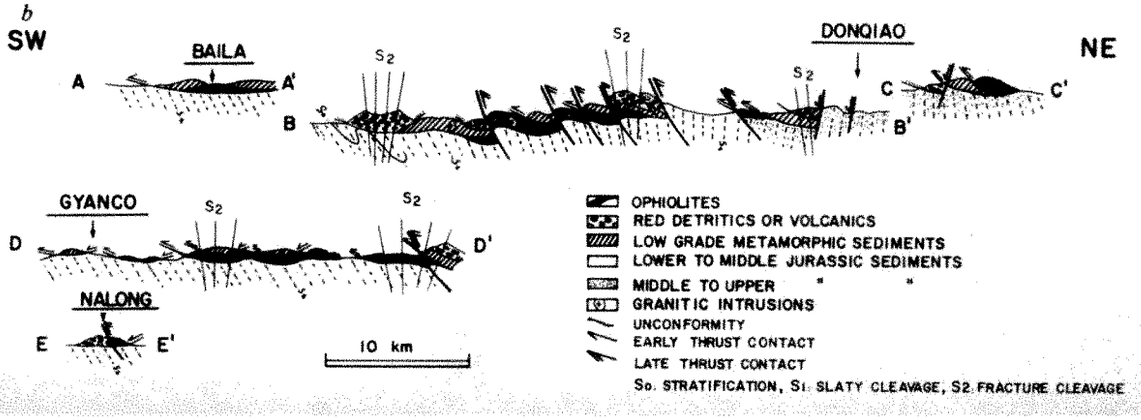
The observations presented here were obtained in the Donqiao–Gyanco area, during the 1981 field work of the 3-yr French–Chinese joint project in Tibet. This area has been mapped at a scale 1:100,000 over a distance of nearly 100 km from north to south between Donqiao and Gyanco, with an approximate EW width of 30 km.

A synthetic cross-section in that area shows, from bottom to top, a succession of Jurassic flysch, and ophiolitic and Palaeozoic metasedimentary series. They all stand in flat tectonic contact with each other and are overlayed in angular unconformity by Aptian–Albian red detritics and volcanics. The lithological and structural characteristics of the different series and their geodynamic significance are discussed.

Lithological and structural characteristics

The most representative outcrops of the Jurassic flysch series can be observed along the eastern side of the Pamu-Co Lake and immediately north of No-Name Lake (Fig. 2*a*). This series consists of rhythmic intercalations of dark shales, slates and yellow sandstones with scarce bio-detritic limestones forming lenses several metres thick. Fossils indicate a Middle Jurassic age (Aalenian) in the Gyanco area (*Gutnicella* (*Lucasella*) *cayeuxi*, *Haurania* sp. and *Nauticulina* sp. in sample XSM 96)

Fig. 2 *a*, Lithological and structural map of the Donqiao–Gyanco area. *b*, NNE–SSW sections through the Donqiao-Gyanco area (location of sections on *a*; topography has not been respected).



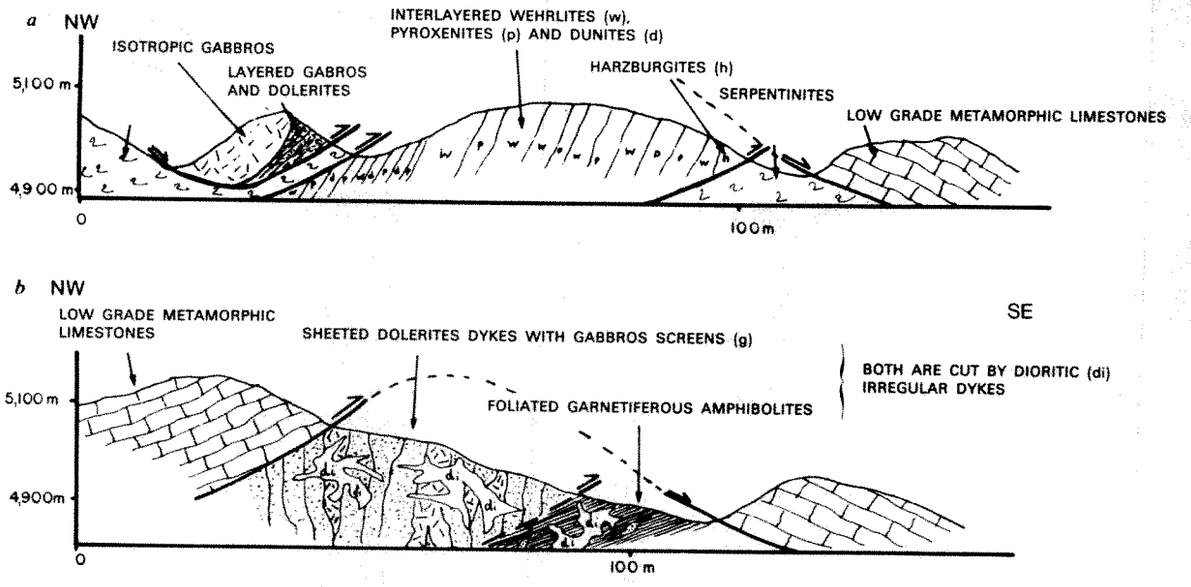


Fig. 3 NW-SE sections through the Baila massif. *a*, Wehrlites and gabbros section; *b*, sheeted dolerites and foliated amphibolite section.

and a Malm age around Donqiao (gastropods, *Sequania* sp.; G. Termier, personal communication).

This sedimentary series has undergone a low-grade metamorphism (sericite–chlorite) and shows N 060 trending asymmetrical folds with subhorizontal axes and a generally north-dipping slaty cleavage S1 (~60°) parallel to their axial planes. In the Donqiao area, this cleavage is vertical or south-dipping (Fig. 2*b*). The F1 folds north of No-Name Lake seem to be overturned to the south. A subvertical late crenulation cleavage S2, related to large symmetrical folds, is locally superimposed on the previous structures.

The ophiolite series tectonically overlies the Jurassic sedimentary series everywhere, their basal contact being marked by low-temperature tectonic breccia, silicified peridotites or schistose serpentinites. Although the ophiolite sequence has been strongly dismembered and scattered, all the different parts of a typical ophiolite suite are found in the Donqiao–Gyanco area. They are:

(1) Residual harzburgites. These largely outcrop in the Donqiao massif and in minor amounts on the western side of Pamu-Co Lake, and in the Baila and Nalong massifs (Fig. 2*a*). Some serpentinites are also observed in the Ilansen area (Fig. 1) and about 20 km east of Amdo (Fig. 1). The harzburgites generally show porphyroclastic structures typical of low-stress high-temperature deformations[12,13]. They are, however, serpentinized and locally schistosed in shear zones related to EW subvertical late northward reverse faults. In some places the harzburgites are rich in dunite patches several metres in size and bear many podiform-type chromite deposits. These deposits, which are surrounded by irregular dunite walls, can reach several tens of metres in size and stand at a steep angle (40°) to the primary foliation plane. The chromite is massive with only a few local interstitial olivines transformed into serpentine. The foliation plane and lineation measured in the harzburgites show nearly constant orientations of about N 060, NW 70 and N 055, NE 17, respectively.

(2) Wehrlites, clinopyroxenites and dunites. These occur only in the Baila massif (Fig. 2*a*), approximately 30 km WNW of Gyanco where they outcrop as a window beneath Palaeozoic metasedimentary rocks (Fig. 2*a*). From south to north a section through this massif (Fig. 3*a*) shows (upwards) a few metres of harzburgites overlain by ~50 m of alternating bands (up to 5 m thick) of wehrlites and clinopyroxenites and ~30 m of more layered rocks composed of 10–30-cm thick layers of dunites, clinopyroxenites and wehrlites.

(3) Troctolites, layered and isotropic gabbros. These have been

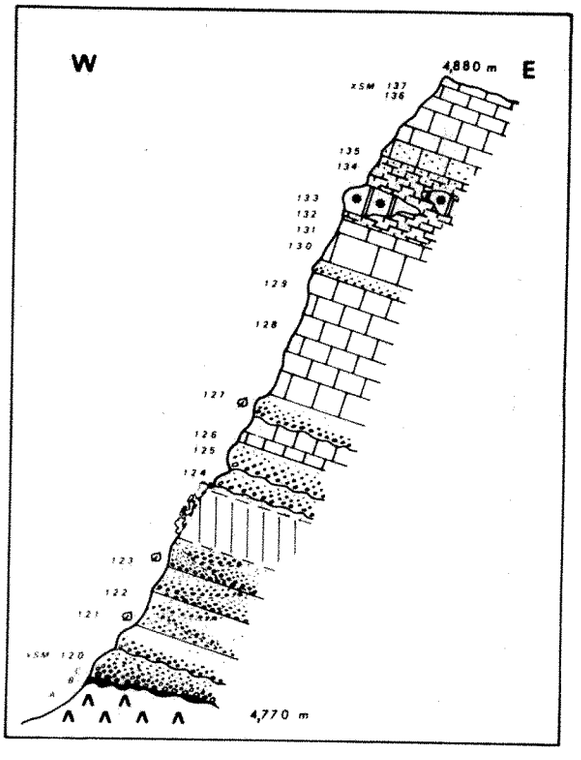


Fig. 4 The transgressive Zigetang formation. From bottom to top: A, serpentinized harzburgites and dunites; B, red silicified encrustings; C, coarse-grained conglomerate with chromite pebbles. Sandstone member, XSM 120 to 127, thick-bedded terrigenous sandstones (cross-bedded laminations and plant remains); calcareous member, XSM 128 to 137, mainly well bedded calcareous mudstones, wackstones, subordonate terrigene influx and patchreefs (XSM 133). Depositional environment: sub-aerial deltaic for XSM 120 to 127 to very shallow marine for XSM 128 to 137. Biostratigraphic content: XSM 122, grainstones with coral remains, *Cladocoropsis* sp. *Pseudochaetetes* sp., algae (Salemoporaceae), *Pycnoporidium lobatum* Y. and T. Foraminifers: *Kurnubia* sp. (or *off. Kurnubia*), *Pseudocyclammina* sp., *Nautiloculina* sp., XSM 133, boundstone with *Cladocoropsis* sp. and Stromatoporidae; XSM 137, mudstone, wackstone with Dasycladacae, *Salpingaporella annulata* Carozzi, *Trinocladus perplexus* Elliot.

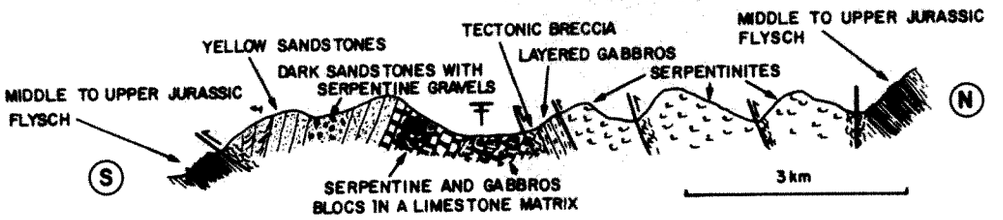


Fig. 5 NS synthetic section across the Ilansen olistostrome formation.

found on the western side of Pamu-Co Lake and in the Baila massif (Fig. 2*a*). Their total thickness does not exceed 50 m. In the Pamu-Co Lake area, troctolites form lenses several metres long and 5–10 m wide which are intercalated with olivine gabbros. They are overlain by a few tens of metres of slightly-layered plagioclase-rich gabbros. In the Baila massif, mafic rocks form a slice in tectonic contact over serpentinized harzburgites and over the previously described wehrlites, clinopyroxenites and dunites (Fig. 3*a*). It consists of approximately 10 m of interlayered fine-grained gabbros and dolerites overlain by 20–30 m of mainly brown amphibole-rich isotropic gabbros and dolerites in minor amounts.

(4) Sheeted dolerites and foliated garnet-bearing amphibolites. Approximately 50 m of sheeted dolerites overlie foliated garnet-bearing amphibolites a few tens of metres thick in the Baila massif area (Fig. 3*b*). The 1-m thick dolerite dykes are intrusive either in scarce isotropic gabbros or in older intrusive dolerite dykes. They generally show two chilled margins. The sheeted dolerites tectonically overlie foliated amphibolites. Both the sheeted dolerites and the foliated amphibolites are intruded by diffuse and interdigitated amphibole-bearing leukocratic diorite. This late undeformed intrusive shows chilled margins with surrounding rocks.

(5) Lava flows and pillow lavas. These outcrop in the Nalong massif, ~20 km east of Gyanco (Fig. 2*a*), and also in the Anduo village (Fig. 1). In the Nalong massif, the volcanics form several tectonic slices limited by late reverse north-dipping faults, and are thrusted to the south over the Aptian–Albian red continental series[14]. The volcanics, which are ~100 m thick, at their base (that is, to the south) comprise mainly massive lava flows several metres thick and, at the top, are formed of pillow lavas with a few hyaloclastite levels. The pillows (40–70 cm diameter in section) are generally variolitic. They display a general northward normal polarity. Lava flows and pillow lavas are cut by rare diabase dykes.

The allochthonous low-grade metamorphic sedimentary series is mostly composed of grey limestones, and of minor yellow-banded quartzites below dark shales. This series is observed in many places in the Donqiao–Gyanco area (Fig. 2*a*). This sedimentary series generally lies over the Jurassic flysch or the ophiolite series but can be locally overthrusted by the ophiolite series, as in the Donqiao area (Fig. 2). We have found no specific

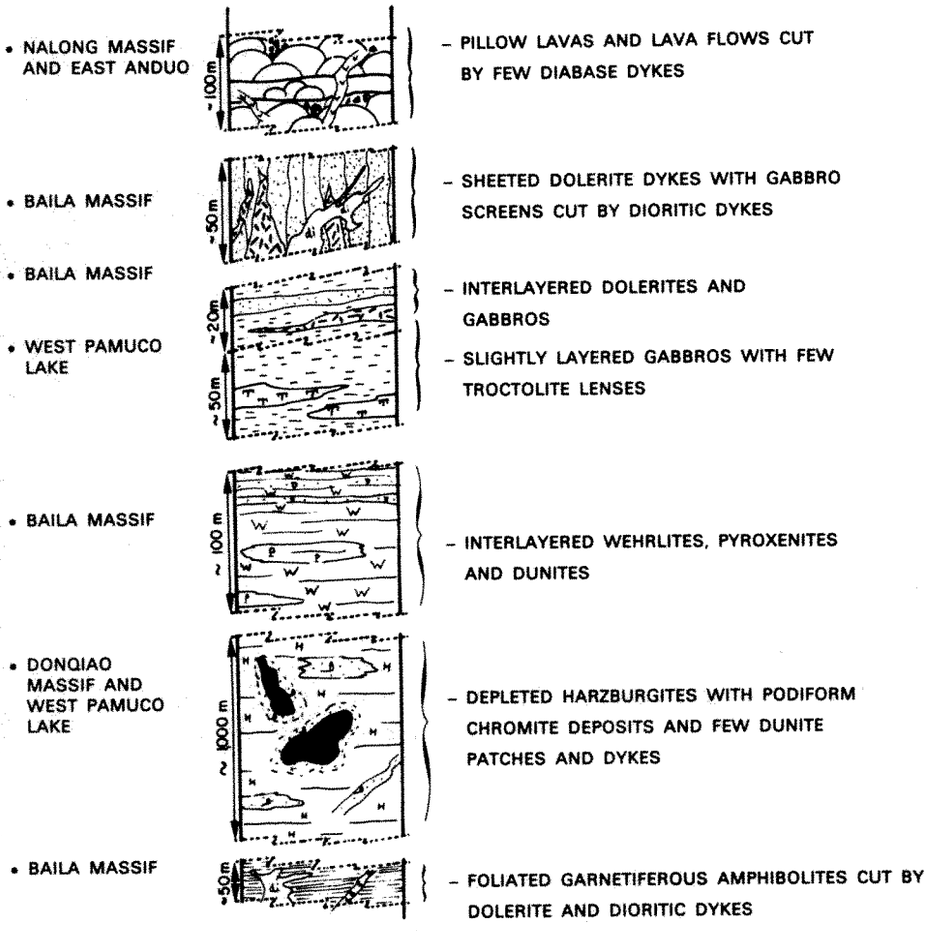


Fig. 6 Restored synthetic section through the Donqiao ophiolitic sequence.

fossils in this low-grade metamorphic formation, which is considered to be Upper Palaeozoic (C. F. Chang, personal communication). This series locally shows south-facing folds. It has been intruded by granites forming locally dykes oriented at about N 120, N 30.

Red detritics comprising conglomerates, red sandstones and shales largely outcrop in the Anduo–Donqiao–Gyanco–Gulu area (Fig. 1). Volcanics occur only north of Pamu-Co Lake where 5-m thick basaltic flows intercalated with thinner vesicular andesitic flows and a few ignimbritic flows lie unconformably above serpentinized peridotites (Fig. 2*a*). These volcanic series, some 10 m thick, are also covered in angular unconformity by red detritics beginning with coarse conglomeratic beds rich in pebbles of volcanics. Upwards, some thin pelite beds and rare ignimbrite flows a few centimetres thick are intercalated within the coarse conglomerate banks. These series, which are ~100 m thick, seem to be covered to the north by a thick yellow volcanic pile.

Biostratigraphical datations have given an Aptian–Albian age for the red sandstones and pelites which outcrop east of Gyanco[14]. The volcanics or red detritics overlie in unconformity the Jurassic flysch series as well as the ophiolitic or Palaeozoic metasedimentary series described above. The volcanics and red detritics are not metamorphic but, in addition to the underlying series, have undergone a symmetrical folding with N 140 axes, accompanied by a fan-shaped spaced cleavage.

North of Donqiao, the chromite-bearing peridotites (Fig. 2*a*) are covered by a transgressive detritic shallow marine formation called the Zigetang formation[15]. It begins with coarse-grained conglomeratic levels containing chromite pebbles transgressing red silicified peridotites (Fig. 4). At the top the conglomerate beds are interbedded with thick terrigenous sandstone beds. This sub-aerial deltaic 40-m thick unit is covered by very shallow marine sediments mainly comprising well bedded calcareous mudstones, wackstones. subordinate terrigene influx and some patchreefs. The thickness of this strongly eroded formation does not exceed 130 m. The numerous foraminifers and algae we have found (see Fig. 4 legend) indicate an uppermost Jurassic–lowermost Cretaceous age.

In the Ilansen area (Fig. 1) the ophiolitic series constitute an olistostrome formation southward thrusted over an Upper Jurassic flysch series. From north to south a section across this massif (Fig. 5) shows (downwards) the stratigraphical succession of yellow sandstones displaying abundant cross-bedded figures indicating a normal polarity to the south, serpentine-bearing sandstones and grey limestones locally rich in angular layered gabbro blocks of various sizes. The contact-between the limestones and the peridotites to the south has not been observed. The grey limestones in which the gabbro blocks are cemented are rich in corals and gastropods, indicating a probable Upper Jurassic age.

The Donqiao ophiolite

A typical ophiolitic sequence, though strongly reduced in thickness (the cumulated thickness is 1,400 m), has been found in the Donqiao–Gyanco area (Fig. 6). This ophiolite is very similar to those previously described elsewhere[16]. It is composed of a mantle part comprising harzburgites which clearly represent residual rocks, wehrlites, pyroxenites and dunites which can be either cumulates or impregnation rocks, and of a crustal part composed of cumulate-layered gabbros, sheeted dolerites and volcanics. The foliated garnet-bearing amphibolites, which lie beneath the sheeted dolerites, could represent remnants of a metamorphic sole, as is seen beneath many ophiolites[17]. The occurrence of large podiform-type chromite deposits is the only peculiarity of this ophiolite sequence. The first geochemical data analysis performed on this ophiolite indicates that it could have been formed in a small ocean in a subduction zone environment and is distinct from the Kigaze ophiolite to the south[18].

Tectonics and geodynamics

Several successive tectonic events can be defined in the different units:

(1) The first tectonic event, D1, has led to folding of the Jurassic flysch series in which south-facing folds accompanied by axial-plane slaty cleavage can be locally observed. This tectonic event has been followed by a large thrusting tectonic event D1-2 corresponding to the southward emplacement of the ophiolites and of the Palaeozoic metasedimentary rocks onto the Jurassic flysch series. Thrusting was from north to south as demonstrated by the NS direction of stretching lineations in limestones beneath the ophiolite series and by subsequent shear structures.

(2) A second folding event, D2, inducing the formation of large symmetrical folds accompanied by vertical fracture cleavage postdates the deposition of the Aptian–Albian red detritics and associated volcanics and deforms all the basal sedimentary or thrust contact planes. It is followed by large reverse faultings producing thrusting of the Upper Jurassic flysch and of the ophiolites over the Lower Jurassic flysch and red detritics, respectively.

The obduction of the Donqiao–Gyanco ophiolite occurred at the Upper Jurassic, as the ophiolite is trangressively covered by uppermost Jurassic–lowermost Cretaceous sub-aerial to shallow marine sediments.

It is therefore likely that the D1 tectonic event observed in the Middle to Upper Jurassic flysch series is due to the obduction of the ophiolite. The obduction probably occurs from north to south as indicated by both the vergence of folds in Jurassic flysch series and the stretching direction and sense determined at the base of the nappes. This obduction can be related to a north-dipping subduction zone which should have been located northwards and could have resulted from the uppermost Jurassic–Lower Cretaceous collision between the Lhasa and the Quantang blocks.

The D2 folding and associated reverse faulting events occurred in the same time span as the folding of the Takena formation to the south (upper part of the Lower Cretaceous) which is probably related to the Eocene India–Eurasia collision[2,6,7].

The Donqiao ophiolite yields new information on the Bangong-Nujiang suture zone. A quite similar suture zone has been described west of Tibet in the Farah Rud, Afghanistan[19,20] and to the east in the Sittang Valley Mytkyina zone in Burma[21,22], where the obduction of the ophiolitic series occurred at the same time (Upper Jurassic to lowermost Cretaceous) during a wide Neo-Cimmerian tectonic crisis along the South Eurasian margin.

We thank J. C. C. Mercier and J. P. Burg for their critical review of the manuscript, G. Termier for fossil determinations and R. Guez for technical assistance. This work has been supported by INAG, through the French–Chinese Three Year Cooperation Project in Tibet. IPG contribution no. 690.

Received 17 June; accepted 29 July 1983.

1. Chang, C. F. & Cheng, H. I. *Scientia sin.* **16,** 257–265 (1973).
2. Tapponnier, P. *et al. Nature* **294,** 403–409 (1981).
3. Nicolas, A. *et al. Nature* **294,** 414–417 (1981).
4. Marcoux, J. *et al. Ophioliti* **6,** Abstr. (1981).
5. Girardeau, J., Nicolas, A., Marcoux, J. & Žao, Yg. *Eclog. Helv.* (submitted).
6. Dewey, J. P. & Bird, J. M., *J. geophys. Res.* **75,** 2625–2647 (1970).
7. Molnar, P. & Tapponnier, P. *Sciences* **189,** 419–426 (1975).
8. Bally, A. W. *et al. U. S. Geological Survey Open Fyle Report,* 80–501 (1980).
9. Chang, C. F., Zheng, X. L. & Pan Y. P. *Chinese Acad. geol. Sci.* **2,** 1–17 (1977).
10. Li, C. Y. *et al. Chinese Acad. geol. Sci.* (1979).
11. Girardeau, J. *et al. Réun. Ann. Sci. Terre. Abstr.* **12,** 179 (1982).
12. Mercier, J. C. C. & Nicolas, A. *J. Petrol.* **16,** 454–487 (1975).
13. Nicolas, A., Boudier, F. & Bouchez, J. L. *Am. J. Sci.* **279A,** 543–576 (1980).
14. Jaegger, J. J. *et al. EOS* **63,** 1093 Abstr. (1982).
15. Chang, C. F. & Pan, Y. S. *Proc. Symp. Qinghai, Xizang, Beijing* (Acad. Sinica, 1981).
16. Coleman, R. G. *Springer Verlag Minerals and Rocks* **12,** (1977).
17. Williams, H. & Smyth, W. R. *Am. J. Petrol.* **279,** 594–621 (1973).
18. Gopel, C. *et al. EOS,* **63,** 1095, Abstr. (1982).
19. Blaise, J., Bordet, P., Carbonnel, J. P. & Montenat, C. *Bull. Soc. Géol. Fr.* **7,** 795–798 (1978).
20. Bassoullet, J. P. *et al. Coll. C5 Memoire BRGM* **115,** 180–198 (1980).
21. Mitchell, A. H. F. *J. geol. Soc. Lond.* **138,** 109–122 (1981).
22. Sengor, A. M. C. *Proc. Symp. Qinghai, Xizang, Beijing,* Vol. 1 (Acad. Sinica, 1981).

High heat flow in southern Tibet

Jean Francheteau*, Claude Jaupart*, Shen Xian Jie†, Kang Wen-Hua‡, Lee De-Lu‡, Bai Jia-Chi‡, Wei Hung-Pin‡ & Deng Hsia-Yeu§

* Institut de Physique du Globe, Université Paris 6, 4 place Jussieu, 75230 Paris Cedex 05, France
† Institute of Geology, Academia Sinica, Beijing, China
‡ Institute of Geomechanics, Chinese Academy of Geological Sciences, Beijing, China
§ Institute of Geophysics, Academia Sinica, Beijing, China

Heat flow measurements have been attempted in two freshwater lakes at altitudes of 4.5 and 5.0 km, south of the Yarlung–Zangbo suture zone in southern Tibet. Probe penetration in the lake sediments was deep enough in the case of eight measurements (5.5–7.2 m) to give reliable temperature gradients. Corrections for seasonal temperature variations, topographic and refraction effects have been applied to the data. In a north–south profile trending perpendicular to the Yarlung–Zangbo suture zone, heat flow is approximately constant at 146 mW m^{-2} over a distance of 30 km and drops to a value of 91 mW m^{-2} in <25 km. The high heat flow and the sharp spatial variation both suggest the existence of a heat anomaly located at relatively shallow depths (no greater than 25 km) in the Tibetan crust, probably due to the recent emplacement of plutonic bodies.

TIBET is a massive plateau with a vast areal extent (2.5×10^6 km^2) at an unusually high elevation (5 km above sea level). It holds a privileged geodynamic position north of the India–Asia collision zone between the Himalaya and Kun Lun mountain ranges. The origin of this remarkable broad regional uplift is still unresolved. Somehow, it must be related to the overly thick (70-km) crust determined by different seismic studies[1,22]. Any model for the plateau uplift must use as a constraint the thermal regime at depth. So far, educated guesses have only been made about a representative geotherm when trying to model the tectonics of Tibet[2] or when trying to recover the uplift rate from fission track analyses in Himalayan rocks[3]. The Tibetan plateau is characterized by strong geothermal activity with numerous boiling springs, hot springs and even geysers scattered over most of its area[4]. Because the rheology of rocks depends strongly on temperature, the thermal structure of Tibet is a most important parameter for understanding the processes active in a continent–continent collision zone. Some predictions have been made. Wang and others[2] assumed a heat flow value of 84 mW m^{-2} on the basis of poorly constrained correlations between heat flow and age in geological provinces[5]. The existence of a thick crust enriched in radioactive elements would also yield a high heat flow. The lack of conductive heat flow measurements leaves the question unsettled. During the last field programme of the French–Chinese cooperation for the study of Tibet and the northern Himalayas which was started in 1980, it was decided to carry out heat flow measurements using oceanographic techniques in the numerous lakes which dot Tibet. The measurements reported here are the first known conductive heat flow measurements in Tibet.

Measurements

Many heat flow measurements have been made in lakes using the oceanic probe technique[6]. They have the obvious advantage that the cost and time required are much less than those needed for boreholes, tunnels or mines. Unfortunately, they are associated with many difficulties. For example, the recent and fast erosion of a lake depression leads to an artificial increase of temperature gradients[7]. These difficulties have been encountered in the Alps where deep lake heat flow data appear systematically higher than tunnel or borehole data[7,8]. There are fewer problems in small and shallow lakes which have been shown to yield results comparable to those from deep boreholes[9]. The ideal lake is probably not too deep, so that the transient erosion effects mentioned above are small, and not too shallow, so that thermal conditions in the lake are rather stable. In Tibet, our main problem has been to find such lakes as well as a suitable vessel from which to conduct the measurements.

We used a pontoon, which could be assembled and disassembled in a few hours, 10×12 m, about 1 m high on the water and powered by three outboard motors. A derrick had been erected in the middle and a gasoline-powered winch permitted the instruments to be lowered on a standard steel cable.

We used seven equally spaced (60 cm) thermistors mounted on outriggers tied either to the 4.5-m long barrel of a standard Kullenberg piston corer or to a thin (48-mm diameter) 6-m long metal rod used in quarry drilling operations. Both the corer barrel or the metal rod were clamped to a Kullenberg coring head with ~250 kg weight. A trigger weight enabled the barrel

Table 1 Heat flow data

Station no.	Lat.	Long.	Water depth (m)	Penetration depth (m)	G_1 (°C km^{-1})	T_1 (°C)	G_2 (°C km^{-1})	Steady-state correction: Topography (%)	Refraction (%)	Warm rim (%)	Heat flow (mW m^{-2})
6	29°10′15″	90°37′00″	40	3.9	/	5.07	185	−13.3	+2.6	−7.2	127
10	29°08′00″	90°41′30″	40	3.9	/	5.08	218	−7.1	+0.8	−3.0	166
14	28°58′45″	90°45′00″	44	6.7	192	5.11	176	−4.3	+1.6	−3.2	138
15	28°51′30″	90°37′30″	33	6.3	294	5.10	299	−1.6	+0.3	−1.3	242
16	28°50′45″	90°36′45″	31	7.0	206	5.11	188	−2.0	+0.4	−1.4	152
17	28°34′00″	90°26′45″	53	7.0	99	4.78	101	/	/	/	88
18	28°33′30″	90°24′45″	57	7.2	101	4.74	103	−1.8	+1.8	−0.3	90
19	28°33′45″	90°28′30″	60	6.9	116	4.88	101	−0.3	+1.0	−0.2	88
20	28°34′00″	90°28′45″	65	7.2	118	4.80	114	−0.2	+1.1	−0.2	100
21	28°34′45″	90°28′15″	60	7.2	131	4.85	101	−0.3	+0.8	−0.4	88

The average heat flow for Yang-Zho-Yung lake (stations 6, 10, 14–16) = 146 ± 17 mW m^{-2} (excluding station 15). Average heat flow for Pu-Mu-Yung lake (stations 17–21) = 91 ± 5 mW m^{-2}. G_1 is the uncorrected temperature gradient which can be estimated from the deepest data points. G_2 is the temperature gradient after correcting for seasonal bottom-lake temperature fluctuations. T_1 is the average bottom-lake temperature, obtained after extrapolating the corrected temperatures.

or rod to free fall through the last 2 m of the water column before penetrating the sediments.

The temperature recorder for the seven sediments and one water temperatures was housed in a basket fastened to the coring head. We used an AANDERAA recorder modified for oceanic heat flow work at the Centre Océanologique de Bretagne, France. Temperatures were recorded internally on magnetic tape which could be read later in our base camp Lang-Ka-Zhe (Fig. 1).

Water depth was monitored by a Furuno FE-400C echo-sounder operating at 50 kHz and good to 480 m. We lacked a 3.5-kHz seismic system so that the sediment thickness was assumed from previous drilling by a Chinese party in one of the lakes (Yang-Zho-Yung lake). Navigation was by means of triangulation on key-features of the shore using large-scale topographic maps (1:100,000) and a hydrographic circle good to 1°.

Conductivity measurements were made on cores using the standard needle-probe method[11].

The two lakes where heat flow work was feasible during our field trip are located south of the Yarlung-Zangbo suture zone (Fig. 1). The northern lake, Yang-Zho-Yung, lies 4,400 m south of a prominent range. It is very tortuous in plan view (Fig. 1) and clearly represents a blocked river valley which used to drain into the Zangbo river and which probably got cut off through tilting in the active extensional tectonic zone striking roughly north–south between Yadong and Gulu[12] (Fig. 1). On the basis of a preliminary description of one core from the deepest part of the lake (core K1), the sediments consist mainly of light-grey, carbonate-rich muds including many layers with grassy mats and seem to have sedimented quietly (K. Kelts, personal communication).

The southern lake, Pu-Mu-Yung, lies at 5,000 m altitude just to the north of the Himalayan mountain range (Fig. 1). Some glaciers feed into the lake. One core (core K4) shows that the sediments are siltier with less admixture of carbonate and fewer diatoms. The core material is being studied for mineralogy, ^{14}C dating, palynology, organic content and ^{210}Pb.

The temperature measurements were conducted by selecting a site away from the shore, lowering the probe 10 or 5 m off the bottom for a few minutes, free-falling the probe into the sediments and waiting for 17 and up to 70 min in the sediments. The thermistors were off scale in the bottom water so that possible instrumental bias between the probes could not be removed.

Results

Four cores about 5 m long were taken before or during the temperature measurements. To check on possible conductivity changes between stations, outrigger sampling was undertaken at 12 stations. A total of 124 conductivity measurements including 24 repeated runs were made within 12 h after samples were retrieved from the lake bottoms.

The results show that thermal conductivity is rather uniform both horizontally and vertically (Fig. 2). There is good agreement between measurements on core and outrigger samples, although a subtle tendency can be detected showing a slight underestimation of conductivity in the outrigger samples, which can be attributed to a slightly different water content. There is no systematic variation of thermal conductivity with depth, except for the lowermost part of the cores (40 cm) where a dramatic increase of k values occurs, presumably due to compaction at the end of penetration (Fig. 2). These high values were omitted from the data set. We therefore used simple average values of 2.00 ± 0.16 (8%) and 2.09 ± 0.20 (9%), in units of 10^{-3} cal cm^{-1} s^{-1} °C^{-1}, for Yang-Zho-Yung and Pu-Mu-Yung lakes respectively.

According to the diffusivity–conductivity relation reported by Von Herzen and Maxwell[11], the thermal diffusivity of these sediments should be $\sim 2.5 \times 10^{-3}$ cm^2 s^{-1}. This value will be used throughout this study.

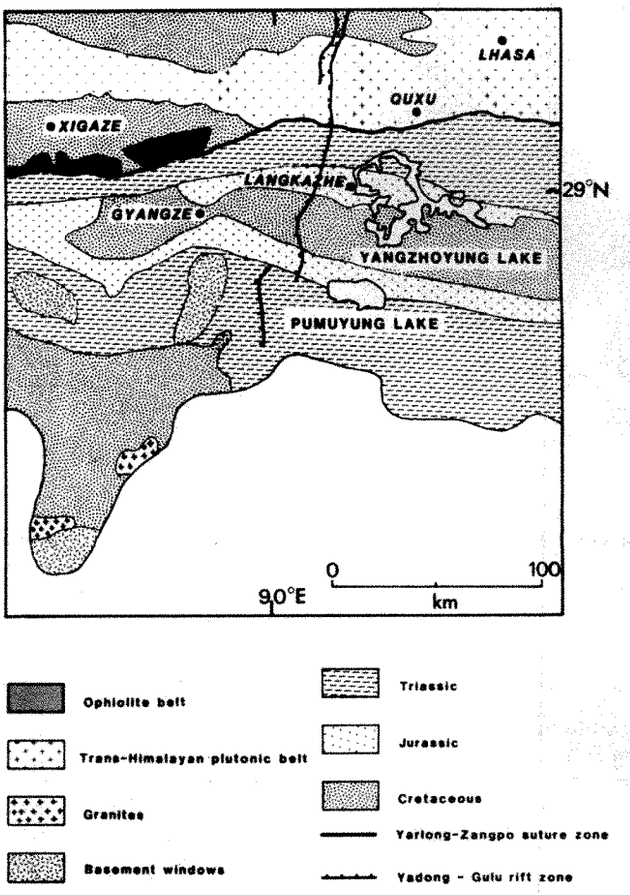


Fig. 1 Schematic geological map of Tibet in the vicinity of Lhasa showing the location of the two lakes where heat flow measurements have been made (modified from ref. 12).

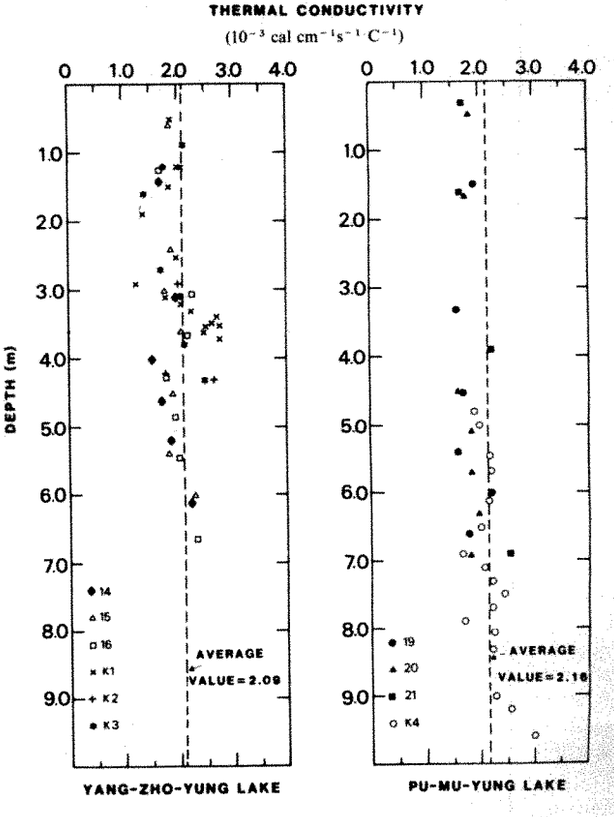


Fig. 2 Thermal conductivity of sediments from the two Tibetan lakes. The average value is that for all measurements.

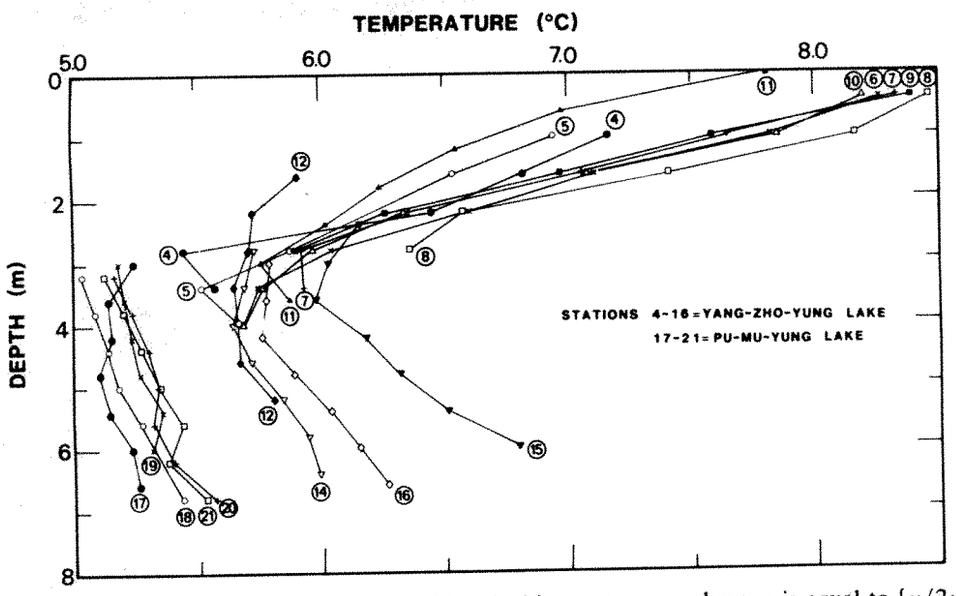


Fig. 3 Temperature versus depth plots for all the heat flow stations of this study.

Seventeen temperature stations were achieved with penetration depths ranging from 2.7 to 7.2 m (Table 1). For most of the stations, the duration of stationing was sufficiently long (>20 min) for equilibrium temperatures to be obtained. All the available measurements can be seen in Fig. 3. Note the good consistency of the data within each lake, and the marked difference between the two lakes. The data are believed to be reliable except for stations 4 and 5 where the measurement duration was too short to yield equilibrium conditions. In stations 11 and 17, the estimates of penetration depths seem to be wrong. Above a depth of 4–5 m, temperatures are obviously affected dramatically by seasonal climatic variations.

We now turn to the various corrections which must be applied to these raw data.

The correction for lake-bottom water temperature fluctuations requires the knowledge of the sediment–water interface temperature variations. In the remote highlands of the Tibetan plateau, none of these data are available or easy to obtain. However, climatic records are available over a 20-yr period from the Lang-Ka-Zhe meteorological station close to our lakes (Fig. 1). Figure 4 shows the evolution of the yearly mean temperature over this length of time, as well as the monthly mean air and ground temperatures. The data can be fitted by a simple cosine function with a period of 1 yr (Fig. 4). The lakes do not freeze in the winter season. The external forcing due to weather conditions is regular and it is likely that the lake water temperature varies with the same 1 yr period. In any case, the 1 yr cycle with 8 °C amplitude must be the dominant component of temperature fluctuations.

Fluctuations with shorter periods are possible due to transient water transport phenomena in the lakes, but would be of smaller magnitude and of much smaller depth penetration. For an amplitude T_0 and a pulsation ω, the resulting amplitude of fluctuations at depth z is (ref. 13, p. 63)

$$T_0 \exp\{-(\omega/2\kappa)^{1/2} z\} \quad (1)$$

Considering the high gradients observed, such fluctuations must be smaller than 0.02 °C in amplitude to be considered as negligible. For the main 1 yr cycle, we found that a good estimate for T_0 was 4 °C. From equation (1), this perturbation has no measurable effect at depths >8.4 m. Shorter period fluctuations must be of smaller amplitudes. Taking for example, a 1 °C amplitude and a 6-month period, we find that the effects are significant down to depths of 4 m only.

As a consequence, we assumed that the major correction to be applied is for a 1 yr cycle, with an amplitude smaller than that of the air temperature (T_0) and a time-delay (ϕ) due to the lake response. The formula used for the temperature fluctuations at depth z is:

$$T_0 \exp\{-\alpha.z\} \cos\{(\Pi/6)t - (7.5/6)\Pi - \phi - \alpha z\} \quad (2)$$

where α is equal to $\{\omega/2\kappa\}^{1/2}$, ω is the pulsation ($\Pi/6$) and t is time (in months). The correction was applied to all stations on a trial basis, varying both the amplitude and the delay, until optimum linear profiles were obtained. Parameters used in establishing the best correction were: amplitudes of 3.2–4.0 °C for both lakes, and small delays of 1.3–1.7 months which are not unexpected since the lakes are rather shallow. The extrapolated sediment–water interface temperatures were checked to be uniform in all cases (Table 1). In each lake, a single type of correction was found to correct most of the stations. The corrected gradients are in good agreement with each other, except for station 15 which yields a very high value. We attribute this anomaly to local refraction and topographic effects because this station is close to a small island in Yang-Zho-Yung lake (Fig. 5). No correction could be applied with reasonable success to several stations with shallow penetrations in Yang-Zho-Yung lake. This is probably due to temperature fluctuations with periods smaller than 1 yr, over which we have no control but which have negligible effects on the deeper data points. These stations do not constitute a heavy loss because they are clustered near stations 6 and 10 where reasonable corrections could be applied. A more detailed discussion of these corrections will be reported elsewhere.

Steady-state corrections are made up of corrections for local topography, thermal refraction at the sediment–basement boundary, and the warm-rim effect of the lake.

These effects were estimated numerically using a finite-element computer program. Calculations were done mostly at the Institute of Geology, Academia Sinica, Beijing and some on a different program in Paris. We took seven profiles between 14 and 30 km long, roughly perpendicular to local topographic features, which covered all heat flow stations. Most of these corrections are of small magnitude (<10%) (Table 1).

Transient corrections involve long-term climatic changes, sedimentation rates and uplift and erosion. Using available climatic models for Tibet[14] and the preliminary observation that the lakes have sedimented quietly, the first two corrections are found to be small (~1.7–3.5% and 1.0–2.6% for climatic and sedimentation effects respectively). The correction for uplift and erosion is more difficult to evaluate because we have no good estimates for erosion and uplift rates. We now discuss the implications of these measurements, with due consideration for the remaining uncertainties.

Discussion

Figure 5 summarizes the heat flow values obtained in this study after all corrections excluding those for uplift and erosion. Note the good consistency of the values within each lake. Given the magnitude of measurement errors which is certainly larger than

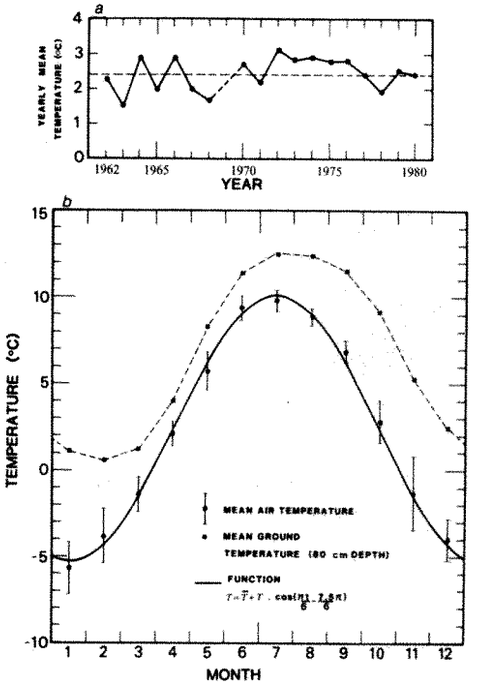


Fig. 4 *a*, Evolution of the yearly mean temperature at the Lang-Ka-Zhe meteorological station. *b*, Evolution of the air and ground temperature (at 80 cm depth) over a period of 1 yr. The air temperature data is made of average monthly values over 20 yr (bars represent ±1 s.d.). Also shown is the simplest cosine function which fits these data.

10% (a representative value for the conventional oceanographic technique), the data do not provide any evidence for a significant variation of heat flow within Yang-Zho-Yung Lake. Our measurements show that heat flow rises sharply from 91 to 146 mW m^{-2} in ≤25 km, and remains approximately constant over a distance of 30 km towards the Yarlung–Zangbo suture zone (Fig. 6). There are *a priori* three possible explanations for such high heat flow values: high heat production, an anomalous heat source (magma), or an anomalous mantle heat flow. A detailed interpretation of the data requires a proper understanding of: (1) uplift and erosion; (2) the regional thermal structure and its evolution with time; and (3) the age of the hypothetical intrusion or melting event. More extensive thermal modelling will be reported elsewhere.

The uplift problem is probably the most crucial one. Several authors have argued on palaeontological evidence and fission track studies[3] that the uplift is recent (2 Myr) and fast (0.2 cm yr^{-1}). Such fast uplift rates could increase the heat flow values by as much as 60%. However, some of these estimates depend on an assumed thermal regime and the reality of such a dramatic pulse remains questionable. The India–Asia collision has been occurring for ~45 Myr (ref. 15) and there is no obvious reason why a major change in tectonic regime occurred 2 Myr ago. Typical epeirogenic uplift rates in active regions are of the order of 0.2 mm yr^{-1} (ref. 16), which would spread out in time the plateau uplifting, making it more compatible with the overall evolution of the region. In such a case, the uplift effect would be much smaller, around 20%. However, these corrections were estimated in the usual fashion (ref. 13, p. 338) and, in the very transient thermal state of Tibet, are of little interpretative value.

Erosion effects must also be taken into consideration. In the absence of any information concerning the shaping of the present terraine morphology, it is impossible to estimate the effects of local and recent erosion, such as those discussed by England[7]. In the plateau environment, they are probably small because local topography is very subdued, as witnessed by the small magnitude of the topographic correction (Table 1). Relief is more pronounced near sites 6 and 10 in Yang-Zho-Yung Lake. However, these stations yield values which are comparable to

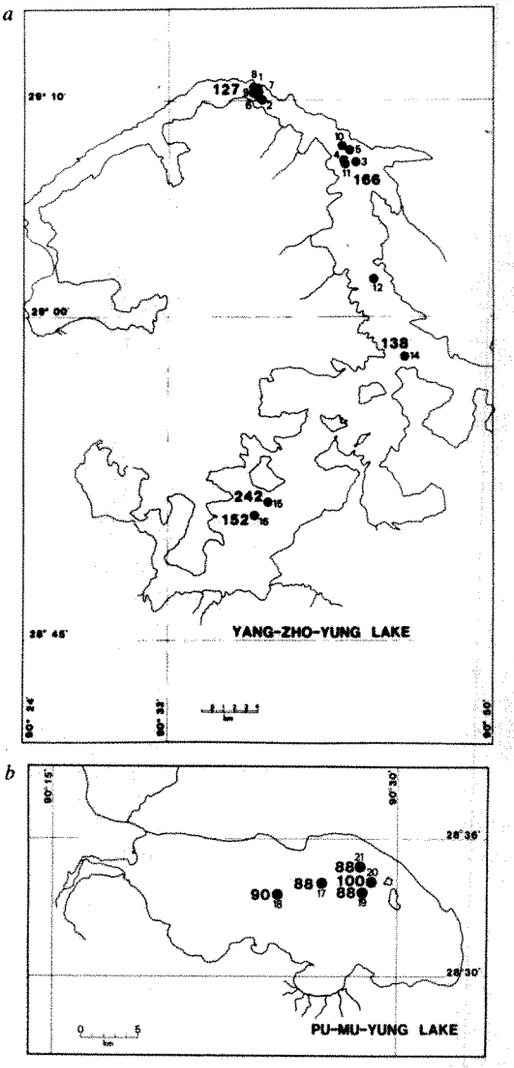


Fig. 5 *a*, Heat flow stations (small numbers) and values (large numbers) in Yang-Zho-Yung lake. Heat flow values are in mW m^{-2} (not corrected for uplift and erosion, see text). *b*, As *a* for Pu-Mu-Yung lake.

those from stations 14 and 16, 20 km away to the South where local topography is negligible (<70 m). We believe that the difference between the heat flow values for the two lakes is real. As will be stated later, electromagnetic measurements yield evidence for a discontinuity at the same location. The regional erosion of the Tibetan highlands is more of a problem. It represents an additional increase of heat flow, as it corresponds to a vertical advection of hot material (see, for example, ref. 17).

Note that in the extreme conditions prevailing here, the usual procedure for 'correction' is not valid. It is designed to account for transient effects which are significant at shallow depths only in otherwise steady-state conditions and to allow the calculation of representative geotherms. In the case of Tibet, we must take into account the full transient thermal evolution of the region, including crustal shortening and the resulting stretching of geotherms[18] as well as regional cooling due to the underthrusting of cold continental lithosphere.

At any rate, such regional phenomena must affect all heat flow values and cannot account for the sharp spatial variation which is observed. Even if the maximum correction of 60% is applied, the Yang-Zho-Yung values would still be high by continental standards: at 58 mW m^{-2} they would clearly stand above 'equilibrium' regions where the mean heat flow is 46 mW m^{-2} (ref. 19). This difference is not due to anomalously high heat production. There are few radioactivity determinations on Tibetan rocks. The available data from basement material

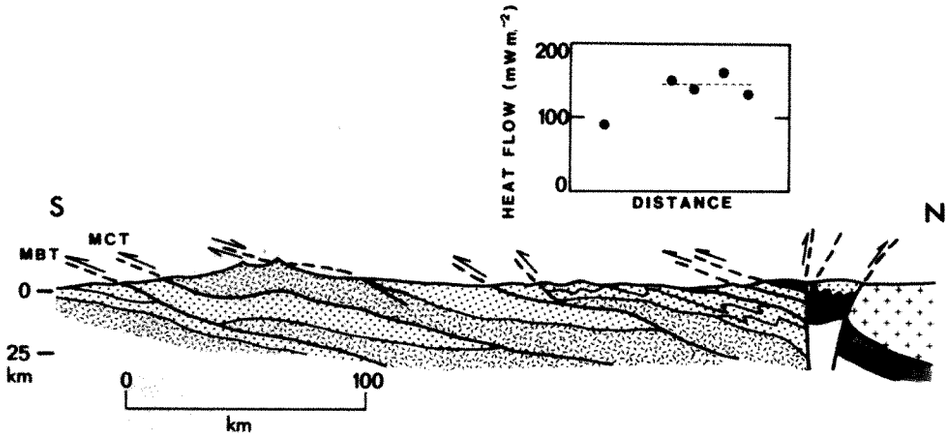


Fig. 6 Schematic cross-section perpendicular to the Yarlung-Zangbo suture zone (from ref. 15). Crosses and dots indicate granitic and sedimentary formations. The stippled and grey patterns are for basement and ophiolite rocks. The heat flow values are shown at their respective locations.

which outcrops to the West of our lakes (Fig. 1) indicate a value of 2.7 $\mu W\,m^{-3}$ for the heat production rate[20]. This value is normal for old continental formations, and, according to empirical heat flow/heat production relations, corresponds to heat flow values around 45 $mW\,m^{-2}$ (see, for example, ref. 19).

One explanation for the high heat flow values would be that because the crust is anomalously thick, there is a larger amount of radioactive elements beneath the plateau. Considering an average heat production of 0.7 $\mu W\,m^{-3}$ for continental crust material[21], it would take a thickness of 140 km to yield a radiogenic contribution of 100 $mW\,m^{-2}$ to the surface heat flow (that is corresponding to an observed average heat flow of 125 $mW\,m^{-2}$). Furthermore, we would also have to explain why heat flow increases so rapidly between the two lakes. Similarly, an anomalous mantle heat flow is not a suitable hypothesis because it should have a regional effect. Even if it was localized in a narrow zone below the lithosphere, it could not create a sharp horizontal variation because of the masking effect of the plate.

The most likely explanation is therefore that there is a source of heat in the crust which terminates abruptly somewhere between the two lakes. Such a discontinuity at this particular spot was also found from magneto-telluric soundings carried out in the same area[21]. This anomalous source of heat is most probably a hot intrusive body. To model the associated thermal effects would require some knowledge about the time of emplacement or melting: a recent event must be shallow to be detectable by surface heat flow measurements. The conductive time scale is $\tau = d^2/4\kappa$, where d is the depth to the anomaly and κ thermal diffusivity. Because the intrusive or melting event must somehow be associated with the underthrusting of India beneath Asia, it must be younger than 45 Myr[15,22,23] and must therefore be shallower than ~70 km (using a κ of $10^{-2}\,cm^2\,s^{-1}$). The Makalu granite in the Himalayan range has been recently dated at about 21 My[24]. This is evidence that melting conditions have been reached in the Tibetan crust after India collided with Asia.

From the value of 146 $mW\,m^{-2}$ and the maximum range in corrections, it is possible to give a rough estimate of the depth to the anomalous body. Assuming steady-state conditions and a standard radioactivity model (see ref. 19), and taking the 600 °C isotherm as a reference for crustal solidus temperatures (for hydrated material such as sediments), we find that this depth must be between 10 and 25 km. Allowing for transient effects, these estimates must be considered as upper bounds. A conservative conclusion is therefore that the heat source must be shallower than 25 km. Such small crustal depths would indeed yield a sharp horizontal variation of heat flow in the vicinity of the body edge. The horizontal distribution of heat flow allows some further constraints on the timing and depth of the intrusive or melting event which will be developed in a future paper.

Our data yield no evidence for any significant heat flow variation over a distance of ~30 km (Fig. 6). This suggests that the heat source is of large horizontal dimensions. If this source is also the cause of the widespread geothermal activity of the plateau, it is not surprising that it has large horizontal dimensions. This would agree with geological observations made in old continental collision zones which typically exhibit tabular granitic formations extending over whole provinces[24].

Sharp horizontal heat flow variations are reminiscent of other tectonically active regions such as the southwestern United States[25,26] or geothermal areas[27]. Finally, note that the heat flow increase occurs at a distance of about 230 km from the main locale of underthrusting (Fig. 6). This is typical of subduction environments where high heat flows and volcanic activity show up abruptly at similar distances from trenches[28].

The measurements reported here suggest that regional melting conditions are reached at relatively shallow crustal depths in Tibet. This represents a powerful contraint for thermo-mechanical models of continental collisions.

The field work was funded by CNRS and INAG (France) and the Chinese Academy of Geological Sciences and Academia Sinica (China). It would not have been possible without the loan of heat flow and coring equipment by CNEXO and GENAVIR. We thank C. Allegré, chief scientist of the French expedition, for his encouragements in setting up this project. Professor Shau Shu Chang was the chief scientist of the Chinese party. We thank H. Lossouarn for help in the field, J. P. Foucher, R. Conogan, C. Toularastel and J. Le Pavec for assistance in preparing the Tibetan cruise and K. Kelts for advice. We also thank the Math Geology Research Group at the Institute of Geology, Academia Sinica, Beijing. We acknowledge the important logistical support provided by the People's Liberation Army and all the individuals who helped us in the field.

Received 5 August; accepted 20 September 1983.

1. Chen, W. P. & Molnar, P. *J. geophys. Res.* **86,** 5937–5962 (1981).
2. Wang Chi-Yuen, Shi Yao-Lin & Wen Hu-Zhou *J. geophys. Res.* **87,** 2949–2957 (1982).
3. Zeitler, P. K., Johnson, N. M., Naeser, C. W. & Tahirkheli, R. A. K. *Nature* **298,** 255–257 (1982).
4. Wang Ji-Yang *et al. J. Volcan. geotherm. Res.* **9,** 57–76 (1981).
5. Chapman, D. S. & Pollack, H. N. *Earth planet. Sci. Lett.* **28,** 23–32 (1975).
6. Hart, S. R. & Steinhart, J. S. *Science* **149,** 1499–1501 (1965).
7. England, P. C. *Earth planet. Sci. Lett.* **39,** 427–434 (1978).
8. Finckh, P. *Bull. geol. Soc. Am.* **92,** 452–514 (1981).
9. Allis, R. G. & Garland, C. D. *Can. J. Earth Sci.* **16,** 1951–1964 (1979).
10. *Geological Map of the Tibetan Plateau,* 1/1,500,000 (Chengdu, China, 1981).
11. Von Herzen, R. P. & Maxwell, A. E. *J. geophys. Res.* **64,** 1557–1563 (1959).
12. Tapponnier, P., Mercier, J. L., Armijo, R., Tong Li-Han & Zhou-Ji *Nature* **294,** 410–414 (1981).
13. Carslaw, H. S. & Jaeger, J. C. *Conduction of Heat in Solids* (Oxford University Press, 1959).
14. Guo, S. in *Symposium on Qinghai-Xizang (Tibet) Plateau,* Beijing, 13–14 (Gordon & Breach, New York, 1980).
15. Allègre, C. J. *et al. Nature* **307,** 17–22 (1984).
16. Blatt, H., Middleton, G. & Murray, R. *Origin of Sedimentary Rocks* (Prentice Hall, Englewood Cliffs, 1980).
17. England, P. C. & Richardson, S. W. *Geophys. J. R. astr. Soc.* **62,** 421–437 (1980).
18. Houseman, G., McKenzie, D. & Molnar, P. *J. geophys. Res.* **86,** 6115–6132 (1981).
19. Sclater, J. G., Jaupart, C. & Galson, D. *Rev. Geophys. Space Phys.* **18,** 269–311 (1980).
20. Vidal, P., Cocherie, A. & Lefort, P. *Geochim. cosmochim. Acta* **46,** 2279–2292 (1982).
21. Van Ngoc, P., Boyer, D. & Therme, P. *Terra Cognita* **3,** 270 (1983).
22. Scharer, U. *Earth planet. Sci. Lett.* in the press (1983).
23. Scharer, U., Xu, R.-H. & Allegré, C. J. *Earth planet. Sci. Lett.* in the press (1983).
24. Nielson, D. L., Clark, R. G., Lyons, J. B., England, E. J. & Borns, D. J. *Geol. Soc. Am. Mem.* **146,** 301–318 (1976).
25. Warren, A. E., Sclater, J. G., Vacquier, V. & Roy, R. F. *Geophysics* **34,** 463–478 (1969).
26. Bodell, J. M. & Chapman, D. S. *J. geophys. Res.* **87,** 2869–2884 (1982).
27. Blackwell, D. D. & Baag, C. G. *Geophysics* **38,** 941–956 (1973).
28. Blackwell, D. D., Bowen, R. G., Hull, D. A., Riccio, J. & Steele, J. L. *J. geophys. Res.* **87,** 8735–8754 (1982).

Evolution of the genes for the β subunits of human chorionic gonadotropin and luteinizing hormone

Karen Talmadge*, Nikos C. Vamvakopoulos* & John C. Fiddes*

Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, New York 11724, USA

Nucleotide sequence comparisons of the single gene for the human luteinizing hormone gene β subunit with two of the seven genes for the human chorionic gonadotropin β subunit suggest that the β human chorionic gonadotropin genes have evolved from an ancestral β luteinizing hormone gene by a series of selected changes with very little neutral drift. Moreover, the 24 amino acid carboxy-terminal extension of the human chorionic gonadotropin β subunit appears to have arisen by a single base deletion that incorporated the 3'-untranslated region of the ancestral β luteinizing hormone gene into the coding region.

HUMAN chorionic gonadotropin (HCG) and luteinizing hormone (LH) are two members of the glycoprotein hormone family. The two hormones are very similar both functionally and structurally, although they are expressed in different tissues and at different developmental stages. Luteinizing hormone is made in the pituitary and has a central role in promoting spermatogenesis and ovulation by stimulating the testes and ovaries to synthesize steroids. In contrast, HCG is made by the first trimester placenta and stimulates the ovaries to synthesize the steroids that are essential for the maintenance of pregnancy. Nevertheless, both hormones bind to the same testicular and ovarian receptors (for a review of the biochemistry of these hormones, see ref. 1).

In common with the other members of the glycoprotein hormone family, HCG and LH are dimeric with two dissimilar, noncovalently associated subunits, α and β. The α subunit is common to all the glycoprotein hormones[1] and, in humans, is encoded by a single gene which has been isolated and characterized[2–4]. Unlike the α subunit, the β subunits are unique to each hormone and confer biological specificity. However, in keeping with their closely related biological functions, β-HCG and β-LH have an 82% amino acid sequence homology[5–9].

We isolated a cDNA clone for the β subunit of HCG[10] and used this to examine the structure and chromosomal organization of the genes for the β subunits of HCG and LH. We found that the β-HCG and β-LH genes were linked[11] and that the latter was encoded by a single gene[12]. In contrast, we found that β-HCG is encoded by seven genes or pseudogenes[11,12] which, together with the single β-LH gene, were shown to have an unusual structural organization of genes arranged in tandem and inverted pairs[11]. Figure 1 summarizes these data.

We present here the complete nucleotide sequences for the single β-LH gene and for two of the β-HCG genes, those numbered 5 and 6, which were chosen because they form one of the two inverted gene pairs (see Fig. 1) and we were interested in the possible implications of this symmetrical arrangement for gene expression. The strategies used to determine the sequences are shown in Fig. 2 and the sequences, aligned with respect to each other, are shown in Fig. 3. Comparison of these sequences suggests that the β-HCG gene evolved from an ancestral β-LH gene by a series of selected changes with very little neutral genetic drift. The sequence comparison also shows that the carboxy-terminal extension of the β-HCG subunit protein arose, as we previously predicted[10], by a readthrough event in which the 3'-untranslated region of the β-LH gene became incorporated into the coding region. This readthrough could have been caused by a single base deletion.

Origin of the HCG gene

Luteinizing hormone is synthesized in the pituitaries of a wide range of species. In contrast, chorionic gonadotropin is found only in the placenta of certain mammals such as horses, baboons and humans[1,13,14], and is thus considered to be the most recently evolved member of the glycoprotein hormone gene family. Due to the strong structural and biological similarity between LH and HCG, it has been suggested previously that the β-HCG gene arose directly from a β-LH or β-LH-like ancestral gene[15].

The β subunit of HCG is 24 amino acids longer than the β subunit of LH. Alignment of the two β subunit amino acid sequences shows that the extra 24 amino acids of the HCG β subunit form a carboxy-terminal extension that is unique to β-HCG. We suggested previously[10] that this carboxy-terminal extension resulted from a readthrough event in which the β-HCG gene evolved from its β-LH gene ancestor by incorporation of the 3'-untranslated region into the coding sequence. This suggestion was based on the observation that the cDNA

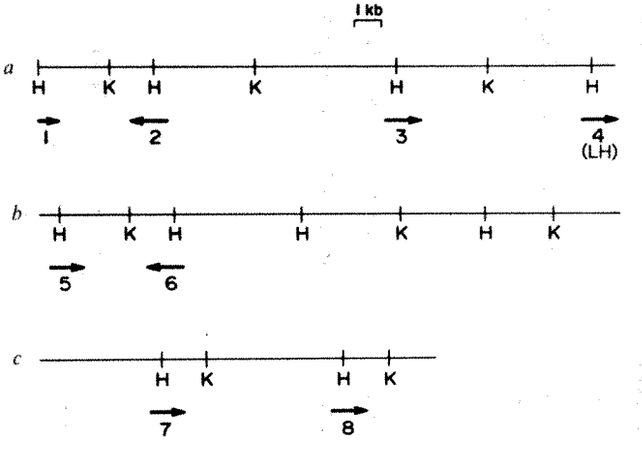


Fig. 1 Organization of the three groups, *a*, *b* and *c*, of cloned sequences that hybridize to the β human chorionic gonadotropin cDNA probe. The positions of the eight genes (4 is the single β luteinizing hormone gene) are shown by arrows pointing in the putative directions of transcription. Locations of sites for the restriction enzymes *Hin*dIII (H) and *Kpn*I (K) are shown. The identification of these genes was established in refs 11 and 12. The map is adapted from ref. 11.

* Present addresses: California Biotechnology Inc., 2450 Bayshore Frontage Road, Mountain View, 94043, California, USA (K.T., J.C.F.); 28 Kononos, Pagrati, Athens, Greece (N.C.V.).

Table 1 Gene sequence comparisons

Gene pairs	Replacement changes No.	Replacement changes %	Silent changes No.	Silent changes %	Intron changes No.	Intron changes %
LH:CG5	19/296.7	6±2	7/101.3	7±3	24/545	4±1
LH:CG6	19/296.7	6±2	8/101.3	8±3	32/545	6±1
CG5:CG6	1/369	0.3±0.3	1/123	1±1	18/545	3±1
HGH:HPL	33.5/482	7±1	18.5/161	11±3	72/726	10±1
RI:RII	8/249.2	3±1	15/80.8	19±5	21/99	21±5
RBG:MBG	35/330	11±2	49/110	44±6	120/267	45±4

Genes compared: LH, β luteinizing hormone; CG5, β human chorionic gonadotropin gene 5; CG6, β human chorionic gonadotropin gene 6; HGH, human growth hormone; HPL, human placental lactogen; RI, rat preproinsulin gene I; RII, rat preproinsulin gene II; RBG, rabbit β globin; MBG, mouse β globin. The values for the RI:RII and RBG:MBG comparisons are taken from ref. 20, all other values were calculated by the method given in ref. 20. The fractions (%) are the number of actual substitutions divided by the number of potential substitutions of a given type. The standard deviations were estimated as the square root of [the fractional change (%) divided by the number of potential changes]. The data are not corrected for possible multiple substitutions. The two LH:CG comparisons were made over the homologous coding regions from the start codons at position 553 up to, but not including, the single base deletion in the CG genes that is responsible for the frameshift. The CG5:CG6 comparison is over the 164 residue signal sequence and mature protein encoding regions. The HGH:HPL comparison is over their 210 residue signal sequence and mature protein encoding regions.

for the β subunit of HCG had a particularly short 3′-untranslated region of 16 nucleotides and that the termination codon of β-HCG was the TAA sequence of the polyadenylation signal AATAAA[16].

We can now identify the nucleotide changes that caused the readthrough. A single base deletion at position 1,540 puts the β-HCG sequence out of frame with the last seven codons of β-LH and allows the 3′-untranslated region to become incorporated into the coding region. In addition to this, a two base pair (bp) insertion at positions 1,612 and 1,613 in the β-HCG sequence allows the carboxy-terminal extension to continue to the termination codon, TAA. Without this 2 bp insertion, the β-HCG carboxy-terminal extension would be eight amino acids shorter, ending with a TGA termination codon. If the 2-base insertion occurred first, it would have been a neutral mutation as the inserted bases would still be part of the 3′-untranslated region. If the single base deletion occurred first, then the β-HCG coding sequence has undergone two carboxy-terminal extensions, but we consider this unlikely. Nevertheless, by either mechanism, the primary event that led to the incorporation of the 3′-untranslated region into the coding region was the single base pair deletion. We note that the β-LH termination codon, put out of frame in the β-HCG gene, has been lost by a single base change, but we do not think that that could have been the initial event leading to the carboxy-terminal extension because there is no other terminator in the 3′-untranslated region in frame with it.

Thus, the β subunit of HCG has acquired 8 amino acids by translation in a new reading frame, and 24 amino acids from a previously untranslated region. This has occurred without any substantial change other than the frameshift mutations as β-HCG genes 5 and 6 are 90% homologous with the β-LH gene over the region encoding the carboxy-terminal extension, nucleotides 1,541–1,634. The function of this carboxy-terminal extension is not known as HCG appears to bind to the same membrane receptors and to elicit the same response as LH. However, HCG is 10 times more stable than LH, and it has been suggested that the carboxy-terminal extension has a role in this (see ref. 13 for a review).

Nucleotide changes

We suggested previously[11,12] that the β-HCG gene arose by a duplication of an ancestral gene. This duplication was followed by a series of changes, including the frameshift mutations, which generated the new β-HCG function. Once this new function was established, the β-HCG gene itself duplicated and rearranged to give the present complex organization (see Fig. 1). The nucleotide sequences we have established here provide information on the series of changes that took place as the newly duplicated ancestral gene evolved into that for β-HCG.

To compare the nucleotide sequence differences between the β-LH gene and the two β-HCG genes 5 and 6, we have calculated the per cent divergence between the introns, and between replacement and silent substitution sites across the homologous coding regions. These data are shown in Table 1. Table 1 also shows similar calculations for a selection of other gene pairs, including the two β-HCG genes themselves, human growth hormone (HGH) and human placental lactogen (HPL) genes[17–19], the two rat preproinsulin genes I and II[20], and the rabbit and mouse β-globin genes[21,22].

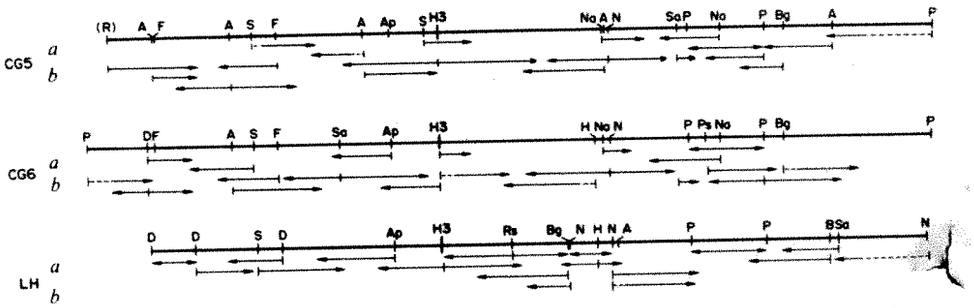


Fig. 2 Nucleotide sequencing strategy for the β-HCG genes 5 and 6 (CG5 and CG6) and the β-LH gene. Arrows mark the restriction sites sequenced from and the extent of the individual sequence determinations, the dotted portions of the arrows indicating where the sequence was not read immediately adjacent to a labelled site. Restriction enzyme sites used in the sequence determination are marked. A, *Ava*I; Ap, *Apa*I; B, *Bgl*I; D, *Dde*I; F, *Hin*fI; H, *Hin*cII; H3, *Hin*dIII; N, *Nco*I; Na, *Nae*I; P, *Pvu*II; (R), *Eco*RI (generated by an *Eco*RI linker); Rs, *Rsa*I; S, *Stu*I; Sa, *Sac*I. The nucleotide sequence of β-LH gene shown in Fig. 3 includes the area marked by solid arrows. The nucleotide sequences of β-HCG genes 5 and 6 shown in Fig. 3 begin about 100 bp after the solid arrows above. Both the chain termination method (*a*) and the chemical degradation method (*b*) were used. For chain termination sequencing[36], purified fragments were blunt-ended and cloned into the unique *Sma*I site of M13mp8[37]. A 17 bp synthetic primer was purchased from P-L Biochemicals. Chemical sequencing[38] was performed on fragments labelled at their 3′ ends with [α-^{32}P]-deoxynucleoside triphosphates and the Klenow fragment of DNA polymerase I. All restriction enzyme sites were overlapped in the β-HCG gene 5, but in the cases of β-HCG gene 6 and the β-LH gene, the overlap at one and two sites respectively was done by analogy with the β-HCG gene 5.

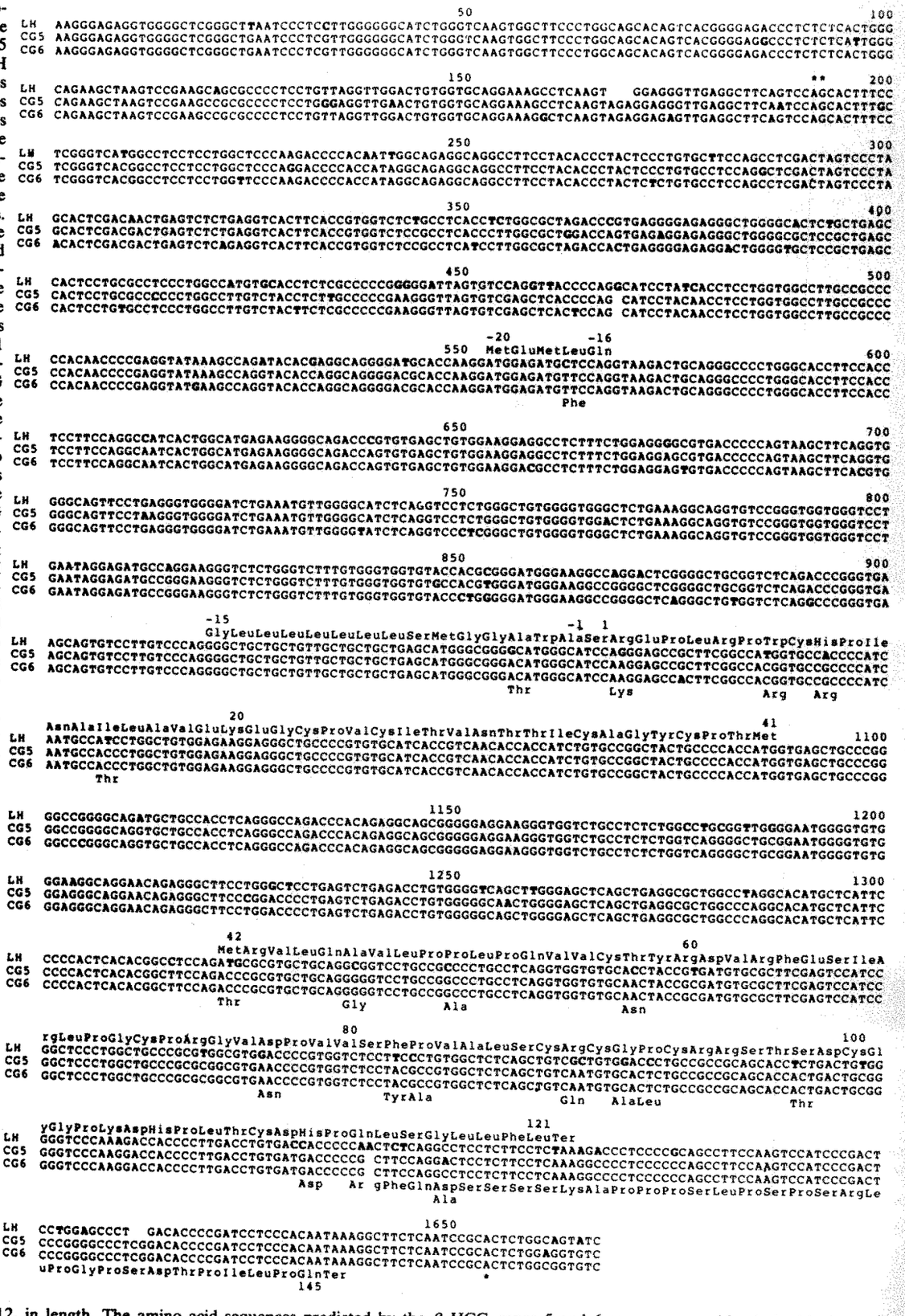


Fig. 3 The complete nucleotide sequences of the β-HCG genes 5 and 6 (CG5 and CG6) and the β-LH gene. The three sequences have been aligned with gaps where there are deletions and insertions, and the nucleotide sequence numbers, immediately above the nucleotide sequences, are not adjusted for deletions. In the alignment of the nucleotide sequences, bold face letters indicate positions where one of the three sequences differs from the other two. The two asterisks at nucleotides 190 and 191 identify the proposed transcriptional start for β-HCG (unpublished data), and the single asterisk at nucleotide 1,654 marks the polyadenylation site. The amino acid sequence of β-LH is shown above the nucleotide sequence. For the β-HCG genes 5 and 6, only the predicted amino acids that differ from those of β-LH are shown below the nucleotide sequences. Amino acid numbers are placed immediately above amino acid names, and negative numbers refer to amino acids in the signal peptide. The β-HCG genes 5 and 6 introns were located by comparison with the β-HCG cDNA sequence[10]. The β-LH gene introns were located by homology with the β-HCG gene sequences. The sequences at the intron–exon boundaries show the expected homologies with those in other systems[39–41]. The β-LH amino acid sequence derived from our nucleotide sequence differs from the published sequence[5,9], in that there is a Met–Met and a Val–Val at positions 41–42 and 55–56, respectively, rather than the single methionine and valine residues previously reported[5,9], bringing the β-LH protein sequence into register with that of β-HCG. Also, the carboxy-terminus is seven amino acids longer than previously reported[9], making the human β-LH sequence more homologous to the ovine, porcine and bovine sequences[42–44] and 121 residues, rather than 112, in length. The amino acid sequences predicted by the β-HCG genes 5 and 6 sequences are identical with the exception of position 117, which is aspartic acid in gene 5 and alanine in gene 6. This position is aspartic acid in both the published amino acid sequences from two groups[7,8] and in our cDNA sequence[10]. The β-HCG genes 5 and 6 have a single silent change in codon 4; our cDNA sequence[10] and β-HCG gene 5 agree. The nucleotide sequence of the β-HCG gene 5 is identical with that of the published cDNA sequence with two exceptions: nucleotide position 529 is a C in the gene sequence and a G in the cDNA sequence (this nucleotide is close to the 5′ end of the cDNA clone, and presumably was misincorporated by reverse transcriptase, as has been observed in other systems[45]), and nucleotide position 1,589 is a T in the gene sequence and a C in the cDNA sequence (this is a silent third position change and is an error in the published cDNA sequence[10]).

Striking differences emerge from these comparisons. For the preproinsulin and β-globin gene pairs, the rates of intron and silent position divergence are similar within each pair, and are four to seven times greater than the rate of replacement changes. Similar results have been obtained with a further 42 gene pairs encoding identical functions and a further 4 gene pairs encoding different functions[23,24]. In marked contrast, the replacement and silent sites of the two β-HCG genes have diverged from those of the β-LH gene at similar rates, between 6 and 8%, whilst the introns of each pair have diverged slightly less, between 4 and 6%. Thus, unlike most other gene pairs, the replacement, silent and intron changes have all occurred at approximately equal rates. The introns and silent sites of the HGH/HPL gene pair have also diverged at rates that are only slightly higher than the rate of replacement changes. It is interesting that the HGH/HPL gene pair is analogous in this respect to the β-HCG/β-LH gene pair: both proteins in each of the pairs have similar functions, but one (HGH/HPL) is synthesized in the pituitary while the other (β-HCG/β-LH) is synthesized in the placenta. A high rate of replacement, compared with neutral, changes has also been observed in a pair of rabbit globin alleles[21,25,26] and a pair of immunoglobulin light-chain genes[27], and in cDNA fragments encoding the carboxy-terminal thirds of two mouse H-2^d antigens[28].

Discussion

How can we explain the high rate of replacement, compared with neutral, changes between the β-LH/β-HCG and the HGH/HPL gene pairs? One theory of evolution holds that observed changes in protein structure are spread through the population by selection[29–31]. In support of this hypothesis, Perler *et al.*[23] cite the two rabbit β-globin alleles[21,25,26], between which there are four replacement changes with no silent changes and only two intron changes, as a clear example of selection. They assumed that these changes were fixed so rapidly by selection that there was no opportunity for neutral changes to appear. This could be the case for the reported immunoglobulin light-chain[27] and H-2^d antigen[28] gene pair comparisons, although the generation of polymorphisms in these systems may be a specialized and, at least in the case of the immunoglobulin light chains, perhaps distinct mechanism. The selection argument of Perler *et al.*[23] can also be applied to the evolution of the new functions in the β-HCG/β-LH and HGH/HPL gene pairs.

An alternative interpretation of the high rate of replacement, compared with neutral, changes in the evolution of the β-HCG from a β-LH gene is that genes evolve first by fixation of the new function (selection), followed by accumulation of neutral changes (drift). By this hypothesis, the evolution of β-HCG is the most recent event yet analysed, more recent than the evolution of HPL. Like the β-chorionic gonadotropin and β-LH genes, the GH and PL genes diverged late in mammalian radiation[32], but which gene family diverged first is not known because systematic cross-species protein comparisons either for chorionic gonadotropin or PL have not been done yet.

The neutral mutation–random drift theory[33–35] holds that most observed changes in amino acid sequence are neutral and are fixed in the population by random drift. Unless the β-HCG genes duplicated very recently, it is difficult to imagine how so many replacement changes could have taken place between the β-HCG and β-LH genes by neutral drift, given that so few replacement changes have been allowed between the two β-HCG genes. It is unlikely that the β-HCG genes duplicated recently compared with the original β-LH gene duplication, because the rate of divergence of the intron sequences of β-HCG genes 5 and 6 approaches those of the β-LH/β-HCG gene pairs.

From the rapid rate of replacement relative to neutral changes in the evolution of β-HCG from β-LH, and the low level of replacement relative to intron changes between the two β-HCG genes, we conclude that selection of a new function is the mechanism most consistent with the observed sequence differences. If stability is truly the only difference between HCG and LH, then the changes across the homologous coding region, and not just the carboxy-terminal extension, must be involved. However, we believe that there may be other, as yet unidentified, functional differences between chorionic gonadotropin and LH that require the observed coding differences. Moreover, because the divergence between the two β-HCG intron sequences approaches the divergence between the introns of the two β-LH/β-HCG gene pairs, we suggest that the β-HCG gene duplications and rearrangements occurred soon after the establishment of the new β-HCG function.

This work was supported by a grant from the NIH. We thank M. DeLuca for technical assistance, Computer Technology, Limited, for the computer print-out of the sequences, our colleagues at Cold Spring Harbor Laboratory for their valuable advice and discussions, and T. Jukes, B. Cordell, P. Kushner and B. McCarthy for helpful comments on the manuscript. K.T. was a postdoctoral fellow supported by a grant from the Ford, Rockefeller and Mellon Foundations.

Received 4 August; accepted 4 October 1983.

1. Pierce, J. G. & Parsons, T. F. A. *A. Rev. Biochem.* **50,** 465–495 (1981).
2. Fiddes, J. C. & Goodman, H. M. *Nature* **281,** 351–356 (1979).
3. Fiddes, J. C. & Goodman, H. M. *J. molec. appl. Genet.* **1,** 3–18 (1981).
4. Boothby, M., Ruddon, R. W., Anderson, C., McWilliams, D. & Boime, I. *J. biol. Chem.* **265,** 5121–5127 (1981).
5. Shome, B. & Parlow, A. F. *J. clin. Endocr. Metab.* **36,** 618–621 (1973).
6. Morgan, F. J., Birken, S. & Canfield, R. E. *J. biol. Chem.* **250,** 5247–5258 (1975).
7. Keutmann, H. T. & Williams, R. M. *J. biol. Chem.* **252,** 5393–5397 (1977).
8. Birken, S. & Canfield, R. E. *J. biol. Chem.* **252,** 5386–5392 (1977).
9. Keutmann, H. T., Williams, R. M., Ryan, R. J. *Biochem. Biophys. Res. Commun.* **90,** 842–848 (1979).
10. Fiddes, J. C. & Goodman, H. M. *Nature* **286,** 684–687 (1980).
11. Boorstein, W. R., Vamvakopoulos, N. C. & Fiddes, J. C. *Nature* **300,** 419–422 (1982).
12. Talmadge, K., Boorstein, W. R. & Fiddes, J. C. *DNA* **2,** 279–287 (1983).
13. Canfield, R. E., Birken, S., Morse, J. H. & Morgan, F. J. in *Peptide Hormones* (ed. Parsons, J. A.) 299–315 (University Park, Baltimore, 1978).
14. Ward, D. N., Moore, W. T. Jr & Burleigh, B. D. *J. Protein Chem.* **1,** 263–280 (1982).
15. Dayhoff, M. O. in *Atlas of Protein Sequences and Structure* Vol. 5, Suppl. 2, 122 (National Biomedical Research Foundation, Washington 1976).
16. Proudfoot, N. J. & Brownlee, G. G. *Nature* **263,** 211–214 (1976).
17. Goodman, H. M. *et al.* in *Mobilization and Reassembly of Genetic Information* (eds Scott, W. A., Werner, R., Joseph, D. R. & Schulz, J.) 155–179 (Academic, New York, 1980).
18. DeNoto, F. M., Moore, D. D. & Goodman, H. M. *Nucleic Acids Res.* **9,** 3719–3730 (1981).
19. Seeburg, P. H. *DNA* **1,** 239–249 (1982).
20. Lomedico, P. *et al. Cell* **18,** 545–558 (1979).
21. Efstradiatis, A., Kafatos, F. C. & Maniatis, T. *Cell* **10,** 571–585 (1977).
22. van den Berg *et al. Nature* **276,** 37–44 (1978).
23. Perler, F. *et al. Cell* **20,** 555–566 (1980).
24. Miyata, T., Yasunga, T. & Nishida, T. *Proc. natn. Acad. Sci. U.S.A.* **77,** 7328–7332 (1980).
25. Hardison, R. C. *et al. Cell* **18,** 1285–1297 (1979).
26. van Ooyen, A., van den Berg, J., Mantei, N. & Weissmann, C. *Science* **206,** 337–344 (1979).
27. Bernard, O., Hozumi, N. & Tonegawa, S. *Cell* **15,** 1133–1144 (1978).
28. Bregegere, F. *et al. Nature* **292,** 78–81 (1981).
29. Clarke, B. *Science* **168,** 1009 (1970).
30. Richmond, R. C. *Nature* **225,** 1025 (1975).
31. Blundell, T. L. & Wood, S. P. *Nature* **257,** 197 (1975).
32. Niall, H. D. in *Prolactin and Carcinogenesis* (ed. Griffiths, K.) 13–20 (Alpha Omega Alpha, Cardiff, 1972).
33. Kimura, M. *Nature* **217,** 624–626 (1968).
34. Kimura, M. & Ohta, T. *Nature* **229,** 467–469 (1971).
35. Kimura, M. & Ohta, T. *Proc. natn. Acad. Sci. U.S.A.* **71,** 2848–2852 (1974).
36. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
37. Messing, J. & Vieira, J. *Gene* **19,** 269–276 (1982).
38. Maxam, A. & Gilbert, W. *Meth. Enzym.* **65,** 499–560 (1980).
39. Breathnach, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **75,** 4853–4857 (1978).
40. Catterall, J. F. *et al. Nature* **275,** 510–513 (1978).
41. Breathnach, R. & Chambon, P. *A. Rev. Biochem.* **50,** 349–383 (1981).
42. Liu, W.-K. *et al. J. biol. Chem.* **247,** 4365–4381 (1972).
43. Sairam, M. R., Samy, T. S. A., Papkoff, H. & Li, C. H. *Archs Biochem. Biophys.* **153,** 572–586 (1972).
44. Maghuin-Rogister, G. & Hennen, G. *Eur. J. Biochem.* **39,** 235–253 (1973).
45. Richards, R. I. *et al. Nucleic Acids Res.* **7,** 1134–1146 (1979).

Recurrent bursts in GBS0526−66, the source of the 5 March 1979 γ-ray burst

S. V. Golenetskii, V. N. Ilyinskii & E. P. Mazets

A. F. Ioffe Physical-Technical Institute, 194021 Leningrad, USSR

The results of the observations of GBS0526−66 during 1979[1–3] have been reviewed[4] recently primarily in the context of its possible identification with the N49 supernova remnant in the Large Magellanic Cloud (LMC). Observations in 1981 and 1982, however, reveal a still more complex pattern of behaviour and show both that the nature of this source of the 5 March 1979 event and its true position in space are far from being clear. Observations from Veneras 13 and 14 reveal that GBS0526−66 continues to generate weak recurrent γ-ray bursts. Thus the transient object which during the 5 March burst behaved for several minutes as a flaring X-ray pulsar[1] has since then been exhibiting a peculiar similarity with a hard X-ray burster.

γ-Ray burst observations from Veneras 13 and 14 started in November 1981. The Konus instrument used is a modified version of the one flown on Veneras 11 and 12 (ref. 5). The time resolution and recording time essential for the burst time profile measurements have been improved. Apart from this, Venera 13 can now measure 16 energy spectra consecutively. While Venera 14 obtains eight consecutive spectra as before, the number of the energy channels in each spectrum has been increased to 30. In both cases the accumulation time for the first two spectra is 0.5 s; the subsequent spectra are averaged over 4 s, as before.

The first two recurrent bursts originating in GBS0526−66 were recorded on 1 December 1981 and 2 January 1982 [6]. The time profiles and energy spectra of these events are shown in Fig. 1, the profiles being drawn with a 1/32 s time resolution. They reveal short rise and decay times. From the available recordings with a 1/256 s resolution one may conclude that the rise times here are $\tau_r < 30$ ms.

No spectral variability has been found within experimental accuracy. Therefore for GB811201 we present a net spectrum summed over three consecutive measurements 0.5, 0.5 and 4 s long, while for the shorter burst GB820102a the sum of the first two spectra accumulated in 0.5 s each is shown. The incident photon spectra were obtained by the usual procedure of unfolding the experimental energy loss spectra using the sensitivity matrix of our detectors. Figure 1 shows the spectra in energy units. The spectra are seen to approximate the relationship $dI(E)/dE \propto \exp(-E/kT)$ with the temperature kT, accordingly, 31 ± 2 and 26 ± 3 keV.

Ten more weak recurrent bursts from the same source have subsequently been recorded on the two spacecraft. The time profiles of some of these bursts are shown in Fig. 2. Because the spectra of these weak events were measured with a poor statistical accuracy, we do not present here the corresponding graphs. However, the data obtained clearly indicate that these spectra resemble one another and correspond to an emission temperature ≈35 keV.

Localization of these bursts was carried out by the usual procedure in the Konus experiment when recording an event at the two spacecraft[5]. The difference in the arrival times yielded a triangulation ring, the section containing the source being determined by analysing the anisotropic angular sensitivity of the detectors. Three events were detected only on Venera 13. The weak burst GB820921 did not trigger the recording on Venera 14. During the two other events, GB830321 and GB830405, the γ-ray burst detector on Venera 14 was switched off. These weak events were localized by the angular anisotropy technique with a low accuracy of ~15°. The crude localization data, the shape of the time profiles and of the spectra suggest that these three events similarly belong to a general sequence of recurrent bursts.

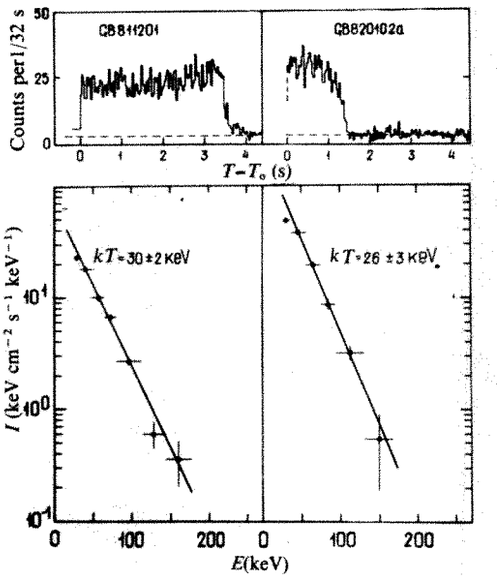


Fig. 1 Time profiles (top) and energy spectra (below) of the γ-ray bursts on 1 December 1981 and 2 January 1982. The horizontal dashed line in the time profiles shows the background level.

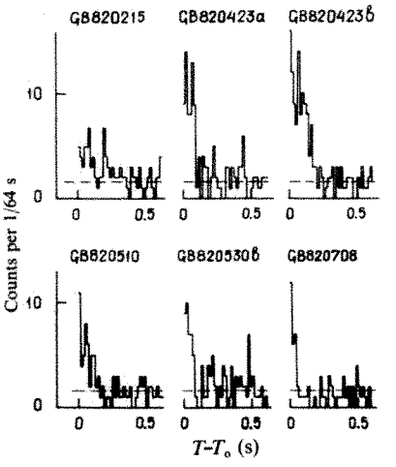


Fig. 2 Time profiles of the weak recurrent bursts from the GBS0526−66, February to July 1982.

The localization regions of 13 recurrent bursts from the GBS0526−66 observed in the Konus experiment on Veneras 11 and 12 in 1979 [7,8] and Veneras 13 and 14 in 1981–83 are presented in Fig. 3. All the localization regions are seen to intersect within a very small area of the sky which contains the most precise position for the 5 March 1979 event[9] denoted with a cross.

The sequence of bursts of 1979 followed the powerful event of 5 March. Between our observations on Venera 11 and 12 and on Venera 13 and 14 there was a break from February 1980 to November 1981. The question may arise of whether the recurrent burst sequence of 1981–83 was preceded by as powerful an event. Apparently this was not the case. The 5

March event was detected on at least nine spacecraft[4]. An analogue of such an event could hardly have escaped observation. More probably, during the break in our observations several weak recurrent bursts occurred. However, a possible loss of a series of recurrent bursts cannot substantially alter the observed pattern of behaviour of the GBS0526−66. We see that the transient object which manifested itself in the 5 March event as a flaring X-ray pulsar reveals afterwards a certain similarity to a hard X-ray burster.

The major characteristics of the recurrent bursts are listed in Table 1 which contains the date and detection time T_0 of an event on the two spacecraft, burst duration T_b, emission temperature kT, peak count rate N_{max} and total intensity S. Also given are the coordinates α, δ of the triangulation ring centre, that is the direction of the line connecting the two spacecraft, the radius of the ring, its width $\theta \pm \Delta\theta$ and the minimum distance Δ to which the ring approaches the most precise position for the 5 March event.

These data reveal two remarkable features. First, the energy spectra of all the recurrent bursts have practically the same shape and are characterized by very close values of the temperature kT. Furthermore, they are very close to the spectrum of the soft component in the two-component spectrum of the narrow initial pulse in the 5 March 1979 burst and to that of the 8-s period pulsating radiation in its late stage[8].

Second, the maximum count rate N_{max} in the recurrent bursts and, hence, the maximum luminosity of the source L_{max} also varies only slightly from event to event. Indeed, N_{max} does not differ within the error limits from the mean over all recurrent bursts which is 13 photons in 1/64 s. This count rate corresponds to the flux $I(>30\ \text{keV}) = 10^{-6}\ \text{erg cm}^{-2}\ \text{s}^{-1}$. Only in the first recurrent burst GB790306 does the maximum flux exceed this value by a factor of two. At the same time the scatter in the values of the total intensity S is much larger, namely by about a factor of 40. These differences are determined essentially only by the different duration of the bursts T_b.

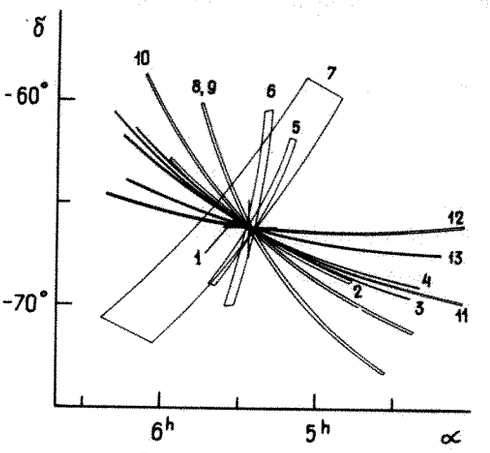


Fig. 3 Localization error boxes for the following events: 1, 5 March; 2, 6 March; 3, 4 April; 4, 24 April 1979; 5, 1 December 1981; 6, 2 January; 7, 15 February; 8 and 9, 23 April a and b; 10, 10 May; 11, 30 May; 12, 8 July; 13, 31 December 1982. The cross shows the most precise localization of the GBS0526−66 (ref. 9).

The 5 March 1979 event and views on its nature have been widely discussed (see review in ref. 4). New observations of the activity of this source show that the burst recurrence represents one of the most essential characteristics of its behaviour. Therefore these new data should prove to be important in the search for an explanation of the nature of the source. In particular, they provide a new argument against its identification with the N49 supernova remnant in the LMC at a distance $d = 55$ kpc.

Table 1 Recurrent bursts from the 5 March 1979 event source

Event	Space-craft	t_0 (UT) (h min s)	T_b (s)	kT (keV)	N_{max} $(1/64s)^{-1}$	$S\times10^{-6}$ (erg cm^{-2})	Triangulation ring (deg) α / δ	θ	Δ
GB790305*	V-11	15 51 39.145	0.25	35±5;	6×10^3	450	44.428	68.522±0.030	0.026±0.030
	V-12	15 51 44.352		520±100			−2.747		
GB790306	V-11	6 17 25.201	1.5	31±5	25	6.5	44.341	68.679±0.085	0.009±0.085
	V-12	6 17 30.457					−2.591		
GB790404	V-11	0 43 31.317	0.2	30±10	15	0.7	43.708	77.142±0.020	−0.006±0.020
	V-12	0 43 37.938					6.015		
GB790424	V-11	18 27 18.241	0.2	30±15	12	0.6	48.038	81.917±0.012	0.013±0.012
	V-12	18 27 24.730					11.971		
GB811201	V-13	9 18 02.424	3.5	30±2	12	6.6	133.165	51.82±0.14	0.08±0.14
	V-14	9 17 59.132					−25.430		
GB820102a	V-13	17 11 11.109	1.5	26±3	15	3.6	153.420	67.21±0.17	0.04±0.17
	V-14	17 11 09.707					−17.049		
GB820215	V-13	10 36 31.071	0.1	40±15	7	0.15	163.819	130.6±1.0	−0.7±1.0
	V-14	10 36 33.279					49.482		
GB820423a	V-13	3 43 16.907	0.1	36±10	13	0.2	205.788	122.489±0.068	0.033±0.068
	V-14	3 43 24.946					20.694		
GB820423b	V-13	16 29 21.315	0.15	45±15	15	0.3	206.113	122.181±0.068	−0.010±0.068
	V-14	16 29 29.350					20.275		
GB820510	V-13	13 27 55.921	0.1		10	0.15	215.867	113.998±0.052	0.042±0.052
	V-14	13 28 03.263					7.886		
GB820530b	V-13	12 46 13.968	0.1	35±15	10	0.15	226.788	104.895±0.043	0.017±0.043
	V-14	12 46 19.305					−4.709		
GB820708	V-13	10 18 18.979	0.1	30±15	12	0.15	251.669	87.438±0.036	0.013±0.036
	V-14	10 18 17.921					−26.155		
GB820921	V-13	18 37 54.138	0.1	27±15	10	0.15			
GB821231	V-13	4 17 21.363	0.18	27±10	14	0.3	61.798	85.235±0.042	0.008±0.042
	V-14	4 17 19.620					17.817		
GB830321	V-13	22 32 11.400	0.15	24±10	12	0.2			
GB830405	V-13	7 58 07.667	1.1	31±6	15	1.6			

* The data presented are for the initial burst pulse, whose spectrum consist of two (one hard and one soft) components. For the pulsating phase $S = 10^{-3}$ erg cm^{-2} (ref. 7), $kT = 35.5\pm1.0$ keV (main pulse sequence) and 31.5 ± 1.0 keV (interpulses)[8].

If one compares the limitation on the maximum source luminosity observed in all the recurrent bursts with the Eddington luminosity limit for a neutron star, $I_{Edd} \sim 10^{38}$ erg s^{-1}, then from the measured maximum fluence in the recurrent bursts, $I = L_{Edd}/4\pi d^2 \sim 10^{-6}$ erg cm^{-2} s^{-1}, one can derive an estimate for the distance to the source, $d \sim 1$ kpc. Moreover, it is only natural to assume that the recurrent bursts, just as the 5 March event, are generated in the vicinity of the neutron star's magnetic poles. In this case the emitting region will constitute only a small fraction of the total surface of the neutron star, $f \sim 10^{-2}$–10^{-3}, the total source luminosity being determined by the quantity $L_{max} = fL_{Edd} \sim 10^{35}$–$10^{36}$ erg s^{-1}. Accordingly, the estimate of the distance to the source will become reduced down to $d \sim$ 30–100 pc.

We conclude that the continuing activity of the GBS0526−66 opens up the possibility of looking for recurrent bursts in other, primarily the soft X-ray and visible spectral regions. If these observations are successful, they may be invaluable for helping to solve one of the most intriguing problems in γ-ray astronomy.

Received 19 April; accepted 4 October 1983.

1. Mazets, E. P. *et al. Nature* **282,** 587–589 (1979).
2. Barat, C. *et al. Astr. Astrophys.* **79,** L24–L26 (1979).
3. Cline, T. L. *et al. Astrophys. J. Lett.* **237,** L1–L5 (1980).
4. Cline, T. L. in *γ-Ray Transients and Related Astrophysical Phenomena, AIP Conf. Proc.* No 77, 17–33 (1982).
5. Mazets, E. P. & Golenetskii, S. V. *Astrophys. Space Sci.* **75,** 47–81 (1981).
6. Golenetskii, S. V. *et al. Pisma Astr. Zh.* **8,** 657–662 (1982).
7. Golenetskii, S. V. *et al. Pisma Astr. Zh.* **5,** 636–640 (1979).
8. Mazets, E. P. *et al. Astrophys. Space Sci.* **84,** 173–189 (1982).
9. Cline, T. L. *et al. Astrophys. J. Lett.* **255,** L45–L48 (1982).

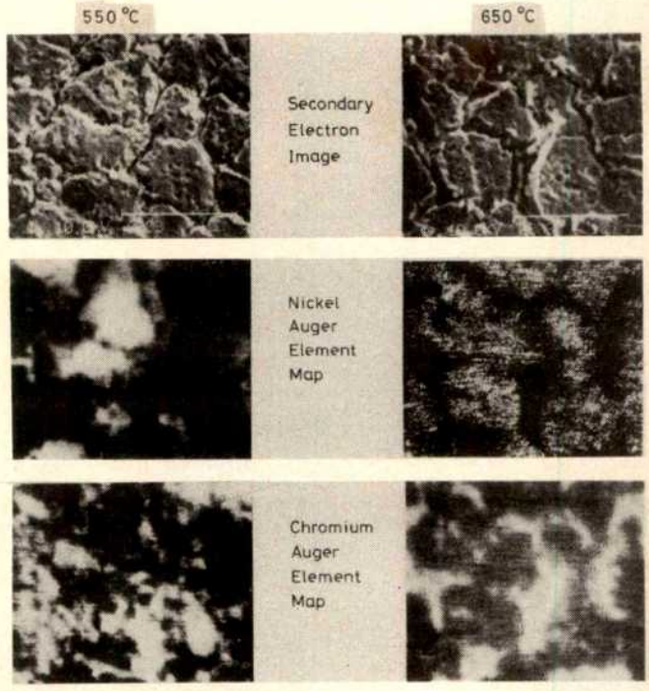


Fig. 1 Scanning electron micrograph and Auger element maps from the surface of a 20% Cr, 25% Ni, Nb stabilized austenitic stainless steel after removal of the oxide layer by sputter ion plating.

The study of metal–oxide interfaces using sputter ion plating

G. C. Allen & R. K. Wild

Central Electricity Generating Board, Berkeley Nuclear Laboratories, Berkeley, Gloucestershire GL13 9PB, UK

The interface between solids has always been difficult to characterize. It is possible to use analytical techniques to probe through an outer surface but matrix effects within the penetrated solid often modify the results to the extent that the interface becomes difficult to detect. The progressive analysis and removal of layers of material from an outer surface until the interface is reached may prove useful but during the removal of thick outer layers the interface becomes indeterminate. A third approach is to dissolve one solid leaving the other intact, but again limitations are encountered. For example, only one side of the interface can be studied and the real boundary may be perturbed or damaged by the dissolving medium. Here we describe a new approach which may be used to study both the metal and oxide side of metal–oxide interfaces. Although this may at first appear to be a rather specialized application, the principles involved may be readily transferred to a number of other systems.

Metal–oxide interfaces may be studied in several ways. For interfaces formed between a relatively thin layer and a bulk material, layers of the thinner entity can be removed by ion sputter-etching the surface. If analysis of the freshly exposed surface is performed simultaneously using Auger electron spectroscopy[1], the surface composition as a function of depth into the specimen may be obtained. This 'depth profiling' capability permits the analysis of a material in three dimensions. When a particular sub-surface feature, such as an interface, is of interest, etching can be stopped when this is reached and a more detailed analysis performed.

A novel technique has been developed by Coad, Tappin and Rivière[2] for stripping oxide layers from metals to expose the interface on both the oxide and metal side. Previously one of the few reliable methods for the removal of an oxide from ferrous materials involved the dissolution of the metal by iodine–methanol solutions. Originally developed by Vernon, Wormwell and Nurse[3], this method has been extended more recently to iron–silicon alloys by Moseley, Tappin and Rivière[4]. Although a profile into the oxide could be obtained, the results suffered from the limitation that contamination of the oxide occurred during the process of dissolution. Coad's method, known as sputter ion plating is not subject to this disadvantage because the oxide is simply pulled away from the metal matrix.

The technique involves the deposition of a layer of nickel, or other suitable metal, onto the oxide surface at the elevated temperature of 300 °C. During the deposition process the oxide surface is subjected to bombardment with 1 kV argon ions at a pressure of 30 torr to encourage a strong nickel–oxide bond and the temperature regulated to prevent modification of the oxide film composition. On cooling to room temperature, stresses are induced between the oxide and its nickel overlayer sufficient to pull the oxide away from its base when the coating is deformed. Experiments using sputter ion plating have been carried out on iron–silicon alloys[2] and in our own laboratory on 20% Cr, 25% Ni, Nb stabilized steel[5].

The current UK civil advanced gas-cooled nuclear reactor uses a thin-walled 20% Cr, 25% Ni, Nb stabilized austenitic steel cladding for the fuel pins. For heat transfer purposes the surface of the steel tubing is roughened by circumferential ribs of 0.31 mm^2 section with a pitch of approximately 2.5 mm. Here we report the examination of two samples of this austenitic alloy (total composition 20% Cr, 25% Ni, 0.6% Nb, 0.6% Si, 0.05% C, balance Fe) which had previously been exposed to a CO_2, 1% CO gas mixture at 40 atm for 4,000 h at 550 °C and 650 °C respectively. An oxide layer of thickness between 0.5 and 2.0 μm was formed at the surface. To study the mode of formation and integrity of the oxide, each sample was coated with a 30-μm layer of nickel metal and oxide–metal partition was induced by bending the oxidized cladding alloy parallel to a surface rib. Since separation of the oxide and metal occurred in air, the metal side was contaminated with an air-formed oxide film. However, sputtering experiments using a beam of argon ions of energy 4 keV showed this film to be no more than a few

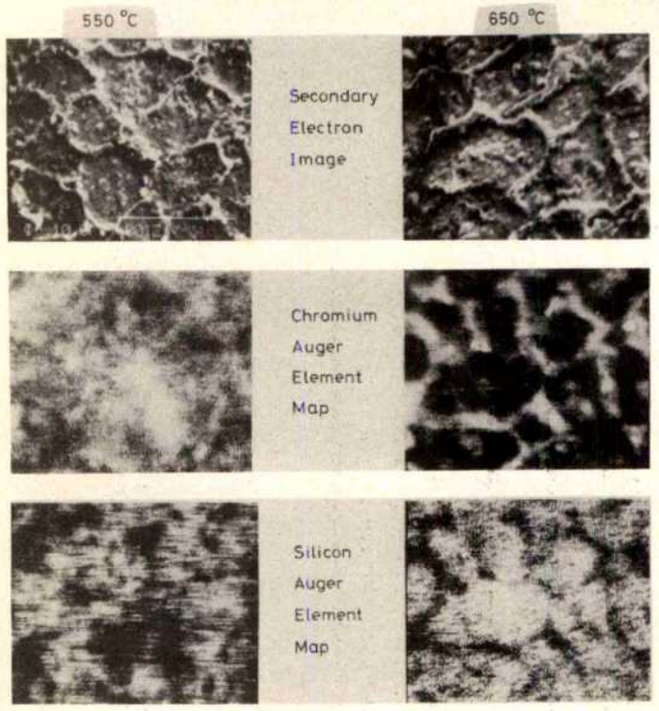


Fig. 2 Scanning electron micrograph and Auger element maps from the oxide side of the metal–oxide interface exposed by sputter ion plating a 20% Cr, 25% Ni, Nb stabilized austenitic steel.

atom layers in thickness and supported the view that separation had occurred at the metal–oxide interface rather than within the oxide.

The alloy side of the oxide–metal interface is illustrated in Fig. 1. It is this surface which cannot be examined readily by other methods. The secondary electron images from the 550 °C and 650 °C samples are very similar. Both show grains of ~20 μm diameter within the polycrystalline alloy which are clearly delineated by grooves at the boundary positions. The grooves corresponded to ridges on the oxide side of the interface which were pulled out from the grain boundaries during the process of separation.

Auger electrons emitted by a given element were measured over the area examined in the secondary electron micrograph to provide a visual display of the lateral distribution of the element in question. Maps of this type obtained using the technique of scanning Auger microscopy are illustrated for the elements nickel and chromium beneath the corresponding secondary electron image. Although not illustrated here, results of this kind were also recorded for silicon and iron. As Fig. 1 indicates, striking differences in the distribution of elements were observed from the two samples. At 550 °C an enrichment of nickel at some grain surfaces was noted, together with rather low concentrations at others but, following exposure at 650 °C, a consistently uniform concentration of this element occurred at grain centres with a corresponding depletion at the boundaries of the grains. Similarly, chromium appeared to be randomly distributed across the surface of the metal oxidized at 550 °C but at the higher temperature a significant enrichment was observed in the grain boundary regions. Maps for silicon and iron identified a similar pattern of behaviour, the higher temperature sample revealing the enrichment of silicon at grain boundaries and iron at grain centres.

Scanning electron micrographs of the oxide side of the interface are shown in Fig. 2, together with the Auger element maps from chromium and silicon. Iron and nickel were rarely detected on the oxide surface, an indication that the deposited nickel layer had not penetrated the oxide to the interfacial region. Detailed studies identified a marked difference in the composition of grain surface and grain boundary regions on this side of the oxide–metal interface. In contrast to the metal, the freshly exposed oxide counterpart of the 650 °C sample showed high levels of silicon at the grain centre and high chromium levels at the grain boundary positions but similar behaviour was not evident in the sample oxidized at 550 °C. Complementary information of this kind can be used to determine the mode of metal–oxide fracture. Results from the experiments at 650 °C, taken together with those from experiments at 850 °C (ref. 6) and argon ion etching, indicated that above the grains at the surface of the metallic structure, separation occurred between the metal crystallites and a very thin layer, ~70 Å across, of silicon-rich oxide. At the crystallite boundary regions within the surface of the polycrystalline metal on the other hand, oxide was formed as a wedge down the grain boundary and into the metal so that fracture occurred across the surface and through the bulk of the keyed oxide. Hence the Auger map from the surface, exposed when the oxide was torn away, revealed the presence of chromium, a major constituent of the bulk oxide, in the grain boundary regions.

At 550 °C simple metal–oxide separation no longer occurred. The intervening silicon-rich oxide was incompletely developed across the interfacial region and the metal and oxide were more strongly bound. In fact, the oxide film was so adherent that in certain areas of the surface, separation was only achieved by removing a portion of the metal substrate with the oxide layer.

Providing separation can be achieved, the sputter ion plating technique thus permits the examination of both the metal and oxide sides of a metal-oxide interface by scanning Auger electron spectroscopy. The data so obtained may be used to determine both the mode of separation and the strength of the metal–oxide interaction.

This letter is published by permission of the CEGB.

Received 13 June; accepted 13 October 1983.

1. Allen, G. C. & Wild, R. K. *CEGB Res.* **11**, 12–30 (1981).
2. Coad, J. P., Tappin, G. & Rivière, J. C. *AERE Rep.* R10285 (1981).
3. Vernon, W. H. F., Wormwell, F. & Nurse, T. F. *J. Iron Steel Inst.* **150**, 81–92 (1944).
4. Moseley, P. T., Tappin, G. & Rivière, J. C. *AERE Rep.* R10042 (1981).
5. Coad, J. P. & Wild, R. K. *Appl. Surf. Sci.* **14**, 321–329 (1983).
6. Allen, G. C. & Wild, R. K. *Phil. Mag.* **A48**, 373–386 (1983).

Last interglacial reef growth beneath modern reefs in the southern Great Barrier Reef

John F. Marshall & Peter J. Davies

Bureau of Mineral Resources, Geology and Geophysics, Canberra City, ACT 2601, Australia

Studies of modern coral reefs have shown that Holocene reef growth is relatively thin, and that in many instances it has developed on an older limestone surface[1–4]. This surface is commonly associated with leaching and pedogenic processes, indicative of subaerial exposure, and has been termed the solution unconformity[5]. Investigations into reef growth history have interpreted the pre-Holocene substrate as an older reef surface[2–4,6–8] that may or may not have undergone karst erosion during periods of lower sea level[6,9]. Uranium-series dating of corals directly below the uppermost solution unconformity at Eniwetok[10] and Mururoa[11] has shown that previous reef growth, before the Holocene, occurred ~120 kyr ago. We present here $^{230}Th/^{234}U$ dates that confirm a last interglacial age for reefal limestones directly beneath the uppermost solution unconformity in the southern Great Barrier Reef.

Previous drilling on the Great Barrier Reef has encountered the topmost solution unconformity at depths ranging from 4 to 22 m below sea level[2–4]. Radiocarbon dating of material below the unconformity has produced ages that are beyond the detection limit of this method, and there has been insufficient suitable

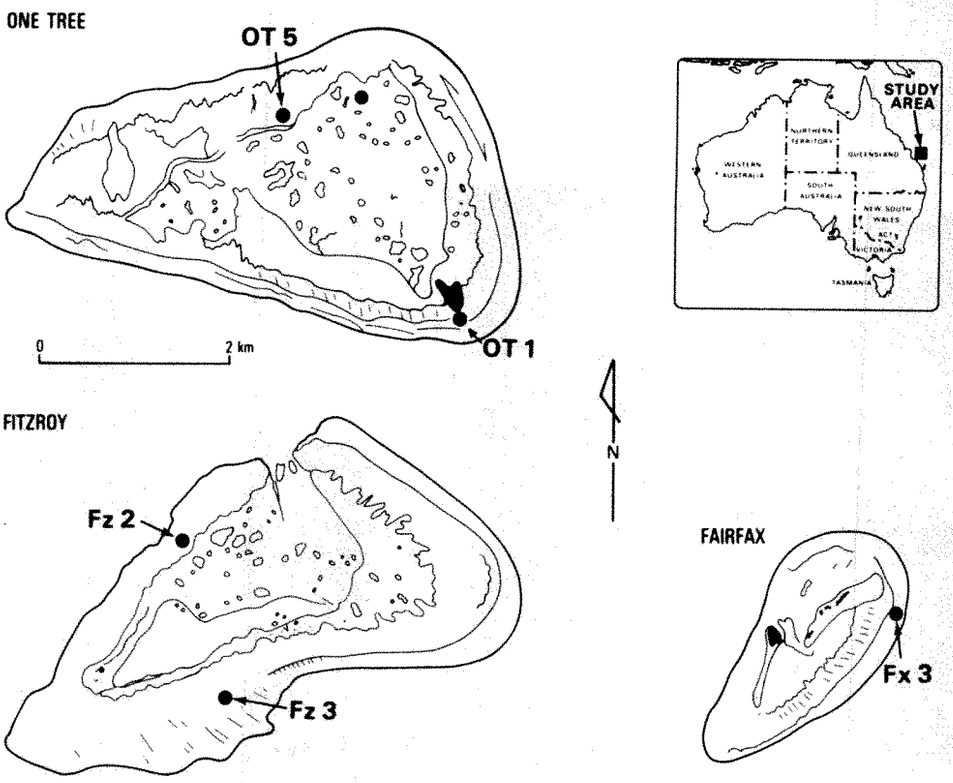


Fig. 1 Locality diagram and physiographic outline of the reefs showing the position of drill holes which penetrated below the solution unconformity.

material to enable absolute age determinations using the uranium-series technique[3]. Since then, shallow drilling operations in the southern Great Barrier Reef have penetrated the solution unconformity at depths ranging from 7.4 to 14.3 m on three reefs (Figs 1 and 2). Previously, seismic refraction profiling on these reefs indicated the solution unconformity at depths ranging from 8 to 23 m (ref. 12). Radiocarbon dating of corals above the unconformity have confirmed that it is Holocene[13–15], whereas below it, unrecrystallized corals produced radiocarbon ages in excess of 30,000 yr BP (ref. 14). Core material below the unconformity is commonly leached and cemented by low-Mg calcite, while in some cores the limestone is impregnated with soil humates or contains cross-cutting stringers of brown calcrete containing numerous rhizocretions. However, while the material below the unconformity shows the effects of subaerial vadose diagenesis, aragonite corals suitable for dating were found in five of the drill holes (Fig. 2).

The results of $^{230}Th/^{234}U$ dating of these corals are shown in Table 1. All the corals obey the closed system criteria, and their uranium contents and $^{234}U/^{238}U$ ratios fall within the expected ranges.

Two anomalous ages of 107 kyr from Fairfax Reef (hole 3) and 172 kyr from Fitzroy Reef (hole 2) are considered to have resulted from these corals not being in their original growth position. The 107-kyr coral from Fairfax Reef occurs directly on top of a pronounced soil horizon that represents the surface of the solution unconformity. Previously, this coral had produced a minimum ^{14}C age of 30,000 yr BP. Its presence above the unconformity suggests that it is not *in situ*. The 172-kyr age from Fitzroy Reef (hole 2) is more difficult to explain. In the absence of age determinations both above and below it, no reliance is placed on its accuracy.

Most corals occur in the age range of 122–145 kyr. This spread in the ages is consistent with results from previously dated last interglacial terraces[16–19] and the submerged foundations of atolls[10,11]. The two ages from One Tree Reef are older than those from Fairfax and Fitzroy Reef (hole 3). This could reflect variations in the timing of reef growth and their respective growth rates, such as seen in the modern reef. However, variations of this type in the Holocene reefs are only of the order of 2,000–4,000 yr, whereas the difference betweeen One Tree and the other reefs is >10,000 yr. Alternatively, it could reflect differing rates of subaerial erosion of the last interglacial reefs during the period of low sea level before the last transgression—a period of some 100,000 yr. The relatively greater depths to the solution unconformity in the One Tree Reef drillholes (Fig. 2) would seem to bear out this latter alternative.

On the assumption that sea level during the last interglacial was ~5 m higher than present, and that the reefs were capable of attaining this high sea level elevation, the present position of

Table 1 Radiochemistry and ages of coral samples beneath the solution unconformity in the southern Great Barrier Reef

Drill hole	Sample no.	Depth (m)	Mineralogy (% aragonite)	U (p.p.m.)	$^{234}U/^{238}U$	$^{230}Th/^{234}U$	Age (kyr)
One Tree Reef							
1	78632219	13.4–13.7	100	3.82	1.15±0.01	0.75±0.02	145±8
5	79632597	15.0–16.7	95	3.38	1.10±0.01	0.73±0.03	138±11
Fairfax Reef							
3	79632955	8.5–8.8	100	2.78	1.14±0.03	0.64±0.02	107±8
3	79632967	11.1–11.2	100	4.03	1.08±0.01	0.70±0.02	127±8
Fitzroy Reef							
2	79632734	8.7–8.9	98	3.67	1.11±0.01	0.81±0.02	172±12
3	79632838	10.6–11.0	100	2.57	1.10±0.01	0.69±0.02	125±6
3	79632856	13.0–13.2	100	3.51	1.10±0.01	0.68±0.02	122±6

$^{232}Th/^{230}Th < 0.05$ for all samples. Errors shown are based on counting statistics $\pm 1\sigma$.

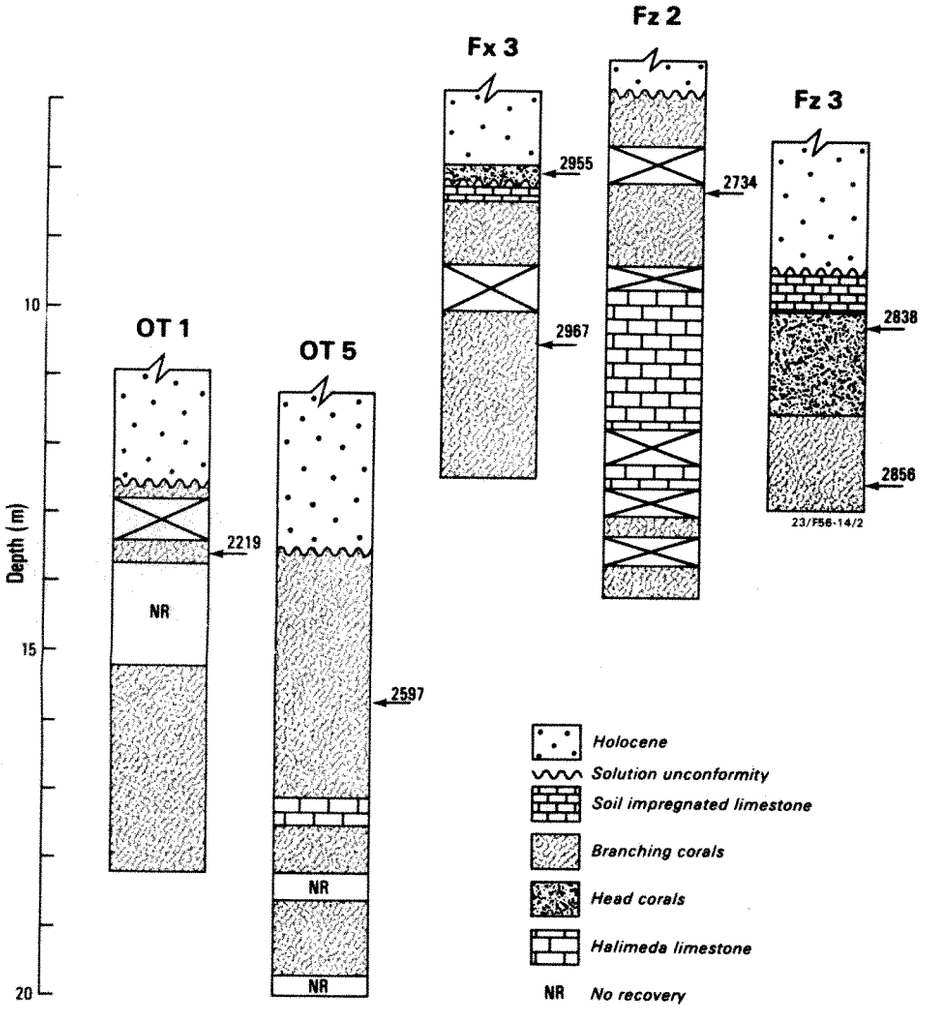


Fig. 2 Lithological logs of the pre-Holocene sections of the drill holes showing the position of the dated coral samples.

the solution unconformity in the drillholes suggests a lowering of the reef's surface of the order of 12–19 m during the intervening period between the last interglacial and the Holocene transgression. The position of last interglacial reefs and other strandline features removed from plate boundaries have verified that the sea level was higher than present by ~4–6 m (refs 19, 20). On the basis of present-day and Holocene growth rates it would have been possible for all the reefs dated here to have reached this high sea level position within the period of the last interglacial. For example, the 127-kyr date at ~11 m in hole 3 at Fairfax Reef (Table 1) indicates that for a uniform vertical accretion rate of 4 m kyr^{-1}, this section of the reef would have reached the position of the last interglacial high sea level by 123 kyr ago. Providing that the subsidence rate of the continental margin (0.05 m kyr^{-1}) has remained uniform[21], the present position of the unconformity would require a rate of erosion of the reefs' surface of the order of 0.07–0.14 mm yr^{-1}, a rate that is not unrealistic for subaerial weathering of reefal limestones[22]. Differential erosion between the perimeters of the reefs and their lagoons, possibly as a result of submarine lithification within the former, would accentuate the original relief in such a manner as to lend credence to the antecedent karst hypothesis[6].

The determination of the exact age and depth of the modern reef substrate has long been considered as crucial to understanding the development of coral reefs. The arguments regarding the age and nature of the substrate go back as far as Darwin's[23] and Daly's[24] theories on reef development, and continue to the present day. Before our results there were only two reefs (Eniwetok and Mururoa) where it had been shown that the foundations of extant Holocene reefs were last interglacial in age. Because these are both atolls, it is encouraging to find a similar age in a different tectonic setting, such as the shelf reefs of the Great Barrier Reef.

We thank G. O'Brien for the age determinations of the two corals from One Tree Reef and T. Torgersen for providing laboratory and counting facilities. H. H. Veeh kindly commented on the manuscript. Published with the permission of the director, Bureau of Mineral Resources.

Received 29 March; accepted 19 October 1983.

1. Adey, W. H. *Atoll Res. Bull.* **187,** 1–67 (1975).
2. Thom, B. G., Orme, S. R. & Polach, H. A. *Phil. Trans. R. Soc.* A**291,** 37–54 (1978).
3. Hopley, D., McLean, R. F., Marshall, J. & Smith, A. S. *Search* **9,** 323–325 (1978).
4. Davies, P. J. & Marshall, J. F. *Search* **10,** 276–279 (1979).
5. Schlanger, S. O. *U.S. Geol. Surv. Prof. Pap.* **160-BB,** 991–1066 (1963).
6. Purdy, E. G. in *Reefs in Time and Space* (ed. Laporte, L. F.) 9–76 (Sepm Spec. Publ. 18, 1974).
7. Halley, R. B., Shinn, E. A., Hudson, J. F. & Lidz, B. *Proc. 3rd int. Coral Reef Symp.* **2,** 29–35 (1977).
8. Emery, K. O., Tracey, J. I. & Ladd, H. A. *U.S. Geol. Surv. Prof. Pap.* **260**-A, 1–265 (1954).
9. Adey, W. H. *Science* **202,** 831–837 (1978).
10. Thurber, D. I. Broecker, W. S., Blanchard, R. L. & Potratz, H. A. *Science* **149,** 55–58 (1965).
11. Lalou, C., Labeyrie, J. & Delibrias, G. *C.r. hebd Séanc. Acad. Sci., Paris* D**263,** 1946–1949 (1966).
12. Harvey, N., Davies, P. J. & Marshall, J. F. *BMR J. Aust. Geol. Geophys.* **4,** 141–147 (1979).
13. Davies, P. J. & Marshall, J. F. *Nature* **287,** 37–38 (1980).
14. Marshall, J. F. & Davies, P. J. *Coral Reefs* **1,** 21–28 (1982).
15. Davies, P. J. & Marshall, J. F. *Coral Reefs* (in the press).
16. Broecker, W. S. *et al. Science* **159,** 297–300 (1968).
17. Bloom, A. L., Broecker, W. S., Chappell, J. M. A., Matthews, R. K. & Mesolella, K. J. *Quat. Res.* **4,** 185–205 (1974).
18. Chappell, J. & Veeh, H. H. *Bull. geol. Soc. Am.* **89,** 356–368 (1978).
19. Ku, T. L., Kimmel, M. A., Eastorn, W. H. & O'Neil, T. J. *Science* **185,** 959–962 (1974).
20. Marshall, J. F. & Thom, B. G. *Nature* **263,** 120–121 (1976).
21. Davies, P. J. in *Perspectives on Coral Reefs* (ed. Barnes, D. J.) 69–106 (Brian Clouston, Canberra, 1983).
22. Trudgill, S. T. *Z. Geomorphol.* **26,** 201–210 (1976).
23. Darwin, C. R. *The Structure and Distribution of Coral Reefs* (Smith Elder, London, 1842).
24. Daly, R. A. *Proc. Am. Acad. Arts Sci.* **51,** 155–251 (1915).

Late Quaternary pollen records from Easter Island

J. R. Flenley & Sarah M. King

Department of Geography, The University, Hull HU6 7RX, UK

Easter Island is the most isolated piece of inhabited land in the world. It exhibited an unique megalithic culture[1–3], involving the sculpting of giant statues (moai) especially between AD ~1400 and ~1680[4] when, for unknown reasons, the culture suddenly collapsed. The island is also of interest in relation to Pleistocene climatic change as CLIMAP[5] predicted no reduction of sea-surface temperatures for this part of the Pacific at 18,000 yr BP. We have obtained fossil pollen records covering the past 37,000 yr from three craters on the island. They suggest that the late Pleistocene climate was cooler and/or drier than the present one. They also suggest the former existence of forest on the island, and its decline in the last millennium. This decline was probably due to deforestation by man and could have caused the cultural collapse.

The island (lat. 27°9′ S; long. 109°26′ W) appears to have been substantially without woody vegetation since its discovery in 1722[6–8]. The present vegetation is mostly grassland. The known native flora consists of 46 vascular species[9] of which only one, the endemic *Sophora toromiro* (Phil.) Skottsb., is a tree. This species is now extinct on the island, but survives in botanical gardens[10]. The only native shrubs are *Lycium carolinianum* Walt. and *Caesalpinia bonduc* (L.) Roxb. The chief herbaceous families represented are the Gramineae and Cyperaceae.

Initial work on the Quaternary palynology of Easter Island, without dating, was carried out by Selling[4] who showed that a woody vegetation had formerly occurred. He found pollen of a palm, which he believed might be *Pritchardia* sp., now extinct on the island.

In the present study, the deposits of three volcanic craters were cored, and single complete cores returned to the laboratory. All three craters hold freshwater, and the deposits are highly organic. The stratigraphy has been described elsewhere[11], and appears to be relatively simple. ^{14}C and ^{210}Pb dating were carried out on samples from the pollen cores. Figures 1–3 outline pollen diagrams; full results will be published later.

Oceanic islands are well known as recipients of pollen from distant sources[12]. In the present work some sparsely represented taxa, such as *Ephedra* and *Casuarina*, are thought to have arrived in this way, but the major pollen contributors, discussed here, are presumed to have grown on the island.

The dating results require comment. The Rano Raraku ^{14}C dates (Fig. 1) are augmented by assay for ^{210}Pb of 33 samples from the top 70 cm. The results suggest a mean accumulation

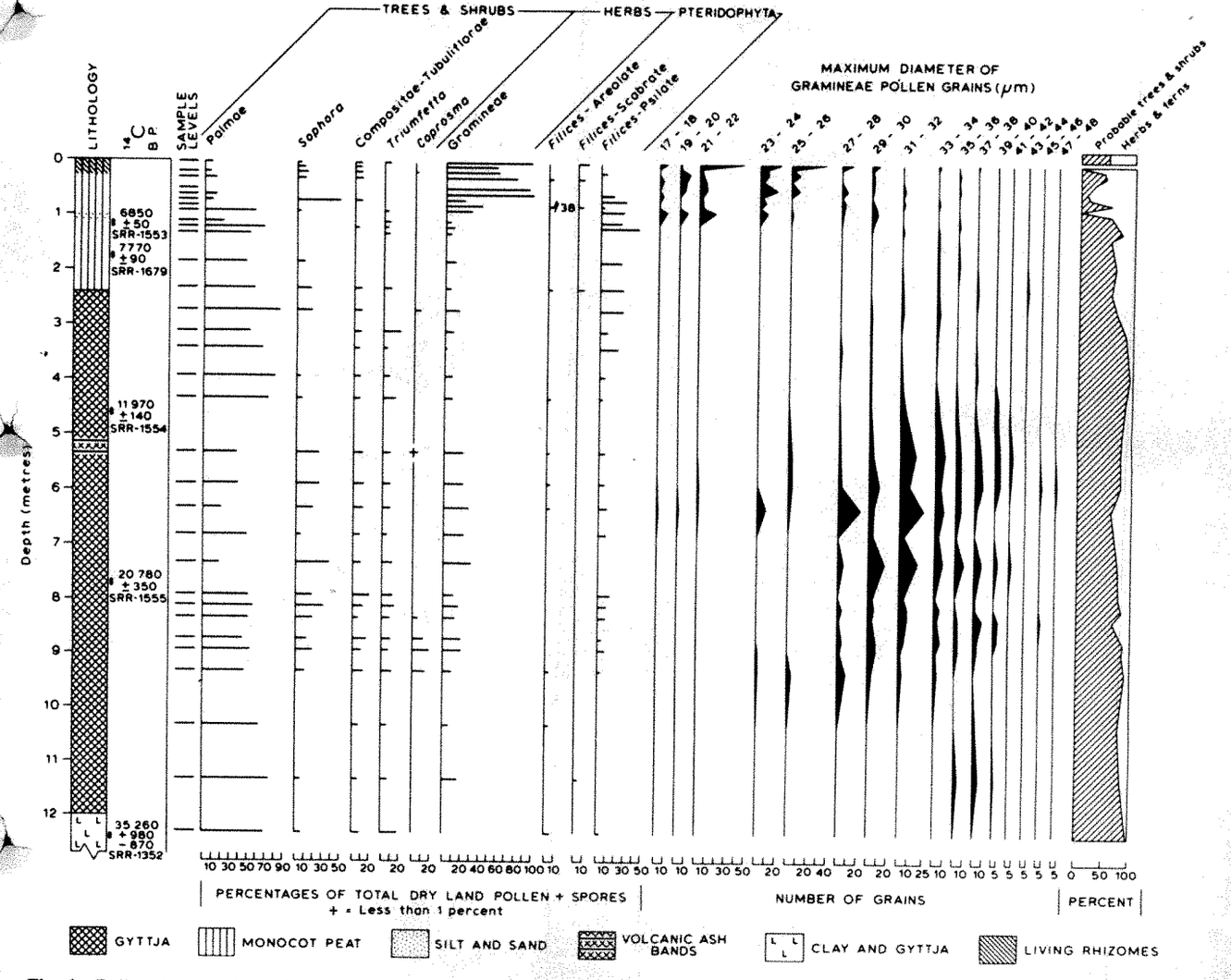


Fig. 1 Pollen diagram from Rano Raraku, alt. ~75 m. Only selected taxa are shown. Unidentifiable and unidentified grains only once represented more than 5% of the total. The summary diagram at right shows percentages of total dry land pollen and spores, not just of the taxa illustrated.

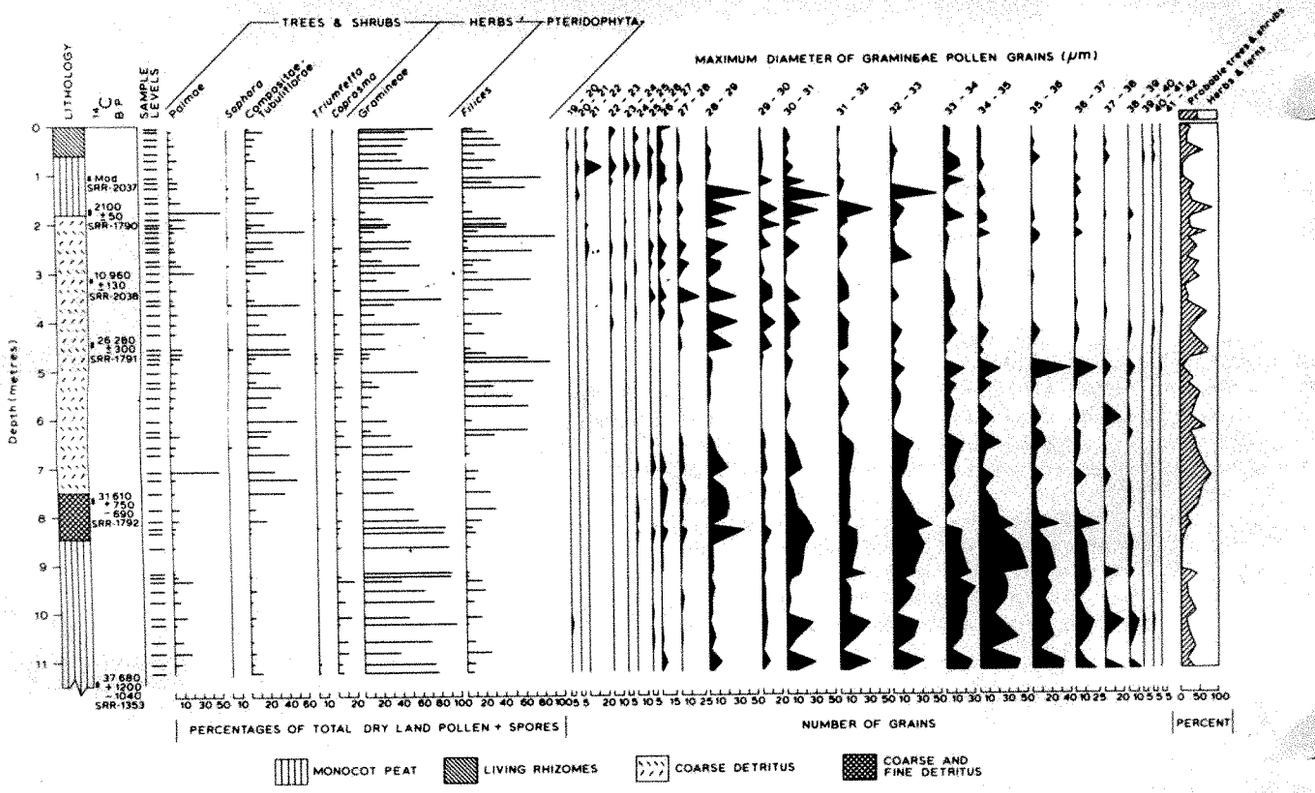


Fig. 2 Pollen diagram from Rano Aroi, alt. ~425 m. Only selected taxa are shown. Unidentifiable and unidentified grains never represented more than 2% of the total. The summary diagram at right shows percentages of total dry land pollen and spores, not just of the taxa illustrated.

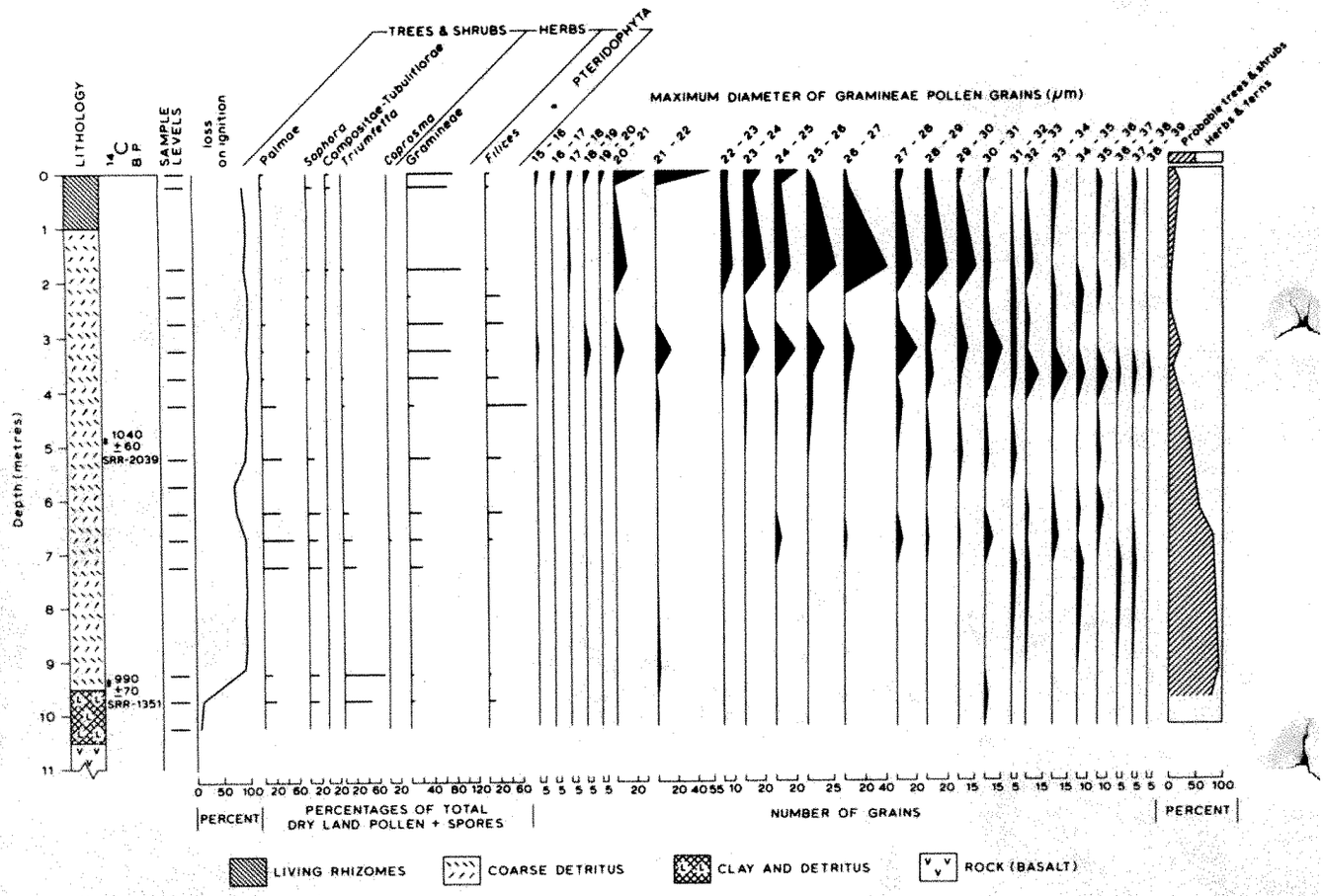


Fig. 3 Pollen diagram from Rano Kao, alt. ~110 m. Only selected taxa are shown. Note the changes of scale. Unidentifiable and unidentified grains were never more than 5% of the total. The summary diagram at right shows percentages of total dry land pollen and spores, not just of the taxa illustrated.

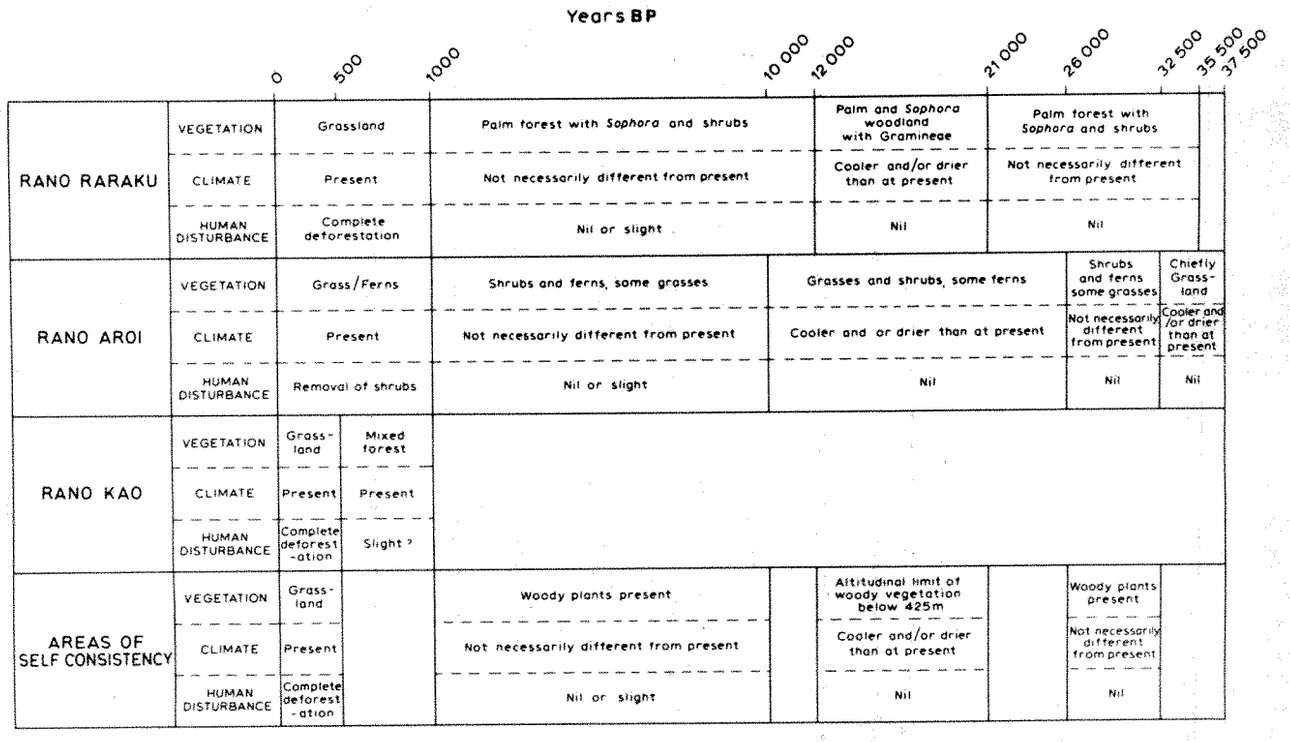


Fig. 4 Tentative conclusions as to former vegetation, climate and human disturbance on Easter Island. The time scale is given as a square root transformation.

rate of ~1 cm yr^{-1} for this part of the deposit. A date of 70 BP for 70-cm depth accords with the lower three dates in the core if a steady rate of accumulation is assumed below 70 cm, but this suggests that SRR-1553 and SRR-1679 are too old. This is feasible because they are close to the layer of inwashed silt and sand at 1.10 m. This probably indicates the inwash of soil carbon, a well known cause of contamination in radiocarbon dating[13]. The linear accumulation rate is therefore preferred for dating purposes. At Rano Kao (Fig. 3), similar contamination is suggested by the drop in loss-on-ignition values at ~6-m depth, and SRR-2039 is consequently rejected. At Rano Aroi (Fig. 2) there seem to have been changes in the rate of deposition. SRR-1790 is stratigraphically close to inwashed material seen in another borehole, and it could therefore be too old.

Figures 1–3 show evidence of the occurrence on the island of taxa which are now extinct there. The palm pollen found by Selling is abundant at all three sites. *Pritchardia*, which occurs on many Polynesian islands[14], has very similar pollen[15], but other possibilities cannot be excluded on pollen morphological grounds. This pollen taxon has been included in 'probable trees and shrubs'. There is also abundant pollen of Compositae, subfamily Tubuliflorae. Pollen morphology places this taxon in the tribe Heliantheae[16]. Plant geographical considerations make the most likely taxa *Chrysogonum* and *Campylotheca*[14–17], both of which comprise trees or shrubs. This pollen taxon has therefore also been included in 'probable trees and shrubs'.

The pollen records do not include all the taxa believed to be native today. Note that the two shrubs, *C. bonduc* and *L. carolinianum* are absent, but as they are both entomophilous, their absence is not particularly surprising. A total of 40 pollen taxa were recorded.

It is theoretically possible that some of the changes seen in the pollen record might be related to changes of facies in the stratigraphy. Thus at Rano Aroi the abundance of Tubuliflorae coincides with the occurrence of coarse detritus. The deposit contains no macroscopic remains of Tubuliflorae, however, which might have been expected if Tubuliflorae had been growing on the swamp. At Rano Raraku the rise of Palmae coincides with the occurrence of two 1-cm thick bands of volcanic ash at ~5-m depth, but it seems unlikely that so minor a volcanic event could have caused a vegetational change lasting several thousand years. The effect of such facies changes is therefore discounted.

For conciseness the vegetational and climatic interpretations will be considered simultaneously. To do this it is necessary to consider now the climatic significance of the major pollen taxa. *Sophora* grew on the island until recently, when it was probably destroyed by felling and grazing, and it may be taken as suggesting a climate similar to the present one. Palmae and Tubuliflorae were also present until recently (according to the pollen record), but Palmae were more abundant at Rano Raraku (~75-m altitude) and Tubuliflorae at Rano Aroi (~425-m altitude). This suggests that Palmae are indicative of warmer temperatures and Tubuliflorae of cooler temperatures, within the range found on the island today.

The Gramineae pollen of the record falls into two main categories. Grains below ~27 μm maximum diameter dominate the recent part of the record and are believed to be chiefly those of the grasses, both introduced and native, which now dominate most parts of the island. Grains above this size occur chiefly in the earlier part of the record and are thought to represent other native grass species which were more frequent before disturbance. They are particularly abundant in the Rano Aroi record and are probably therefore to be associated with the lower temperatures within the range found on the island.

It is certainly possible that both Gramineae and Palmae would have been favoured by drier conditions on the island in the past, as judged by the known ecological preferences of these families, but we have as yet no information on this point in relation to Easter Island taxa.

Bearing all these considerations in mind and interpolating where necessary between dates, it is possible to draw the tentative conclusions as to former vegetation, climate and human disturbance shown in Fig. 4. These will have to be revised as further dates and pollen and chemical analyses become available. Already, however, there emerges a fairly coherent picture. The chief conclusions are: (1) the island was probably formerly forested; (2) deforestation occurred, probably over a

considerable time span, but since 990 ± 70 yr BP at Rano Kao; (3) the climate was cooler and/or drier than at present during the period ~21,000–12,000 yr BP.

The chief interest arising from conclusion (3) is the general correlation in time and direction with late Pleistocene climatic changes elsewhere in both hemispheres, and the possible contrast with CLIMAP conclusions[5]. It is hoped that further work will clarify and quantify the climatic shift involved. Conclusions (1) and (2) are of archaeological interest, and do not conflict with the hypothesis[18] that the decline of the megalithic culture was associated with total deforestation. The latter could have been effective through shortage of food resulting from non-availability of fertile forest soils. Alternatively, or additionally, large timbers may have been essential for the moving of the moai[18,19] and deforestation could, therefore, have made it pointless to continue to carve them.

We thank Dr D. D. Harkness of the Scottish Universities Research and Reactor Centre for the ^{14}C assays, Mr J. D. Eakins of the Atomic Energy Research Establishment, Harwell, for the ^{210}Pb assays, Mrs M. Martin for the pollen preparations, and Mr K. Scurr for drawing of the figures. This work was financed by NERC research grant GR3/3316.

Received 11 July; accepted 12 October 1983.

1. Routledge, K. *The Mystery of Easter Island: The Story of an Expedition* 165–199 (Sifton, Praed, London, 1919).
2. Metraux, A. *Easter Island: A Stone-Age Civilization of the Pacific* pp. 149–170 (Andre Deutsch, London, 1957).
3. Metraux, A. *Bernice P. Bishop Mus. Bull.* No. 160 (1971).
4. Heyerdahl, T. & Ferdon, E. N. Jr (eds) *Reports of the Norwegian Archaeological Expedition to Easter Island and the East Pacific* Vol. 1 (Forum, Stockholm, 1961).
5. CLIMAP, *Science* **191,** 1131–1137 (1976).
6. Gonzalez, D. F. *The Voyage of Captain Don Felipe Gonzalez in the Ship of the Line San Lorenzo, with the Frigate Santa Rosalia in Company, to Easter Island in 1770–01* (Hakluyt Society, Cambridge, 1908).
7. Cook, J. *A Voyage towards the South Pole and Round the World, 1772–75,* 1 (Strahan & Cadell, London, 1777).
8. La Perouse, J. F. de G. *Voyage de La Perouse Autour du Monde,* Paris, 1797 (English translation) (Johnson, London, 1798).
9. Skottsberg, C. *The Natural History of Juan Fernandez and Easter Island* Vol. 1, 193–438 (Almqvist & Wiksells, Uppsala, 1956).
10. Alden, B. *Nouveau Regard sur L'ile de Paques,* 119–122 (Moana, Corbeil, 1982).
11. Flenley, J. R. *Asian Perspect.* **22,** 33–40 (1979).
12. Hafsten, U. *Arbok Univ. Bergen, Mat-Naturv.* Ser. 20, 1–48 (1960).
13. Pennington, W., Cambray, R. S., Eakins, J. D. & Harkness, D. D. *Freshwater. Biol.* **6,** 317–331 (1976).
14. Balgooy, M. M. J. van *Plant Geography of the Pacific, Blumea Suppl.* **6,** 1–222 (1971).
15. Selling, O. H. *Bernice P. Bishop Mus. Spec. Publ.* 37–39, Honolulu (1946–48).
16. Salgado-Labouriau, M. L. *Grana* **21,** 97–102 (1982).
17. Brown, F. B. H. *Bernice P. Bishop Mus. Bull.* No. 130 (1935).
18. Mulloy, W. *J. Archaeol. phys. Anthrop. Oceania* **5,** 1–23 (1970).
19. Heyerdahl, T. *Aku-Aku* (Allen and Unwin, London, 1958).

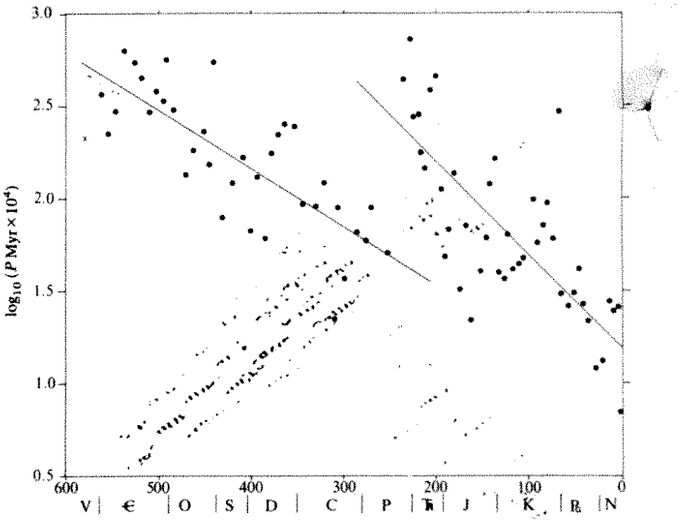


Fig. 1 Probabilities of extinction per Myr (P_{Myr}) through geological time, on a semilogarithmic scale. The abscissa is scaled in Myr BC and by standard symbols for geological periods (Table 1). The plotted regressions exclude the Vendian, because the datum plotted (×) includes soft-bodied forms, and the Maestrichtian, because of its uniquely divergent position. For the left regression, through the Leonardian, $y = -0.00313x + 0.904$, $r = 0.775$, $s_{yx} = 0.235$. For the right regression, from the Guadalupian, $y = -0.00502x + 1.191$, $r = 0.800$, $s_{yx} = 0.275$. (If the Maestrichtian is included, $y = -0.00478x + 1.240$, $r = 0.749$, $s_{yx} = 0.308$.)

A resetting of Phanerozoic community evolution

Leigh M. Van Valen

Biology Department (Whitman), University of Chicago, 915 East 57th Street, Chicago, Illinois 60637, USA

The early part of an adaptive radiation is thought[1] to show more taxonomic turnover than later stages. I show here that the effect on probability of extinction is one of exponential decrease, and that in the case of marine families this regular decline was interrupted by the great Permian extinction. A new exponential decrease followed, at a somewhat faster rate. The Cretaceous–Palaeogene extinction is the only other one detectably different from the scatter, and it had no apparent effect on extinction in the Cenozoic. These regularities support an interactive view of large-scale community evolution but do not uniquely determine the nature of the interaction.

Sepkoski[2] has critically compiled the known geological ranges, by stage whenever possible, of all marine families of invertebrates and vertebrates. I used his summary for the families which are readily preservable in the fossil record.

My analysis is based on probability of extinction[3] rather than absolute numbers of extinctions, the more common procedure. These approaches give different results when the total numbers of taxa differ among the set of taxa in a comparison. An epistemic (or a subjective or propensity) interpretation of probability gives a meaning to the probabiiity of a single event like an extinction, unlike the standard frequentist interpretation of probability. Probability of extinction is appropriate both for single taxa and for communities or broader groups; the same number of extinctions has a smaller expected effect when more taxa are present, just as for the same number of deaths in differently sized populations of individuals.

Probability of extinction decreased rather regularly throughout the Palaeozoic (Fig. 1). However, the late Permian extinction was somewhat larger than even those of the Cambrian, and even in the early and middle Triassic the probability was still about that for the Ordovician. Thus a new decrease was initiated, which has continued to the present. All but two of the post-Palaeozoic probabilities lie above an extension of the Palaeozoic regression. The scatter is homogeneous above and below the regressions and between the two regressions, and normally distributed about the regressions, but the later regression is significantly ($P < 0.02$, $P < 0.05$ if Maestrichtian included) steeper than the earlier one.

Other than the late Permian extinction with respect to the Palaeozoic regression, only the Maestrichtian (late Cretaceous) extinction is more than two standard deviations above the appropriate regression. This does not mean that all other extinctions are causally homogeneous, but any heterogeneity cannot be detected from this sort of analysis (*contra* Raup and Sepkoski[4]). It is therefore not feasible to estimate frequencies of collisions with large meteoroids, as has been done[12].

Some aspects are better seen in Fig. 2. The high Cambrian probabilities were preceded by a rise, ostensibly from no extinction at all in the Vendian. (The probability including soft-bodied forms is artificially high because such forms are almost never found in the early Cambrian; its true value could even be zero also.) A clustering of high probabilities occurs in the early Cambrian, late Devonian, late Permian, late Triassic and late

Table 1 Probabilities of extinction (ext.), by stage or the equivalent, of normally fossilizable marine families

Stage	*P* (ext. per stage)	*P* (ext. per Myr)	Stage	*P* (ext. per stage)	*p* (ext. per Myr)
Middle and upper Vendian (V)			Triassic (Tr)		
Norm. preserved	0	0	Induan	0.0831	0.0277
All	0.591	0.0215	Olenekian	0.0977	0.0279
Cambrian (€)			Anisian	0.0628	0.0179
Early Tommotian	0.218	0.0363	Ladinian	0.0802	0.0146
Late Tommotian	0.157	0.0224	Carnian	0.1472	0.0391
Atdanian	0.265	0.0294	Norian	0.2058	0.0457
Botomian	0.813	0.0625	Rhaetian	0.0781	0.0112
Lenian	0.373	0.0533	Jurassic (J)		
Early middle Cambrian	0.317	0.0453	Hettangian	0.0148	0.0049
Late middle Cambrian	0.326	0.0296	Sinemurian	0.0407	0.0068
Early late Cambrian	0.230	0.0383	Pliensbachian	0.0677	0.0135
Middle late Cambrian	0.167	0.0334	Toarcian	0.0227	0.0032
Late late Cambrian	0.224	0.0560	Bajocian	0.0418	0.0070
Ordovician (O)			Bathonian	0.0205	0.0022
Tremadocian	0.331	0.0301	Callovian	0.0278	0.0040
Arenigian	0.160	0.0133	Oxfordian	0.0363	0.0061
Llanvirnian	0.183	0.0183	Kimmeridgian	0.0356	0.0119
Llandeilan	0.116	0.0232	Tithonian	0.0814	0.0163
Caradocian	0.137	0.0152	Cretaceous (K)		
Ashgillian	0.267	0.0534	Berriasian	0.0159	0.0040
Silurian (S)			Valanginian	0.0183	0.0037
Llandoverian	0.0945	0.0079	Hauterivian	0.0326	0.0065
Wenlockian	0.1315	0.0120	Barremian	0.0249	0.0042
Ludlovian	0.1632	0.0163	Aptian	0.0315	0.0045
Devonian (D)			Albian	0.0384	0.0048
Gedinnian	0.0602	0.0067	Cenomanian	0.0792	0.0099
Siegenian	0.0654	0.0131	Turonian	0.0346	0.0058
Emsian	0.0548	0.0061	Coniacian	0.0289	0.0092
Eifelian	0.1204	0.0172	Santonian	0.0377	0.0094
Givetian	0.1534	0.0219	Campanian	0.0491	0.0061
Frasnian	0.2015	0.0252	Maestrichtian	0.1492	0.0298
Famennian	0.2427	0.0243	Palaeogene (Pg)		
Carboniferous (C)			Danian	0.0154	0.0031
Tournaisian	0.1157	0.0093	Middle and late Palaeocene	0.0172	0.0026
Viséan	0.1111	0.0089	Early Eocene	0.0140	0.0031
Namurian	0.0981	0.0123	Middle Eocene	0.0205	0.0041
Bashkirian	0.0224	0.0022	Late Eocene	0.0192	0.0027
Moscovian	0.0539	0.0037	Early and middle Oligocene	0.0109	0.0022
Stephanian	0.0816	0.0065	Late Oligocene	0.0094	0.0012
Permian (P)			Neogene (N)		
Asselian	0.0383	0.0059	Early Miocene	0.0118	0.0013
Sakmarian	0.0616	0.0088	Middle Miocene	0.0128	0.0028
Leonardian	0.1320	0.0050	Late Miocene	0.0140	0.0025
Guadalupian	0.4423	0.0442	Pliocene	0.0082	0.0026
Dzhulfian	0.3606	0.0721	Pleistocene	0.0013	0.0007

Each probability was estimated as the number of extinctions (last appearances) in the stage, divided by the average number of taxa at risk. The latter was estimated as the number of taxa at the beginning of the interval, minus half the extinctions, plus half the originations (first appearances) in the interval. This assumes that extinctions and originations are evenly distributed in the interval, which is not always true and can be easily corrected for when so desired, but even when the assumption is untrue the method provides an average value for the whole stage. For the middle and late Vendian this procedure is inappropriate because of the rapidly increasing diversity with few extinctions likely within the period; I estimated the taxa at risk as the number of extinctions plus half the number of survivors and used half the duration of the period as the time at risk. Probability per stage is appropriate if extinctions are concentrated at the end of stages. Questionable records were ignored, and the data include vertebrates as well as invertebrates. Data with accuracy less a stage were divided equally among stages, a procedure which undoubtedly makes at least the Cretaceous–Palaeogene extinction seem somewhat less extreme than it really was. Standard errors of the probabilities cannot be determined but are certainly less than the standard deviations about the two regressions in Fig. 1. Pseudoextinctions are negligible; in fact, I can think of no real case of pseudoextinction in the fossil record at the family level, which would require the last surviving species of an earlier family to give rise to the next family.

Jurassic, with some suggestions of rising probabilities before most of these local maxima.

The 'Great Dying' at the end of the Cretaceous would be unusually low for the Cambrian, and the late Devonian extinction (including more than just the Frasnian, *contra* the usual view) would be ordinary for the Ordovician. Cambrian (biomere) extinctions as well as that of the Cretaceous were mostly concentrated at the ends of stages, so probabilities per stage rather than per time are a better measure (Table 1). Apart from the Maestrichtian, the three largest deviations from the regressions are below rather than above. If these and similar deviations are real rather than artefacts of inaccuracy of the data (including stage lengths), they deserve study for their own sake. What causes intervals of low extinction? Such an interval, like one of stasis, is a real datum.

Darwin[5] and many others have proposed that organisms increase their absolute fitnesses, the average increasing through geological time. However, this does not appear to explain the pattern of Fig. 1 because there are two declines in probability of extinction, not just one. The Permian extinction reset the clock of community evolution, and it resumed a little faster than before. Similarly, control by the physical environment does not explain the bipartite distribution.

A decrease in probability of extinction can also be explained by an increase in the ratio of positive to negative interactions among taxa. Most plausibly this would involve a decreasing overlap of the adaptive zones[6] of taxa through time, a pattern which has independent supporting evidence[1]. The steeper post-Palaeozoic slope supports this hypothesis because many families did survive into the Triassic and presumably retained the broader aspects of Palaeozoic community structure. Readjustment should then be more rapid than from the initial establishment of animal communities in the Vendian and Cambrian.

The constancy of overall community fitness which is central to the Red Queen hypothesis[3,7] predicts an approximate constancy in evolutionary rate[3,8]. That is seen here at the community level, because the ratio of probabilities of extinction among successive equal intervals fluctuates about a constant value. This

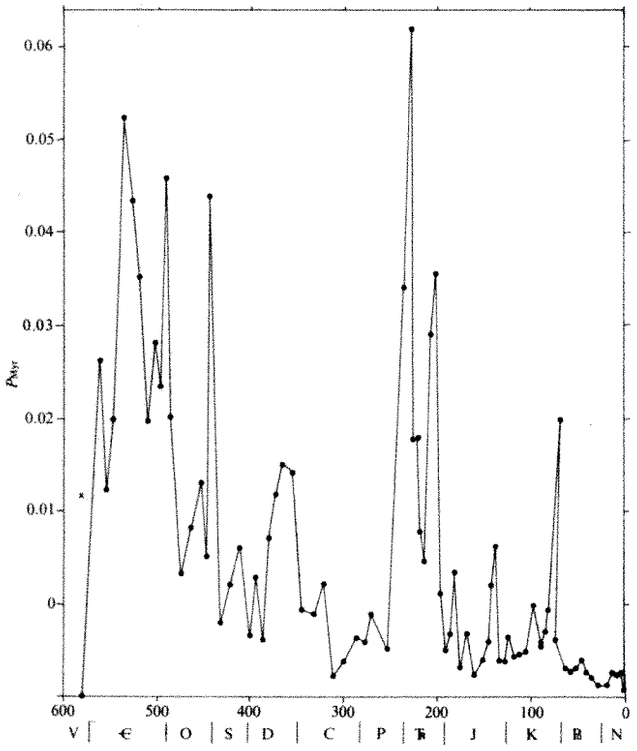


Fig. 2 Probabilities of extinction per Myr (P_{Myr}), as in Fig. 1, on an arithmetic scale. × is an artificially high value including soft-bodied forms. Abbreviations as in Table 1.

value is determined by the slopes of the regressions in Fig. 1; as it would differ numerically for intervals of different lengths, the value of an average ratio itself has little interest. However, in this way the exponential nature of the decline in probability can be explained in a natural way.

As a subsidiary point, all three radiations (angiosperms[9], placental mammals[10] and marine animals) for which data are available show unusually low probabilities of extinction, ostensibly zero in each case at the resolution possible, at the very beginning of the major radiation. Both predation[10] and competition were probably unusually low in each of these cases, although density-dependent regulation of population (or metapopulation) density more or less requires that some competition occur among taxa expanding into new adaptive zones[11]. Thus, initially it is perhaps easier to avoid competition by evolving a new adaptation than it is to stand and fight, evolving adaptations for competition. This should be testable experimentally for suitable organisms in a suitably complex environment.

A cladistic classification would preclude any analysis like that of the present paper.

I thank J. J. Sepkoski Jr for the summary of his data and for comments; A. Hoffman, J. A. Kitchell, and J. W. Valentine for comments; and V. C. Maiorana for help.

Received 11 July; accepted 6 October 1983.

1. Simpson, G. G. *The Major Features of Evolution* (Columbia University Press, 1953).
2. Sepkoski, J. J. Jr *Milwaukee Publ. Mus., Contr. Biol. Geol.* **51**, 1–125 (1982).
3. Van Valen, L. M. *Evol. Theor.* **1**, 1–30 (1973).
4. Raup, D. M. & Sepkoski, J. J. Jr *Science* **215**, 1501–1503 (1982).
5. Darwin, C. *On the Origin of Species* (Murray, London, 1859).
6. Van Valen, L. M. *Evolution* **25**, 420–428 (1971).
7. Van Valen, L. M. *Evol. Theor.* **4**, 289–300 (1980).
8. Van Valen, L. M. *J. molec. Evol.* **3**, 89–101 (1974).
9. Doyle, J. A. in *Patterns of Evolution* (ed. Hallam, A.) 501–546 (Elsevier, Amsterdam, 1977).
10. Van Valen, L. M. *Evol. Theor.* **4**, 45–80 (1978).
11. Van Valen, L. M. in *Conceptual Issues in Ecology* (ed. Saarinen, E.) 323–343 (Reidel, Dordrecht, 1982).
12. Alvarez, L. W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 627–642 (1983).

Air pollution increases *Aphis fabae* pest potential

G. P. Dohmen, S. McNeill & J. N. B. Bell

Department of Pure and Applied Biology, Imperial College, Silwood Park, Ascot, Berkshire SL5 7PY, UK

Many observations have been made of increased population densities of herbivorous insects in areas subject to air pollution[1–3]. However, in the absence of experimental investigations, the significance of most of these observations is not clear and any underlying causal mechanisms remain obscure. The only published studies designed to investigate experimentally the impact of pollutants on plant–herbivore systems involve fumigations with either hydrogen fluoride (HF)[4] or sulphur dioxide (SO_2)[5,6] of *Phaseolus vulgaris* and *Glycine max* plants, infested with the Mexican bean beetle, *Epilachna varivestis*: in general the results showed that HF reduced beetle performance whereas SO_2 stimulated it, but the relative importance of direct and indirect effects of the pollutants was not elucidated. We show here stimulatory effects of SO_2 and NO_2 and ambient London air on the growth of the black bean aphid, *Aphis fabae*, and demonstrate that these have been mediated entirely via the host plant.

Annual surveys of *A. fabae* abundance in south-east England have shown consistently higher levels of infestation on field beans, *Vicia faba*, in an area downwind of London and in the proximity of heavy industry, than predicted on the basis of the number of eggs recorded on the winter hosts (M. J. Way, personal communication). We hypothesized that this might be caused by air pollutants drifting into the area and established an experimental programme to investigate the phenomenon.

Fumigation experiments were conducted in a pair of 0.7 m^3 Perspex chambers, situated inside a heated glasshouse. The chambers were ventilated with four changes per minute of charcoal-filtered air, and SO_2 or NO_2 was added from a cylinder to give a controlled constant level of pollutant in one chamber. *A. fabae* was reared in culture in a constant temperature room and alatae were removed to clip cages attached to *V. faba* leaves. Two-day-old offspring were removed from the cages for use in each experiment. Subsequent performance of the nymphs was assessed by calculating either final fresh weight or mean relative growth rate (MRGR) on the basis of increases in fresh weight. For each pollutant, two types of experiment were carried out, designed to separate direct and indirect effects on the aphids, respectively. Effects mediated via the plants were investigated by fumigating three-leaf-stage seedlings with 200–500 $\mu g\ m^{-3}$ of SO_2 (mean 400 $\mu g\ m^{-3}$) or 400 $\mu g\ m^{-3}$ NO_2 for 7 days; the plants were then transferred to a controlled temperature room at 20 °C and one nymph was placed on top of each and its MRGR measured over the succeeding 3 days. The possibility that the pollutants had a direct effect on the insects was examined by carrying out 400 $\mu g\ m^{-3}$ fumigations with either pollutant while the insects were feeding on artificial diet sachets. At the end of the 3-day fumigation the mean fresh weight per aphid on each sachet was measured and compared between treatment and control.

Table 1 shows MRGR of the aphids grown on post-fumigation plants. Both SO_2 and NO_2 caused significant increases in aphid performance, with MRGR being stimulated by 6.5% and 8.4%, respectively. In contrast, direct fumigation of aphids feeding on the artificial diet produced no significant differences in growth (SO_2 treatment, 126 ± 4 μg; control, 118 ± 6 μg; NO_2 treatment, 90 ± 7 μg; control, 93 ± 5 μg). The MRGR of the diet-fed aphids was not measured directly, because of the problems of disturbance inherent in making repeated measurements on the same animals. However, by taking a mean value for the initial

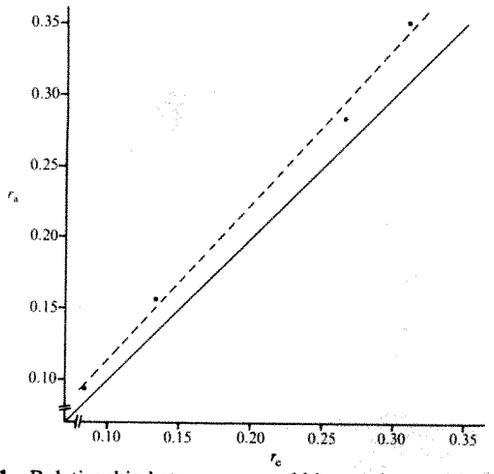


Fig. 1 Relationship between rates of biomass increase for *Aphis fabae* on *Vicia faba*, grown in either ambient London (r_a) or charcoal-filtered (r_c) air. Dashed line, $r_a = 1.086r_c + 0.006$; solid line, $r_a = r_c$.

weight of 2-day-old aphids, the MRGR of diet-fed animals was estimated and shown to be almost identical between the fumigation and control treatments, for both pollutants (Table 1). Although growth was slower overall on the artificial diet, as found by other workers[7], we consider that the difference is sufficiently small to provide a valid comparison between indirect and direct effects of the pollutants on the aphids.

The fumigations described here used SO_2 and NO_2 concentrations which are higher than the mean annual levels recorded currently in British cities. Therefore, to determine the effect of ambient urban air pollution on the performance of *A. fabae*, another pair of 0.7 m^3 chambers was set up on a roof at Imperial College in South Kensington, central London. These were ventilated with ambient air or the same air which had been passed through a charcoal filter, which removed at least 70% of SO_2 and 50% of NO_2 but no nitric oxide (NO) (S. M. Usher, personal communication). Pots containing three three-leaf-stage *V. faba* plants were infested with *A. fabae* by enclosing each pot in a gauze cage with nine adult alatae. The caged plants were then placed in the chambers, where they were grown for 9–12 days in four separate experiments between May and July 1983. The daily SO_2 concentrations measured at the nearest National Air Pollution survey site (500 m distant) ranged between 34 and 145 $\mu g\, m^{-3}$ during the experimental periods (S. T. Mossman, personal communication). No local measurements of nitrogen oxides are available, but on the basis of data from elsewhere in central London, the ratio of SO_2:NO_2 has been estimated[8] to be about 1:0.7, which would give concentrations of NO_2 at South Kensington of 20–100 $\mu g\, m^{-3}$. At the end of each experiment, the aphid offspring in each chamber were counted and weighed and the rate of biomass increase (r) was calculated ($B_t = B_0.e^{rt}$, where B_t = final biomass, B_0 = initial biomass and t = duration of the experiment in days). Different climatic conditions resulted in considerable variation in population growth between experiments, precluding direct comparisons of biomass. Figure 1 shows the relationship between the rates of biomass increase for the ambient air (r_a) and charcoal-filtered air (r_c) treatments. The regression line is significantly greater than a slope of 1 ($P < 0.01$), and there was a 12% increase in r_a over r_c throughout the series of experiments.

The results presented here provide strong evidence that two of the principal phytotoxic air pollutants occurring in the UK can enhance the performance of a serious agricultural pest. Such seemingly small increases in productivity of the insect can, because of the logarithmic nature of population growth, result in large increases in pest populations over small time periods. They will also tend to lead to more frequent escape from control by natural enemies[9]. The fumigation experiments demonstrate that these effects on aphid performance are indirect and mediated via changes in the plant. We have carried out a preliminary investigation on the nature of these changes by analysing the amino acid spectrum of *V. faba* plants subjected to the 7-day SO_2 fumigation treatments used in the present work. Consistent changes, both negative and positive, were detected for several key amino acids. It is well established that the amino acid composition of a plant has the potential to influence insect performance[10,11]. However, the significance of the observed changes for *A. fabae* growth is uncertain and will be the subject of future research.

Table 1 Mean relative growth rate over 3 days of *Aphis fabae* subjected to SO_2 or NO_2, either directly on artificial diet or indirectly via prefumigated plants

		Pollutant	Control	Significance
SO_2	Plant*	0.540	0.507	$P < 0.01$
	Artificial diet†	0.395	0.394	NS
NO_2	Plant‡	0.575	0.530	$P < 0.01$
	Artificial diet§	0.369	0.370	NS

Mean relative growth rate ($\mu g\, \mu g^{-1}\, d^{-1}$) = (ln(final weight) − ln(initial weight))/no. of days growth. NS, not significant.

* Mean of 5 experiments with 15 replicates per treatment.

† Mean of 2 experiments with 15 replicate sachets per treatment, each with a mean of 10 aphids.

‡ Mean of 4 experiments with 15 replicates per treatment.

§ One experiment with 15 replicate sachets per treatment, each with a mean of 10 aphids.

We consider that the results described here indicate a strong possibility that air pollution has a serious economic impact on crop productivity, as a result of increased pest incidence, as well as exerting the more generally recognized direct phytotoxic effects. It is necessary for such work to be extended to a much wider range of pest types and host species to assess the overall importance of air pollution/insect/crop interactions in the field.

We acknowledge the support of the Commission for the European Communities Environment Programme in providing G.P.D. with a studentship, and thank Mr I. H. M. Fosbrooke, Dr D. J. Galley and Mr M. E. Cammell for advice and the provision of technical facilities.

Received 15 August; accepted 17 October 1983.

1. Wiackowski, S. K. *Folia for. pol.* **A23,** 175–187 (1978).
2. Port, G. R. & Thompson, J. R. *J. appl. Ecol.* **17,** 649–656 (1980).
3. Villemont, C. *Envir. Pollut.* **A24,** 245–262 (1981).
4. Weinstein, L. H. *J. occup. Med.* **19,** 49–78 (1977).
5. Hughes, P. R., Potter, J. E. & Weinstein, L. H. *Envir. Ent.* **10,** 741–744 (1981).
6. Hughes, P. R., Potter, J. E. & Weinstein, L. H. *Envir. Ent.* **11,** 173–176 (1982).
7. Van Emden, H. F. *Aphid Technology* (Academic, London, 1972).
8. Lane, P. I. thesis, Univ. London (1983).
9. Lawton, J. H. & McNeill, S. in *Population Dynamics* (eds Anderson, R. M., Turner, B. D. & Taylor, L. R.) 223–224 (Blackwell, Oxford, 1979).
10. McNeill, S. & Southwood, T. R. E. in *Biochemical Aspects of Plant and Animal Coevolution* (ed. Harborne, J. B.) 77–98 (Academic, London, 1978).
11. Mattson, W. J. *A. Rev. Ecol. Syst.* **11,** 119–161 (1980).

Direct transfer of carbon between plants connected by vesicular–arbuscular mycorrhizal mycelium

R. Francis & D. J. Read

Department of Botany, University of Sheffield, Sheffield S10 2TN, UK

Mycorrhizal infection of plants in many natural vegetation systems arises when uninfected roots make contact with mycelia spreading from infected roots[1,2]. The result of the infection is that connections are present between the roots of individual plants. It has been shown by autoradiography that these interconnections provide a direct pathway for the transfer of carbon between ecto-mycorrhizal plants[2,3]. Inter-plant transfer of carbon[4] and phosphorus[5–7] has been reported in plants with vesicular–arbuscular (VA) mycorrhizas, but in the absence of

comparable evidence it remains a possibility that in these cases the pathway is indirect, uptake by the fungus of the 'receiver' plant occurring only after leakage of the nutrients from roots of the 'donor'. We now show using autoradiography that transfer of carbon between plants connected by VA mycorrhizal mycelium occurs primarily by the direct hyphal pathway. It is further shown that the magnitude of the transfer is strongly influenced by shading of 'receiver' plants indicating that movement is governed by source–sink relationships.

Seedlings of *Plantago lanceolata* were used as 'donors' both of infection and of labelled carbon. They were first grown in either the non-mycorrhizal (NM) or mycorrhizal (M) condition in trays of dune sand partially sterilized (PS) by irradiation. Two categories of M plants were produced, one by inoculation with field collected root pieces (unspecific inoculum) and the other with surface-sterilized spores of *Glomus caledonium* (specific inoculum). After 5 weeks both the M and NM donor plants were transferred to fresh PS sand in transparent plastic Petri dishes, to which also were added three aseptically grown seedlings of *Festuca ovina*. Twelve dishes of each category of donor (NM, M field inoculum, M specific inoculum) were established. Inspection of *Festuca* roots in randomly selected dishes after 6 weeks revealed infection levels of 30–50% in M non-specific and 15–20% in M specific inoculum categories. The sand surface was then coated with warm molten paraffin wax, to ensure a seal around the bases of all plants and around the dish circumference. Immediately before application of isotope to donor plants, the receiver plants in six dishes of the NM and six of the field inoculum categories were subjected to shade treatments. Darkness was obtained by loosely covering *Festuca* shoots with aluminium foil, half-shade by covering the shoots with muslin cloth, the remaining dishes being exposed to full illumination. Shoots of donor plants were then sealed in transparent plastic boxes into each of which 30 μCi of $^{14}CO_2$ was released. The plants were exposed to the isotope for 2 days after which time the shoots were excised, and the root systems in three dishes of each of the three categories of plants were prepared for autoradiography by gently removing sand under a fine jet of water. Roots, and in the case of M plants, their mycelial interconnections were then photographed through the transparent dish base before autoradiographic stripping film was applied to the roots which were exposed to the film for 2 days. The remaining M field inoculum and NM plants were harvested, ground in liquid nitrogen and digested in NCS tissue solubilizer before ^{14}C levels of their roots and shoots were determined quantitatively by liquid scintillation counting.

Roots of *Plantago* and *Festuca* can be readily distinguished on the basis of size (Figs 1*a*, 2*a*) and in the inoculated dishes a network of hyphae is seen to form frequent interspecific linkages between plants (Figs 2, 3). Autoradiographs reveal that whereas the roots of NM donor plants are strongly labelled no activity is detected in those of *Festuca* even where they are in

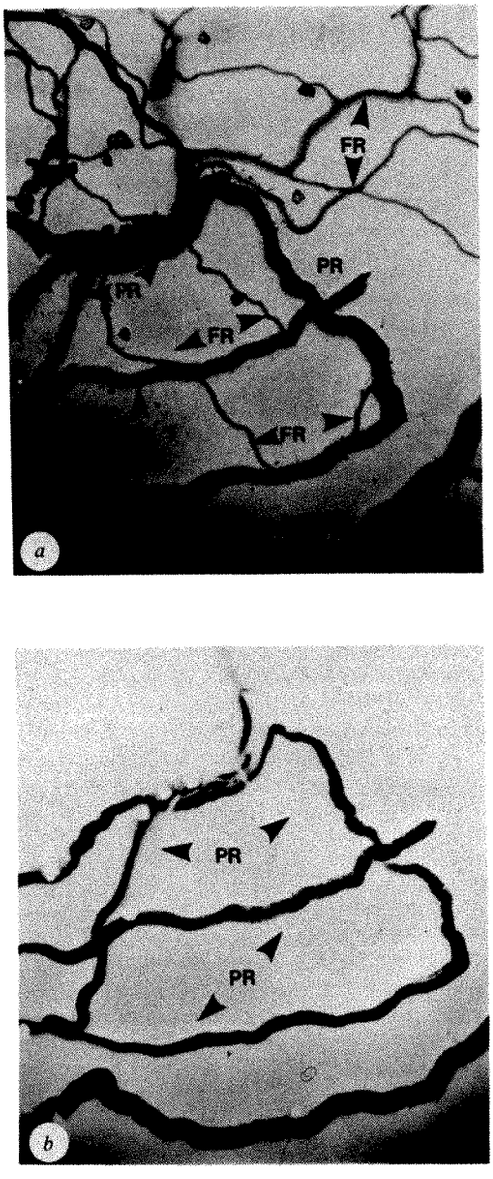


Fig. 1 *a*, Light micrograph of roots of an NM system after removal of sand showing closely intertwined roots of *Plantago* (PR) which are relatively robust, and *Festuca* (FR) (×15). *b*, Autoradiograph of same area as *a* showing accumulation of radioactivity in the donor, *Plantago* (PR), and complete absence of detectable transfer even into the most closely associated *Festuca* roots (×15).

Table 1 Radioactivity in shoots and roots of receiver plants (*Festuca ovina*) under three light regimes

Treatment	Receiver category: *Festuca* mycorrhizal			*Festuca* non-mycorrhizal		
	Activity in root (d.p.m. per mg dry wt)	Activity in shoot (d.p.m. per mg dry wt)	Activity in whole receiver plant as % of that in donor	Activity in root (d.p.m. per mg dry wt)	Activity in shoots (d.p.m. per mg dry wt)	Activity in whole receiver plant as % of that in donor
Full light	8,908* ± 3,530	363†‡ ± 242	0.015	656‡ ± 403	147‡ ± 194	0.0019
Half Light	18,072* ± 7,647	479†‡ ± 75	0.048	264‡ ± 365	229‡ ± 372	0.0010
Dark	57,218* ± 12,372	51‡ ± 38	0.112	171‡ ± 100	ND†	0.0005

Counts are expressed as d.p.m. mg dry weight (with 95% confidence limits) and as percentage of the total activity in the donor plants at harvest ($n = 6$). ND, no counts above background detected.

* Significant difference between this and all other values of activity at both treatment and category levels at $P < 0.05$.

† Significant difference between this and other value so marked at $P < 0.01$.

‡ Value not significantly different from other treatments in the same category.

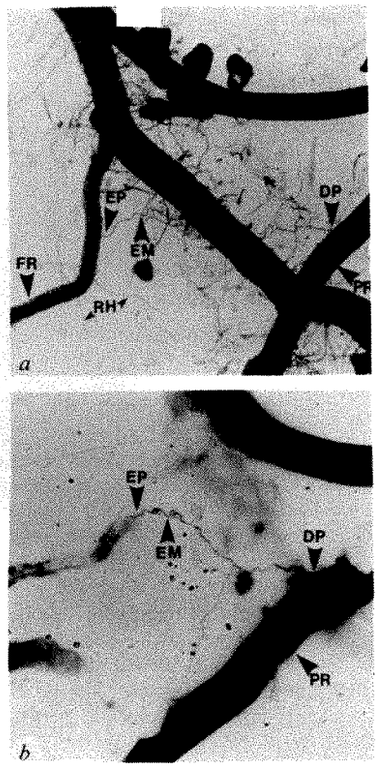


Fig. 2 *a*, Light micrograph of roots in a specific inoculum system showing *Plantago* 'donor' root (PR), *Festuca* 'receiver' root (FR) with many root hairs (RH), and interconnecting endophyte mycelium (EM) of *Glomus caledonium* (×40). *b*, Autoradiograph of same area as *a* showing heavy labelling of donor root (PR) and of endophyte mycelium (EM). Mycelial linkage can be seen from departure point (DP) on donor root to entry point (EP) on receiver root. Radioactivity in receiver roots is restricted to area of developing infection around the fungal entry point (EP) (×40).

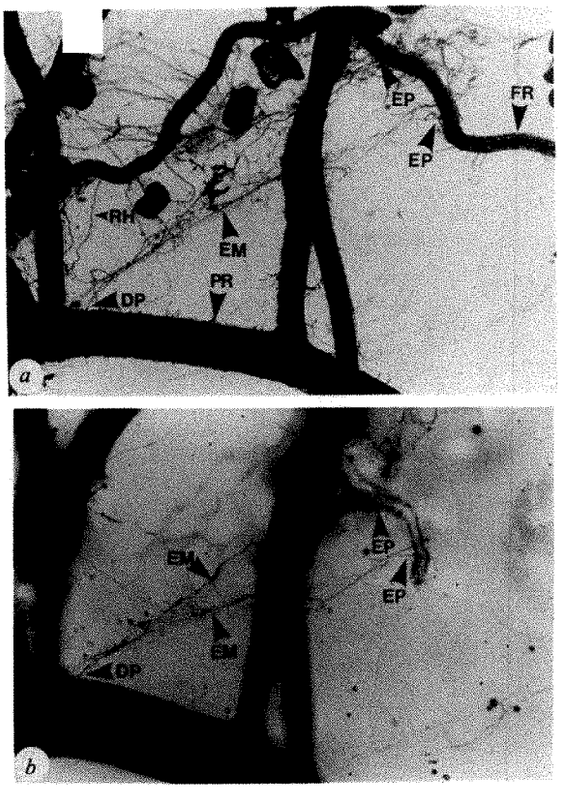


Fig. 3 *a*, Light micrograph of roots of a field inoculum system with typical fascicle of hyphae (EM) passing from departure point (DP) on 'donor' root (PR) and branching to form an entry point (EP) in the upper *Festuca* root (FR) (×40). *b*, Autoradiograph of same area as *a* showing accumulation of radioactivity in the hyphal fascicle (EM), at the entry point (EP) and in the infected part of the *Festuca* root (×40).

close physical contact with *Plantago* (Fig. 1*b*). Indeed, there is little evidence of loss of carbon through leakage from roots of either NM or M donor plants. In the M systems, however, label has moved from donor roots into the external mycelium and to connected receiver roots where it has accumulated around newly formed infections of the *G. caledonium* systems (Fig. 2*b*) and in the vesicles and arbuscules of the more advanced field inoculum infections (Fig. 4*b*). Labelling of mycelium is most pronounced in the coarse hyphae of *G. caledonium* (Fig. 2*b*). The finer hyphae of the field inoculum systems are often grouped together in fascicles running between roots. Chlamydospores developing on these hyphae become sinks for accumulation of label (Fig. 4*a*, *b*) confirming an earlier report[8], which was based upon counting methods. Again, only infected roots accumulate label (Fig. 4*b*) most of which can be shown by subsequent staining to be restricted to vesicles and arbuscules. This pattern of tracer movement indicates that in VA, as in ectomycorrhizas[2], transfer of carbon between plants occurs primarily by a direct hyphal pathway. Slow leakage may yield some assimilate to scavenging mycorrhizal hyphae and so provide an additional indirect or facilitated uptake by receiver plants but the significance of such a pathway is likely to be relatively small not only because leakage is restricted but because of intense competition for such materials amongst the microorganisms of the rhizosphere.

Quantitative analysis of radioactivity in the plants (Table 1) confirms the occurrence of assimilate transfer between mycorrhizal seedlings. While label is scarely detectable in NM plants, levels of activity in M receiver roots of both categories is high and in darkened M plants is six times greater than in those which are fully illuminated. Since the quantity of label moved is strongly influenced by shading it is likely that levels of transfer are largely governed by source–sink relationships. Relatively little activity is detected in shoots of either M or NM receiver plants, probably not only because of the evident capacity of the endophyte mycelium in the root to accumulate labelled material but also because the period of exposure to the isotope was short. Nevertheless the fact that levels of carbon in whole receiver plants can reach over 0.1% of those in donors, even in this limited time span, indicates that whole plants may be to some extent interdependent in terms of assimilate supply. The detection of isotope in the receiver does not, by itself, prove net movement of carbon to these plants, and longer term studies, now being carried out, are required to quantify further the assimilate transfer patterns.

Since seedlings frequently spend their early lives in adverse light environments these observations are likely to be of great ecological and physiological significance. It has been suggested previously[9–11] that the mycorrhizal fungus may form a vigorous sink for host assimilate in the individual infected plant, but in nature plants occur in communities in which individuals are interconnected at both intra- and interspecific levels, through their mycorrhizas. Our results indicate that carbon will move between these plants along gradients caused by differences of illumination and hence of sink strength. Early mycorrhizal infection would provide enhanced nutrient supply but could be critically disadvantageous in terms of the carbon drain imposed by the fungus. This study shows that seedling infection may be sustained by direct transfer of assimilates from neighbouring mature plants which are more likely to be fully illuminated and

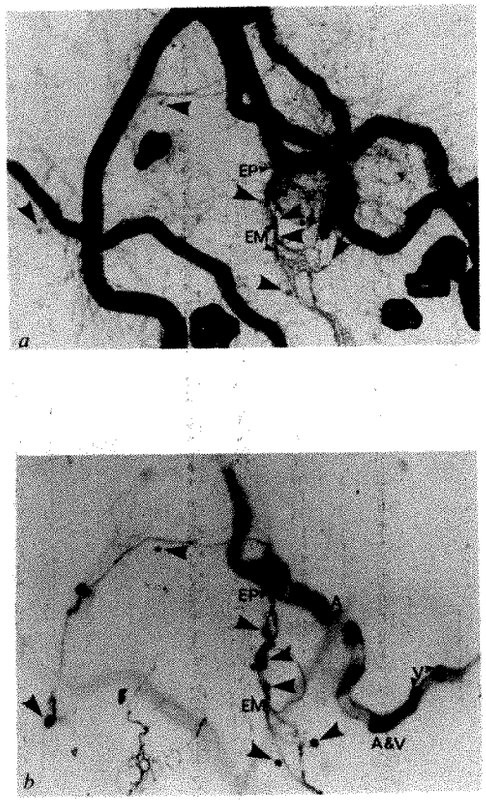


Fig. 4 *a*, Group of *Festuca* roots from field inoculum system showing infection point (EP) arising from a fascicle of hyphae (EM) in which numerous chlamydospores (arrowed) are developing (×40). *b*, Autoradiograph of this group showing accumulation of radioactivity at the entry point (EP), in vesicles (V) and arbuscules (A) along the infected root, in the mycelial fascicle (EM) and in the developing spores. Uninfected roots contain little or no labelled material (×40).

from which infection has spread. There is the further possibility that during the critical establishment phase the entire seedling may live for a period in partial heterotrophic dependence upon its neighbours. The extent of this dependence will be revealed in experiments now being conducted over a longer time period.

We thank Dr F. E. Sanders and Mr L. Reed for supplying spores of *G. caledonium*.

Received 29 July; accepted 1 November 1983.

1. Read, D. J., Koucheki, H. K. & Hodgson, J. H. *New Phytol.* **77**, 641–651 (1976).
2. Brownlee, C., Duddridge, J. A., Malibari, A. & Read, D. J. *Pl. Soil* **71**, 433–443 (1983).
3. Reid, C. P. P. & Woods, F. W. *Ecology* **50**, 178–187 (1969).
4. Hirrel, M. C. & Gerdemann, J. W. *New Phytol.* **83**, 731–738 (1979).
5. Heap, A. J. & Newman, E. I. *New Phytol.* **5**, 173–180 (1980).
6. Whittingham, J. & Read, D. J. *New Phytol.* **90**, 277–284 (1982).
7. Chiariello, N., Hickman, J. C. & Mooney, H. A. *Science* **217**, 941–943 (1982).
8. Ho, I. & Trappe, J. M. *Nature* **244**, 30–31 (1973).
9. Cox, G. Sanders, F. E., Tinker, P. B. & Wild, J. A. in *Endomycorrhizas* (eds Sanders, F. E., Mosse, B. & Tinker, P. B.) 297–312 (Academic, London, 1975).
10. Buwalda, J. G. & Goh, K. M. *Soil. Biol. Biochem.* **14**, 103–106 (1982).
11. Snellgrove, R. C., Splittstoesser, W. E., Stribley, D. P. & Tinker, P. B. *New Phytol.* **92**, 75–87 (1982).

X-ray diffraction evidence that actin is a 100 Å filament

E. H. Egelman

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

R. Padrón

Laboratorio de Biofísica del Músculo, Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Caracas 1010A, Venezuela

The shape of the actin monomer has been determined by X-ray crystallography[1] and image analysis[2] to be a prolate ellipsoid consisting of two domains. A key structural question is how this monomer is assembled into the biologically important actin filament. Two completely different classes of models of F-actin have recently appeared. In one, the long axis of the monomer is oriented parallel to the filament axis[1,2], while in the other it is nearly perpendicular to that axis[3–5]. Fowler and Aebi[6] have argued that a proper assessment of the diameter of the actin filament can distinguish between the correctness of these models, since the 'perpendicular' model is about 95 Å in diameter, while the 'parallel' model is about 75 Å in diameter. Using electron microscopy, they have determined the diameter to be between 70 and 80 Å, consistent with their model. Egelman and DeRosier[3] also provided evidence from electron microscopy that the filament was about 95 Å, consistent with their perpendicular model. However, all specimens prepared for electron microscopy are susceptible to various artefacts induced by the preparation procedure. X-ray diffraction of specimens in solution allows one to examine objects in their native state. Data from X-ray diffraction of actin filaments in live muscle are presented here which show that a reasonable value for the diameter of F-actin is between 95 and 100 Å.

The strongest feature in the muscle X-ray pattern arising from the thin filament is the 59 Å layer line (the measured amplitude distribution is shown in Fig. 1*A*). Because of the different helical symmetries of actin and myosin in muscle, the 59 Å layer line arises only from the thin filament and is related to the distribution of density along the 59 Å left-handed one-start helix of the actin filament. Since the long-pitch strands of tropomyosin are nearly perpendicular to this short-pitch actin helix, tropomyosin will make almost no contribution at low resolution to the diffracted intensity along this layer line[7]. Therefore, by using the phases determined by electron microscopy of negatively stained filaments[3] one can use the X-ray amplitudes to generate a projection of the actin density along this 59 Å helix. Computed transforms of negatively stained filaments showed that the phase was relatively flat across the single broad amplitude peak (considerations of phase continuity show that this must be the case for an object the approximate diameter of F-actin). The resulting helical projection is presented in a one dimensional form in Fig. 1*B*. The X-ray data used were of limited resolution (extending out to 0.020 $Å^{-1}$ from the meridian), but it is clear that any higher resolution data (out to 0.030 $Å^{-1}$) are weak and therefore could not dramatically change the distribution. A comparable helical projection (using the same resolution cutoff) obtained from the model of Egelman and DeRosier is also shown.

The model data match the X-ray data well, the only difference being that the observed data extend out further in radius than the model. The X-ray data shown are consistent with a filament having significant mass extending out to a 100 Å diameter, and are completely inconsistent with a 70–80 Å diameter filament. A comparable plot to Fig. 1*B* (data not shown) has been made using the recently published layer line intensities from a relaxed smooth muscle[8]. The intensity distribution shown by Tajima *et*



Circle No.20 on Reader Service Card.

ANTI ASIALO GM1 (RABBIT)

SPECIFITIES: Reacts with mouse and rat natural killer cells, a part of mouse monocytes and immature mouse fetal thymocytes

USES: The quantitative determination of NK activities
Elimination of asialo GM1 positive cells (dependent complement)
Study of Human all

Following items are also available from WAKO:

Achromopeptidase
Anti-Human Macrophages
Aphidicolin
Mycotoxin Standards
Lysyl Endopeptidase
Nylon Wool Fibre

Wako

Wako Chemicals GmbH
Nissanstr. 2, 4040 Neuss 1
West Germany
Telephone: (02101) 39706
Telex: 8517001

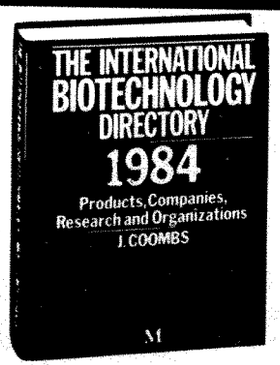
Wako Pure Chemical Industries, Ltd.
10 Doshomachi 3-Chome,
Higashi-Ku
Osaka 541 Japan
Telephone: (06) 203-3741
Telex: 65188 wakoos j

Wako Chemicals USA, Inc.
12200 Ford Road, Suite 212
Dallas, Texas 75234 U.S.A.
Telephone: (214) 484-7518
Telex: 79-1969

Circle No.11 on Reader Service Card.

EXAMINE ON FREE APPROVAL WITHOUT COMMITMENT

TWO NEW reliable, up-to-date and complete biotechnology information resources.



Detailed information on every aspect of biotechnology worldwide

An indispensable guide to:
★ Products, services and research
★ Companies ★ Information sources.
★ Government and international agencies

THE INTERNATIONAL BIOTECHNOLOGY DIRECTORY 1984 —
- is thoroughly international
- features a unique BUYER'S GUIDE to products, research and services
- covers both the old and new biotechnologies

The Directory tells you:
★ Who your competition is, and what they are doing.
★ Who are potential customers and suppliers.
★ Where to buy equipment, chemicals and services.
★ Where culture collections are located and what cultures they hold.
★ Who imports/exports biotechnology products in over 20 countries.
★ Which government departments are associated with biotechnology.

All compiled by the eminent biotechnologist Dr. Jim Coombs and now available to you on **FREE APPROVAL.**

Macmillan Paperback 320pp **September 1983**
ISBN 0 333 36178 4 **£40.00/$80.00**

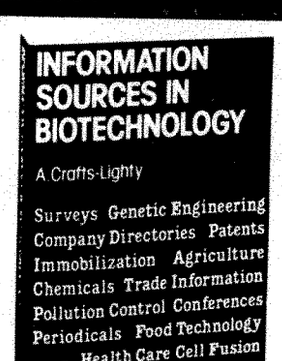
The ONLY guide to the selection of cost effective biotechnology information sources

★ covers both old and new technologies
★ covers technical and commercial literature
★ covers difficult and hard to trace sources
★ includes over 20 at-a-glance evaluation tables
★ provides an Index of publishers and their addresses

Dr. Crafts-Lighty, Head of Information and Library Services at Celltech Limited, systematically and critically reviews all the important information sources:
• books • conferences • trade periodicals • research periodicals • computer databases • patent • market surveys • directories • organisations • secondary sources

Macmillan Hardcover 400pp **November 1983**
ISBN 0 333 35140 1 **£50.00/$100.00**

Send no money now.
Examine on FREE APPROVAL
You can take these new books for 30 days examination without commitment. If you are not completely satisfied, just return the book(s) in good condition within 30 days and we will cancel your invoice.



Globe

Globe Book Services Limited.
Brunel Road, Houndmills,
Basingstoke, Hants RG21 2XS, England
Telephone: Basingstoke (0256) 26222
A DIVISION OF MACMILLAN PUBLISHERS LTD

The Nature Press.
15 E 26 Street, New York, N.Y. 10010
Telephone: 212-532-4811

FREE APPROVAL ORDER FORM

To: Ian Jacobs, Globe Book Services Ltd., FREEPOST, Brunel Road, Houndmills, Basingstoke, Hants RG21 2BR, England.
The Nature Press, 15 E 26 Street, New York. N.Y. 10010. Credit card callers call toll — free 800-221-2123 (or in New York: 212-532-4811)

Please send me on 30 days FREE APPROVAL _____ copies of *The International Biotechnology Directory 1984* at £50.00/$100.00 each, and/or _____ copies of *Information Sources in Biotechnology* at £40.00/$80.00 each.

If I am not entirely satisfied, I may return any book in good condition within 30 days and you will cancel my invoice.

Postage and Packing
UK subscribers £2.00 (Overseas £3.00)
US Subscribers $1.50

Tick preferred method of payment
☐ Bill me. I need send no money now.
☐ I enclose cheque for £/$ _____
☐ Charge my credit card on publication £/$ _____
☐ Visa ☐ Access/Mastercharge
☐ Am. Express ☐ Diners

Card No. _____
Expiry Date _____
Signature _____
(Orders cannot be accepted without a signature)
Name _____
(PLEASE PRINT)
Address _____
_____
Postcode/Zip Code _____

Books will be delivered to you on publication.

UK Subscribers POST TODAY — NO STAMP NEEDED N2

Circle No.63 on Reader Service Card.

Summer Courses at the MBL

June 10–August 25, 1984

Biology of Parasitism John David, Harvard Medical School, Director

This unique program emphasizes the enormous potential utility of the parasitic protozoa and helminths as models for the study of the regulation of the immune response, the alteration of cell membranes, and the genetic controls of the induction of new antigens. Faculty: M. Perkins, G. Cross, Rockefeller; A. Dessein, D. Harn, D. Fearon, Harvard Medical School; C. Gitler, Weizmann Inst. of Science; M. Pereira, Tufts N.E. Medical Center; K.P. Chang, Chicago Medical School; D. Wirth, A. Spielman, S. Landfear and P. Rossignol, Harvard School of Public Health.

Embryology: *A Modern Course in Developmental Biology*
Bruce Brandhorst, McGill and William Jeffery, Texas, Directors

An analysis of the regulatory mechanisms which operate during early embryonic development. Topics to be considered are: Molecular and Cellular Aspects of Fertilization, the Activation of the Program of Gene Expression, the Generation of Embryonic Polarity, the Localization of Macromolecules and Developmental Potential, the Control of Gene Expression During Embryogenesis, and Developmental Genetics. Faculty in 1983 included L. Jaffe, Connecticut; Y. Masui, Toronto; R. Maxson, Stanford; G. Schultz, Calgary; and M. Winkler, Texas.

Microbiology: *Molecular Aspects of Cellular Diversity*
Harlyn O. Halvorson, Brandeis, Director

The primary focus is to investigate, at the molecular level, structural and functional relationships of diverse microorganisms found in nature. Emphasis will be placed on the organisms in their natural habitats with particular attention to the molecular basis of the morphological, physiological, and genetical responses to environmental changes. Faculty: E.P. Greenberg, Cornell; A. Keynan, Hebrew U.; H. Kornberg, Cambridge; J.S. Poindexter, Public Health Res. Inst.; and J. Romesser, DuPont Co.

Physiology Robert D. Goldman, Northwestern, Director

The most advanced approaches used in cell and molecular biology will be treated. The goal is to teach those techniques which are used to relate eukaryotic cell structure with function. Topics to be covered include biochemical procedures for the isolation and characterization of cytoskeletal and contractile proteins associated with microtubules, intermediate filaments and microfilaments, gene transcription, RNA processing, *in vitro* protein synthesis, light and electron microscopy, and immunocytochemistry. Faculty: Thoru Pederson and Richard Vallee, Worcester Foundation; and James Spudich, Stanford.

Costs: Tuition, $1,100; room and board from $68–$105 per week.

Financial aid is available from a variety of sources, including NI

Deadline for All Courses: February 24, 1984. For further information and application material

Circle No.16 on Reader Service Card.

Neuroscience Courses at the MBL

Spring/Summer 1984

Biophysics of Neural Function Daniel Alkon, NINCDS/MBL, Director May 7–June 3

Membrane channels, analyzed in laboratory exercises with current-, voltage-, and patch-clamp techniques, are considered in the context of functioning neural systems which underlie behavior and behavioral change. Research tutorials arranged on an individual basis. Faculty: R. Barlow, Syracuse; M. Brightman, NIH; J. Connor, Bell; C.K. Govind, Toronto; E. Kaplan, Rockefeller. Lecturers: H. Atwood, Toronto; R. Aldrich, R. DeLorenzo, H. Rasmussen, and G. Shepherd, Yale; M. Brodwick, Texas; L. DeFelice, Emory; J. Dowling and E. Kravitz, Harvard; R. French, Maryland; C. Gilbert, Rockefeller; H. Lecar, NIH; G. Pappas, Illinois; and T. Weiss, MIT. —Tuition, $600; room and board, approximately $350.

Neural Systems and Behavior Ronald Hoy, Cornell and Eduardo Macagno, Columbia, Co-Directors June 10–July 28

The central theme is the analysis of behavior in terms of the functional and anatomical organization of the nervous system. Particular emphasis is given to the plasticity and development of the nervous system and to the ontogeny of behavior. Faculty: R. Calabrese, Harvard; D. Kelley, Columbia; M. Nelson and R. Harris-Warrick, Cornell; R. Menzel and J. Erber, Free Univ. of Berlin; and B. Zipser, Cold Spring Harbor.

—Tuition $750; room and board from $68–$105 per week.

Neurobiology John Hildebrand, Columbia and Thomas Reese, NIH/MBL, Co-Directors June 10–August 25

A diversity of conceptual approaches, biological materials, and research techniques are combined to confront current problems in cellular and molecular neurobiology. There are four successive sections: physiology, biochemistry, developmental neurobiology, and neurocytology. Faculty: B. Battelle, NIH; G. Burd, Rockefeller; K. Dunlap, Tufts; G. Fischbach, Washington U.; E. Furshpan, M. Goy, D. Landis, S. Landis, S. Matsumoto, R. Nishi, D. Potter and R. Zigmond, Harvard; L. Hall, Albert Einstein; S. Huttner and P. O'Lague, UCLA; and D. Shotton, Oxford.

—Tuition $1,100; room and board from $68–$105 per week.

Neurobiology of the Leech John G. Nicholls, Biocentrum, University of Basel, Director July 31–August 20

By studying techniques using this single nervous system, students develop skills for the study of cellular neurobiology. The properties, projections, and synapses of sensory neurons, motor neurons, and interneurons are examined using electrophysiological recording and intracellular staining. Students participate in the culture of neruons, use neuron-specific monoclonal antibodies and stain monoamine-containing neurons, prepare identified neurons for electron microscopy and carry out individual projects. Lectures and discussions deal with neural development, rhythmical behaviors and their underlying circuitry, plasticity and regeneration of synapses, monoclonal antibody techniques, and optical recordings. Faculty: K. Muller, Univ. of Miami School of Medicine; W.J. Thompson, Texas; and D. Weisblat, Berkeley. —Tuition, $600; room and board, approximately $350.

training grants, other federal grants, and private scholarship funds.

contact Admissions, Marine Biological Laboratory, Woods Hole, MA 02543 (617) 548-3705.



NEW POST-ELECTION EDITION!

How Britain runs its science...

This highly successful wallchart has now been revised and updated to take account of changes in structure and personnel since the previous edition was published in 1981. It includes the ministerial and other changes which followed the British General Election on 9 June, 1983.

The guide provides a comprehensive survey of the administration of British science, showing the interlocking relationship between science, engineering and medical establishments. It gives a full account of the funding of science in Britain, with details of money spent by government, research councils, industry and universities.

Designed as a fold-out sheet, the 75 × 95cm chart can be carried easily to meetings or hung as a wall poster for permanent reference. The new 1983 edition is available at £3.50 or US$7.95 from Nature's offices in Basingstoke or New York.

In the UK and elsewhere: Nature, Brunel Road, Houndmills, Basingstoke, Hants, RG21 2XS, England.

In the US and Canada: Nature, Suite 1503, 15 East 26th Street, New York, NY 10010, USA.

Cheques should be made payable to Nature.

Please send me

____ Chart(s) of **'How Britain Runs its Science'**

I enclose a cheque for _____

Name_____

Address_____

_____

_____

_____

Please allow 6 to 8 weeks for delivery.

nature

Circle No.68 on Reader Service Card.

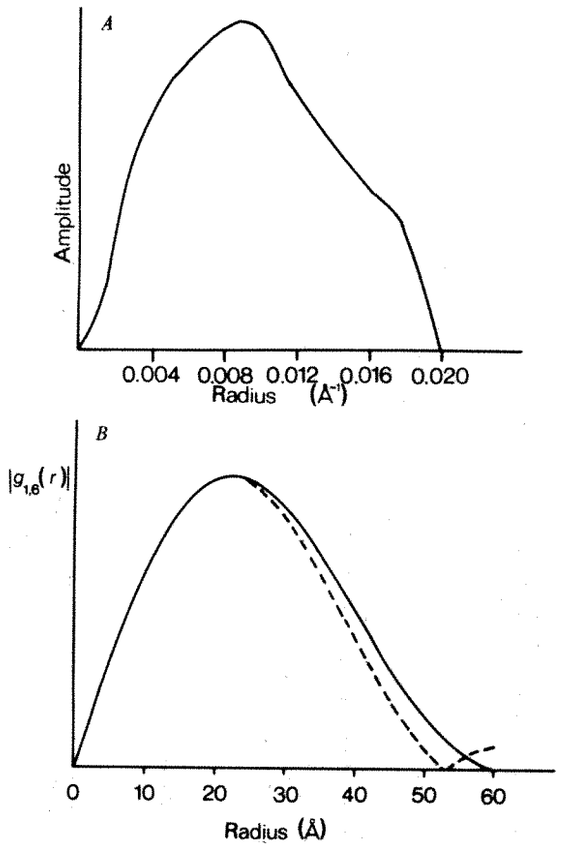


Fig. 1 *A*, The integrated intensity of the 59 Å layer line from the X-ray diffraction pattern of live relaxed frog sartorius muscle was measured, and a background was subtracted. The resulting amplitude distribution is shown. *B*, The function $g_{1,6}(r)$ (ref. 15) has then been computed using Fourier–Bessel inversion of the data shown in (*A*) (solid curve). A corresponding plot from the model of Egelman and DeRosier[3] is also shown (dashed curve). This function is proportional to the modulation of F-actin density when the filament structure is projected along the 59 Å left-handed helix. Because the true radial density distribution of the filament (derived from the equator) has not been added in, this function is zero at the filament axis. The density distribution for the model extends beyond the 49 Å outer radius of the model because of the resolution cutoff which has been imposed to make the two plots comparable.

al.[8] extends out to 0.024 Å^{-1} on the 59 Å layer line, and the resulting helical projection agrees even better with the model. This suggests that the cutoff of our observed intensity at 0.020 Å is responsible for the difference which exists between the model helical projection and the X-ray derived projection.

A second line of X-ray evidence concerns the radius of gyration of the actin filament. This quantity is equal to the square root of the second moment of the average cross-sectional density distribution:

$$\left(\frac{\int r^2\rho(r,\theta)r\,dr\,d\theta}{\int \rho(r,\theta)r\,dr\,d\theta}\right)^{1/2}$$

It has been measured to be 25 ± 2 Å (ref. 9 and R. Mendelson, personal communication). A homogeneous cylinder (whose mean radial density distribution is given by a box function) will have a radius of gyration equal to $r_{max}/\sqrt{2}$, where r_{max} is the outer radius of the cylinder. Thus, the 25 Å value obtained implies that the radius of gyration of actin is the same as that of a homogeneous cylinder of maximum radius $\sqrt{2}\times 25$ Å, or 71 Å in diameter. This value was cited by Fowler and Aebi[6] as being consistent with their 70–80 Å model. But we know, in fact, that actin is far from a solid cylinder. For one thing, it has a deep helical groove along the 59 Å helix which gives rise to the strong and broad peak along the 59 Å actin layer line. This

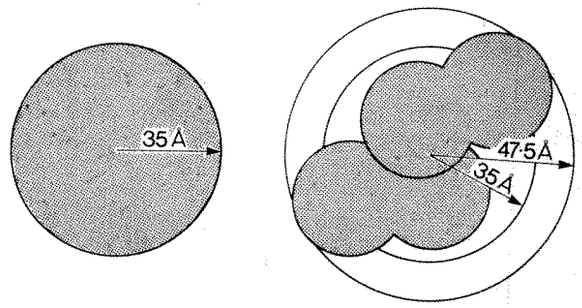


Fig. 2 The radial density distribution of two different models having the same radius of gyration (25 Å) as that experimentally determined for actin[9] are compared. *A*, A homogeneous solid cylinder. *B*, Two actin subunits from the model of Egelman and DeRosier[3], looking down the filament axis. The model uses two symmetrical spheres in the absence of any better information on which domain is the larger. The model is basically enclosed by a cylinder of 95 Å in diameter. The model's radius of gyration (25.4 Å) is equal to that of the solid cylinder shown in *A*.

is the most characteristic feature of both electron microscope transforms and X-ray patterns of actin. A consideration of how the actin subunit can be packed into a filament having such a groove suggests that proportionately more mass has been removed from the 'ideal' homogeneous cylinder at higher radius than at lower radius. This means that the mean radial density distribution will fall with radius. Therefore, the fact that actin has a radius of gyration equivalent to that of a homogeneous cylinder of 71 Å diameter is a strong argument that the maximum diameter of the actin filament is significantly greater than 71 Å. Figure 2 compares the radial density distribution of the 'perpendicular' actin model with that of a homogeneous cylinder having the same radius of gyration. The model of Egelman and DeRosier shown has a radius of gyration of 25.4 Å, in complete agreement with the measured value.

The third avenue of X-ray evidence arises from the early observations[10,11] of the existence of a sixth layer line meridional reflection in the X-ray diffraction pattern of pure F-actin gels. Such a reflection is 'forbidden' by helical symmetry, and must arise from a perturbation of the axial distribution of mass in the actin filament. Lednev interpreted this feature in terms of subunits within the actin filament being in different states. Egelman and DeRosier[12] extended this, using evidence from Mg^{2+} paracrystals and the bending of stereocilia[13] to show that the subunit within the actin filament possessed a variable tilt. They also argued that this feature, mediated by cross-bridging proteins in the stereocilia, arose only from inter-filament packing interactions in Mg^{2+} paracrystals. It is therefore important that Lednev first observed the existence of this reflection when the mean inter-filament spacing in a pure F-actin gel was slightly over 100 Å (ref. 10). At larger spacings this reflection disappeared. This was originally interpreted by Lednev to mean that this deformation of the filament structure occurred when filament surfaces were about 40 Å apart, assuming a 70 Å diameter filament (a traditional assumption). This observation is more consistent with a picture of direct contacts between 'fat' actin filaments inducing tilts of subunits within the filament. The original explanation, of filament conformation being mediated by long range forces, seems much less plausible.

Thus our determination of actin filament diameter using data from X-ray diffraction shows that the actin filament must have a diameter of about 100 Å, and that the perpendicular arrangement of subunits in the filament is required to account for this large outer diameter. Further, this orientation of the monomer is supported by image analysis of isolated actin filaments[3], S-1 decorated actin[4], and single-layered paracrystals of thin filaments[5]. The most likely origin of the parallel model of Smith *et al.*[2] remains the superposition problem associated with multiple layer paracrystals[14].

We thank Dr H. E. Huxley for support and Dr L. Amos for helpful discussions. E.H.E. is a Fellow of the Jane Coffin Childs

Memorial Fund for Medical Research. This investigation has been aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

Note added in proof: The radius of gyration of F-actin has recently been remeasured and found to be 25.7±0.5 Å (P. Matsudaira, J. Bordas and M. Koch, perconal communication). This is in agreement with the cited value[9], but with a significantly smaller error.

Received 4 August; accepted 19 October 1983.

1. Suck, D., Kabsch, W. & Mannherz, H. G. *Proc. natn. Acad. Sci. U.S.A.* **78,** 4319–4323 (1981).
2. Smith, P. R., Fowler, W. E., Pollard, T. D. & Aebi, U. *J. molec. Biol.* **167,** 641–660 (1983).
3. Egelman, E. H. & DeRosier, D. J. *J. molec. Biol.* **116,** 623–629 (1983).
4. Taylor, K. A. & Amos, L. A. in *Actin: Its Structure and Function in Muscle and non-Muscle Cells* (eds dos Remedios, C. & Barden, J.) 25–26 (Academic, Sydney, 1983).
5. O'Brien, E. J., Couch, J., Johnson, G. R. P. & Morris, E. P. in *Actin: Its Structure and Function in Muscle and non-Muscle Cells* (eds dos Remedios, C. & Barden, J.) (Academic, Sydney, 1983).
6. Fowler, W. E. & Aebi, U. *J. Cell Biol.* **97,** 264–269 (1983).
7. Parry, D. A. D. & Squire, J. M. *J. molec. Biol.* **75,** 33–55 (1973).
8. Tajima, Y., Kamiya, K. & Seto, T. *Biophys. J.* **43,** 335–343 (1983).
9. Hartt, J. & Mendelson, R. *Fedn Proc.* **39,** 1728 (1980).
10. Lednev, V. in *Structural Basis and Regulation of Biological Movement* [Russian] (Nauka, Moscow, 1980).
11. Hanson, J., Lednev, V., O'Brien, E. J. & Bennett, P. M., *Cold Spring Harb. Symp. quant. Biol.* **37,** 311–318 (1972).
12. Egelman, E. H. & DeRosier, D. J. in *Actin: Its Structure and Function in Muscle and non-Muscle Cells* (eds dos Remedios, C. & Barden, J.) 17–24 (Academic, Sydney, 1983).
13. Tilney, L. G., Egelman, E. H., DeRosier, D. J. & Sanders, J. C. *J. Cell Biol.* **96,** 822–834 (1983).
14. Egelman, E. H., Frances, N. & DeRosier, D. J. *J. molec. Biol.* **116,** 605–622 (1983).
15. Klug, A., Crick, F. H. C. & Wyckoff, H. W. *Acta crystallogr.* A **11,** 199–213 (1958).

Direct observation of motion of single F-actin filaments in the presence of myosin

Toshio Yanagida, Michiyuki Nakase, Katsumi Nishiyama & Fumio Oosawa

Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka, Japan

Actin is found in almost all kinds of non-muscle cells where it is thought to have an important role in cell motility. A proper understanding of that role will only be possible when reliable *in vitro* systems are available for investigating the interaction of cellular actin and myosin. A start has been made on several systems[1–4], most recently by Sheetz and Spudich who demonstrated unidirectional movement of HMM-coated beads along F-actin cables on arrays of chloroplasts exposed by dissection of a *Nitella* cell[5]. As an alternative approach, we report here the direct observation by fluorescence microscopy of the movements of single F-actin filaments interacting with soluble myosin fragments energized by Mg^{2+}-ATP.

Nagashima and Asakura reported that single filaments of F-actin decorated with heavy meromyosin (HMM) or S-1 are directly visible under an optical microscope with dark-field illumination, although the filaments without HMM or S-1 decoration are not visible[6]. Recently, S. Asakura (personal communication) found that single filaments of F-actin, without HMM or S-1 decoration, are made visible under a fluorescence microscope by labelling with fluorescein isothyocyanate. Using the fluorescent dye, phalloidin–rhodamine, we could clearly and continuously observe the movement of single filaments of F-actin or F-actin-tropomyosin (TM)–troponin (TN) complex for more than 5 min without photobleaching. Phalloidin–rhodamine is most convenient for the present purpose, because it is specifically bound to F-actin without any influence on physiological functions of F-actin[7,8] and its fluorescence intensity is enhanced 4.2-fold by the binding.

When G-actin is polymerized in the presence of phalloidin–rhodamine, many filaments become visible under a fluorescence microscope. Figure 1 shows micrographs of phalloidin–rhodamine-labelled F-actin and F-actin–TM–TN complex in various conditions. All fluorescent filaments have the same brightness and the brightness is uniform along the filament. The number of filaments visible in the field of microscope agrees with the number expected from the concentration of F-actin. The visible filaments are not bundles of F-actin but single filaments of F-actin.

Table 1 Flexural rigidity (ε) of F-actin and F-actin–TM–TN complexes

Sample	Medium	λ (μm^{-1})	ε (10^{-17} dyn cm^2)
FA	+Ca	0.032	6.5
FA+HMM	+Ca	0.042	4.9
	+Ca+ATP	0.059*	3.5*
FA+TM+TN	−Ca	0.028	7.4
	+Ca	0.040	5.2
FA+TM+TN+HMM	−Ca	0.025	8.3
	+Ca	0.045	4.6
	−Ca+ATP	0.027	7.7
	+Ca+ATP	0.053*	3.9*
FA-PM	+Ca	0.033	6.4

The concentration of HMM was 5 mg HMM ml^{-1}. +Ca, rigor buffer +0.2 mM $CaCl_2$; −Ca, rigor buffer +1 mM EGTA; +Ca+ATP, activating buffer; −Ca+ATP, relaxing buffer. The flexural rigidity, ε, was determined by measuring the end-to-end distance, R. About 50 values of R were measured for each filament in a 2–10-s period and the mean square end-to-end distance, $\langle R^2 \rangle$, was related to a parameter of flexibility, λ, by the equation $\langle R^2 \rangle = [2\lambda L - 1 + \exp(-2\lambda L)]/2\lambda^2$ (ref. 16). The most probable values of λ were obtained from a least-square fit to the plots of L versus $\sqrt{\langle R^2 \rangle}$ of more than 10 different filaments 5–10 μm in contour length, L. The flexural rigidity, ε, was calculated from the value of λ by the equation $\varepsilon = kT/2\lambda$ (ref. 17). FA-PM, F-actin cross-linked by *p*-phenylene-*N,N'*-bis(maleimide) by the method of Knight and Offer[14].

* In the activating buffer, the motion is not a thermal one, but the apparent values of λ and ε determined by the above procedure are shown for convenience to compare the extent of bending (see text).

The thermal bending motion of the filaments is observable under the microscope. Table 1 gives the flexural rigidity of F-actin and F-actin–TM–TN complex estimated by the statistical analysis of the end-to-end distance of a number of filaments. The values of the flexural rigidity are in good agreement with those of unlabelled F-actin and F-actin–TM–TN measured by different methods[6,9–11]. This indicates that the binding of phalloidin does not affect the dynamic property of F-actin although it stabilizes F-actin against depolymerization at low ionic strength or high concentration of KI[12]. The binding of Ca^{2+} decreased the rigidity of the complex and the binding of HMM further decreased the rigidity in the presence of Ca^{2+}.

When both HMM and Mg^{2+}-ATP are added to the F-actin–TM–TN complex in the presence of Ca^{2+}, the bending motion of long filaments becomes faster and larger in amplitude, and the tumbling motion of shorter filaments becomes conspicuous. During such motions, long filaments are often broken into fragments of up to a few micrometres (Fig. 1*d*). Figure 2 shows sequences of micrographs taken to compare the bending motion of the F-actin–TM–TN filaments in a solution containing HMM and Mg^{2+}-ATP, in the absence (Fig. 2*a*) and presence (Fig. 2*b*) of Ca^{2+}. The filaments in Fig. 2*a* and *b* have same length, 10 μm. In the absence of Ca^{2+} (Fig. 2*a*), the bending motion of the first order mode has an apparent period of 3–6 s, and that of the second order mode has a period of about 0.3 s. Previously, by using dynamic light scattering, the relaxation time of the bending motion of F-actin of 2.5 μm in length was determined to be about 10 ms[9]. The above value, 3–6 s, for the filaments of 10 μm length is reasonable because the relaxation time, τ_n, of the bending motion is proportional to the fourth power of the length, as given by

$$\tau_n = \pi^{-4}(n+(1/2))^{-4}\,\zeta\,L^4\,\varepsilon^{-1} \qquad (1)$$

where n is the mode number, L the contour length of the filament, ζ the frictional constant for a structural unit and ε the flexural rigidity[9].

In the presence of Ca^{2+} (Fig. 2*b*), the apparent period of the bending motion of the first mode decreased to 1–3 s. If the bending is due to thermal brownian motion, the flexural rigidity in the presence of Ca^{2+} must be about twice as large as that in its absence, according to equation (1). However, as found in

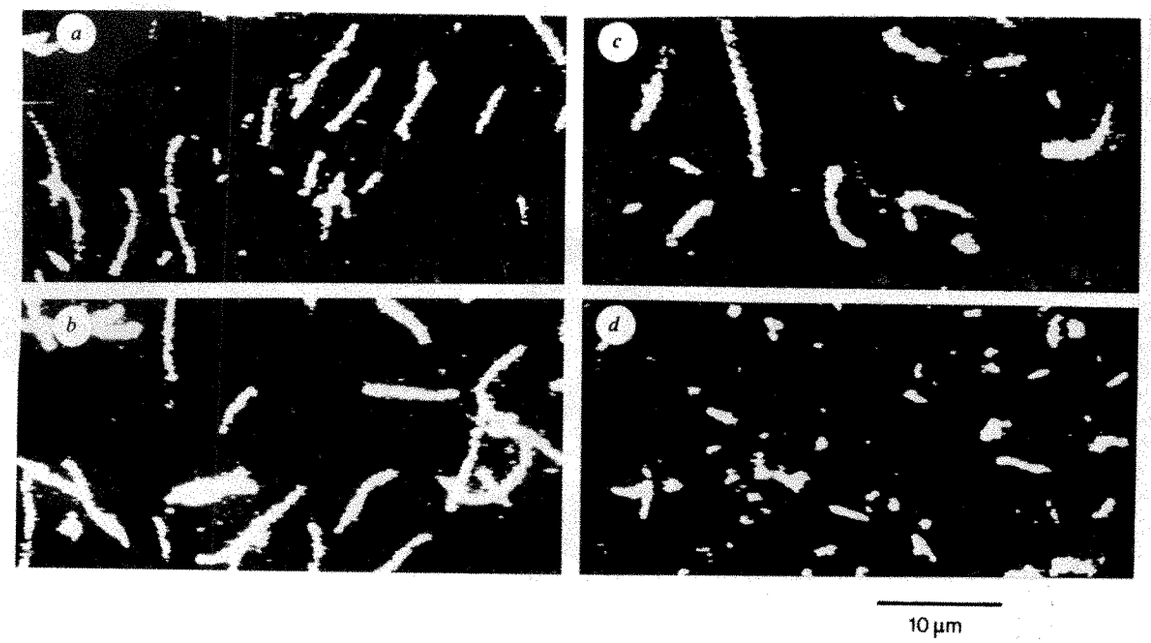


Fig. 1 Micrographs of phalloidin–rhodamine-labelled F-actin: *a* and *b* are for the F-actin in rigor buffer (0.1 M KCl, 5 mM $MgCl_2$ and 10 mM MOPS *p*H 7.0) in the absence and the presence of 10 mg HMM ml^{-1}, respectively. *c* and *d* are for the F-actin–TM–TN complexes in relaxing buffer (rigor buffer +1 mM EGTA, 3 mM ATP, 5 mM creatine phosphate and 2 mg creatine kinase per ml) and in activating buffer (rigor buffer +0.2 mM $CaCl_2$, 3 mM ATP, 5 mM creatine phosphate and 2 mg creatine kinase per ml) containing 10 mg HMM ml^{-1}, respectively. All proteins were obtained from rabbit skeletal muscle and purified according to the method described previously[10]. G-actin of 1 μM was polymerized in rigor buffer containing 1 μM phalloidin–rhodamine overnight at room temperature. TM and TN were added to the F-actin solution at a weight ratio of 1:4, respectively. For observation the solutions were diluted 50-fold in buffer and put in a gap between a glass slide and a coverslip of 50 μm in thickness. Final concentration of actin was 20 nM. β-Mercaptoethanol of 0.1–0.5% (v/v) which did not affect actomyosin ATPase activity or muscle contraction was added to the solutions to suppress photobleaching. Fluorescent F-actin or F-actin–TM–TN complexes were observed with a Nikon VFD-TR microscope equipped with epifluorescence optics, a Nikon UV-F ×100 objective (oil immersion, NA 1.3), a 100–200-W mercury arc lamp and a Nikon rhodamine filter set ($\lambda_{EX} \sim 540$ nm, $\lambda_{EM} > 580$ nm). Fluorescent images were recorded on videotape with a high-sensitivity television camera (Ikegami CTC-900) and a video recorder (National Time Laps NV8030).

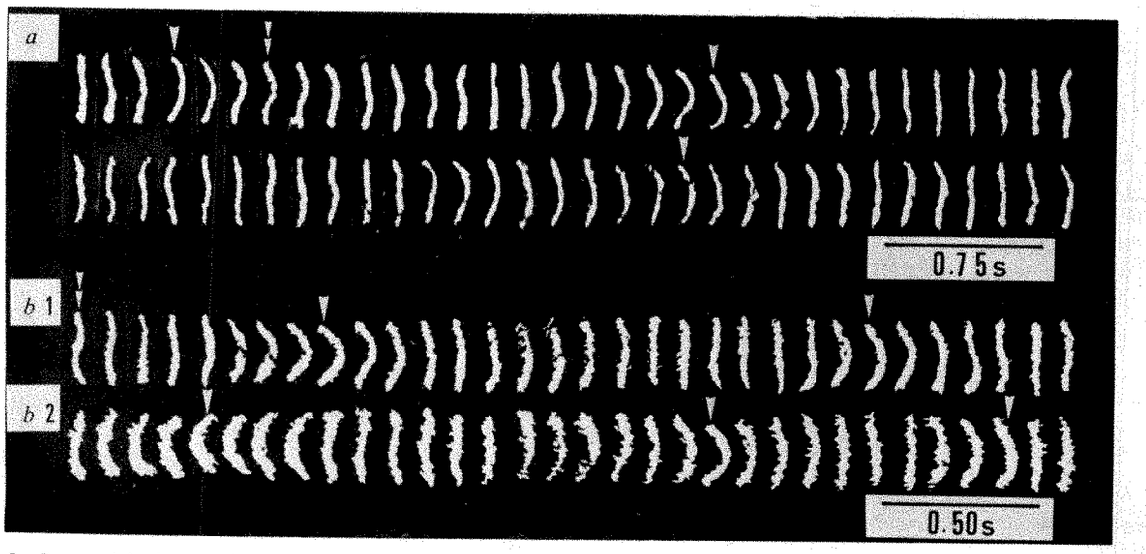


Fig. 2 Sequential micrographs of phalloidin–rhodamine-labelled F-actin having bound TM and TN in relaxing buffer +5 mg HMM ml^{-1}, (*a*) and in activating buffer +5 mg HMM ml^{-1} (*b*, 1, 2). The intervals between successive images were 0.15 s (*a*) and 0.10 s (*b*, 1, 2), respectively. The contour length of both filaments was 10 μm. Single arrows indicate first-order mode bending motion and double ones second order mode bending motion. As measured by a semiconductor thermometer, the temperature of the solution was 25 °C during illumination. No increase in the temperature of the solution during ATP hydrolysis by HMM, S-1, acto-HMM or acto-S-1 was detectable. The resolution of TV camera (1/60 s) was not high enough to record the rapid motion of filaments, especially in activated state (*b*).

Figure 2*a* and *b*, the average amplitude of bending of the filaments is distinctly larger in the presence of Ca^{2+} than in its absence. Therefore, we conclude that more rigid filaments in the presence of Ca^{2+} were forcibly bent by interaction with HMM and ATP. Conversely, it is also possible to conclude that the bending motion of the filament which is more flexible in the presence of Ca^{2+} was accelerated by interaction with HMM and Mg^{2+}-ATP. The latter case is more likely because the F-actin–TM–TN complex is more flexible in the presence of Ca^{2+} as shown in Table 1. In the present conditions (0.1 M KCl, 20 nM actin and 15 μM HMM), the rate of hydrolysis of ATP is estimated to be about 1 mole per mole actin per s (ref. 13). That is, each actin monomer is attacked by energized HMM (HMM–ADP–P_i) about once per second. The energy liberated by the hydrolysis may be (partially) transferred to F-actin to induce its active motion.

Similar results have been obtained by using pure F-actin instead of the F-actin–TM–TN complex and we have preliminary evidence that S-1 has the same effects as HMM on the motion of F-actin and the F-actin–TM–TN complex. The two-headed structure of HMM is not absolutely necessary to produce faster and larger bending motion of F-actin and the complex.

The effect of cross-linking of actin subunits by *p*-phenylene *N,N'*-bis(maleimide) (PM) on the motion of F-actin was also examined. Previously, by applying this reagent, Knight and Offer showed that the cross-linking did not change the ability of F-actin to activate myosin ATPase and induce superprecipitation, and suggested that relative motion of actin subunits was not required for the activation[14]. According to our direct observation, however, the cross-linking by this reagent does not restrict the motion of F-actin and the cross-linked F-actin has the same value of the flexural rigidity as before cross-linking (Table 1).

The present finding suggests that in an activated state, myosin heads induce local conformational changes in F-actin such as distortion or rotation of actin monomers and loosening of actin–actin bonds[8,15], which result in fast and large bending in F-actin filaments. Since F-actin has a structural polarity, the motion in F-actin could be asymmetric, if it occurred during interaction with HMM or S-1 in the presence of Mg^{2+}-ATP. However, the interaction between single F-actin filaments and soluble myosin fragments did not produce directional movement. When F-actin filaments function in living cells, they are usually found as assemblies with same polarity and often supported by some backbone such as membrane. Therefore, higher organizations of F-actin such as polar bundles may be indispensable to transformation of the activated motion in F-actin into an effective sliding force.

We thank Dr Th. Wieland for supplying us with phalloidin-rhodamine, Drs K. Hotani, O. Ohara and E. Pròchniewicz-Nakayama for many useful discussions and Dr T. J. Herbert for reading the manuscript.

Received 28 July; accepted 11 October 1983.

1. Oplatka, A. & Tirosh, R. *Biochim. biophys. Acta* **305,** 683–688 (1973).
2. Yano, M. Yamamoto. & Shimizu, H. *Nature* **299,** 557–559 (1982).
3. Kuroda, K. & Kamiya, N. *Proc. Japan. Acad.* **51,** 774–777 (1975).
4. Higashi-Fujime, S. *J. Cell Biol.* **87,** 569–578 (1980).
5. Sheetz, M. P. & Spudich, J. A. *Nature* **303,** 31–35 (1983).
6. Nagashima, H. & Asakura, S. *J. molec. Biol.* **136,** 169–182 (1980).
7. Wulf, E., Deboben, A., Bautz, F. A., Faulstich, H. & Wieland, Th. *Proc. natn. Acad. Sci. U.S.A.* **76,** 4498–4502 (1979).
8. Pròchniewicz-Nakayama, E. Yanagida, T. & Oosawa, F. *J. Cell Biol.* (in the press).
9. Fujime, S. *J. Phys. Soc. Japan* **29,** 751–759 (1970).
10. Yanagida, T. & Oosawa, F. *J. molec. Biol.* **126,** 509–524 (1978).
11. Yoshino, S. Umazume, Y., Natori, R. Fujime, S. & Chiba, S. *Biophys. Chem.* **8,** 317–326 (1978).
12. Wieland, Th. & Faulstich, H. *CRC crit. Rev. Biochem.* **5,** 185–260 (1978).
13. Eisenberg, E. & Moos, C. *J. biol. Chem.* **242,** 2945–2951 (1967).
14. Knight, P. & Offer, G. *Biochemistry* **19,** 4682–4687 (1980).
15. Yanagida, T. in *Cross-bridge Mechanism in Muscle Contraction* (eds Pollack, G. H. & Sugi, H.) (Plenum, New York, in the press).
16. Landau, L. D. & Lifshitz, E. M. *Statistical Physics,* 478–482 (Pergamon, London, 1958).
17. Harris, R. A. & Hearst, J. E. *J. chem. Phys.* **44,** 2595–2609 (1966).

Microtubules with more than 13 protofilaments in the dividing nuclei of ciliates

U. Eichenlaub-Ritter

Lehrstuhl für Zellmorphologie, Ruhr-Universität Bochum, Postfach 10 21 48, D-4630 Bochum 1, FRG

J. B. Tucker

Department of Zoology, The University, St Andrews, Fife KY16 9TS, UK

Microtubules are largely composed of proteins called tubulins. These are stacked in linear arrays called protofilaments (p). Most microtubules have precisely 13p (ref. 1). The 'incomplete' B and C microtubules (10 or 11p) of cilia, flagella, basal bodies and centrioles are widespread exceptions[2,3]. Very few examples of 'complete' microtubules with more, or less, than 13p have been found[4–7]. However, the 'ciliate cell' includes a larger number of highly differentiated types of microtubule arrays than most other cell types[8–10]. The present study was undertaken to ascertain whether there is variation in p number in two ciliates. In both, all complete cytoplasmic microtubules examined have 13p but microtubules with 13–16p are present in the nucleoplasm of dividing nuclei. These features are probably common to ciliates in general because the free-living hymenostone *Paramecium tetraurelia* and the parasitic heterotrich *Nycotherus ovalis* are not closely related in terms of taxonomic criteria[11] or life-style.

During the division of a ciliate, two main categories of microtubule arrays can be distinguished: cytoplasmic and nucleoplasmic. Nuclear envelopes remain intact and large numbers of aligned nucleoplasmic microtubules are associated with the marked elongation of dividing nuclei (Fig. 1)[12,13]. Protofilaments can usually be detected in cross-sections of microtubules fixed in the presence of tannic acid[3]. However, ciliates possess a substantial pellicle, and nuclear envelopes provide an additional barrier to tannic acid access so far as nucleoplasmic microtubules are concerned. Because of this we have had to devise a protocol for making the organisms permeable without loss of alignment or disassembly of microtubules prior to fixation (Fig. 2).

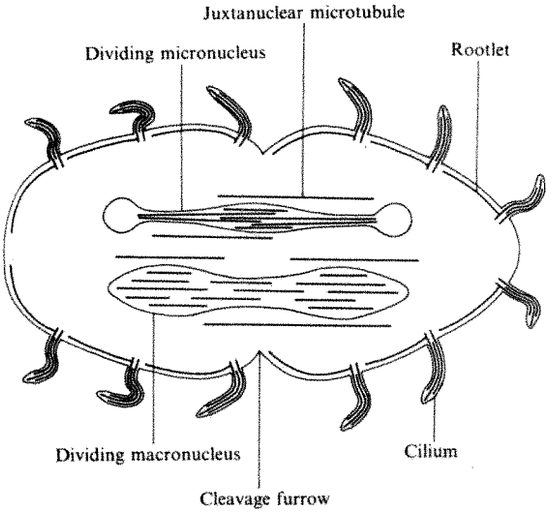


Fig. 1 The general distribution of cytoplasmic and nucleoplasmic microtubules in a dividing ciliate. The cell membrane and nuclear envelopes are depicted by thin lines. The thicker lines show the locations and orientations of microtubules. For clarity, specialized cortical organelle complexes that include microtubules such as mouthparts have been omitted.

Cytoplasmic microtubules with 13p include those that contribute to the cortical cytoskeletal complex such as cytopharyngeal microtubules[14] (Fig. 2*a*) and the A and central pair microtubules of cilia, as well as the more deeply situated juxtanuclear microtubules (Fig. 1) that run very closely alongside the outer surfaces of dividing nuclei (Fig. 3*f*). In *Paramecium* there is a set of cortical 'cytospindle' microtubules[15] that is probably a type of basal body rootlet array. Like most nucleoplasmic microtubules, and unlike most of the other cytoplasmic microtubules, they are only present during cell division and apparently promote elongation [of the dividing cell body][8,15,16]. Nevertheless, they have 13p.

The p number that predominates in dividing micronuclei changes as mitosis proceeds. This is correlated with a marked switch in the organization of nucleoplasmic microtubules during micronuclear mitosis. During the earlier stages (prophase–metaphase) spindle architecture is similar to that of mitotic spindles

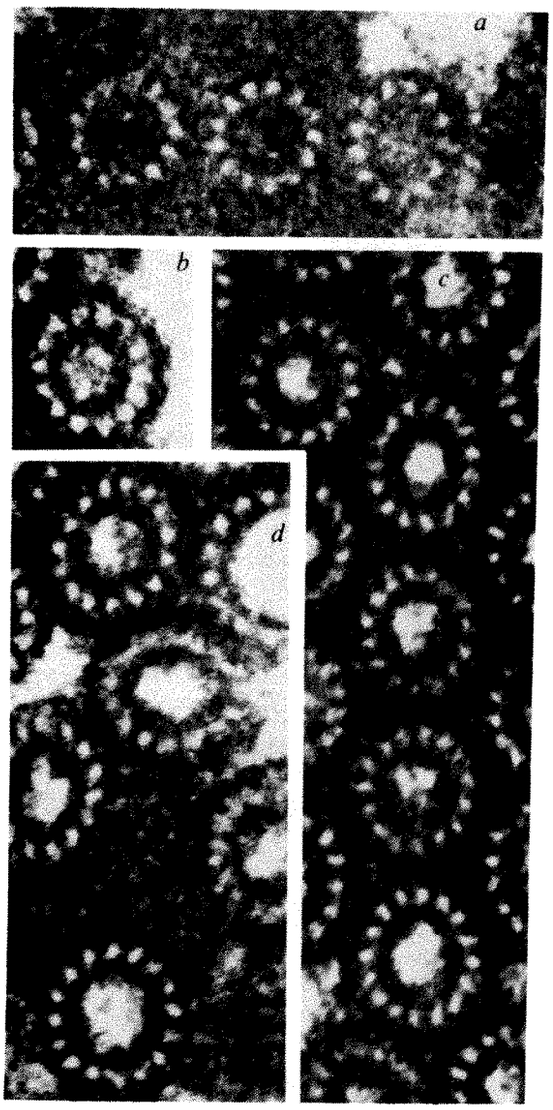


Fig. 2 Cross-section of microtubules in *P. tetraurelia*. All ×625,000. Organisms were permeabilized for 90 s by suspension in a solution of the non-ionic detergent NP40 (0.05%) buffered with the microtubule-stabilizing buffer PM2G[26] and then fixed for 1 h in a solution buffered with cacodylate (0.15 M, *p*H 7.2) containing glutaraldehyde (2.5%) and tannic acid (8%). After rinsing with five changes of cacodylate buffer at 4 °C for 12 h the organisms were post-fixed for 30 min in cacodylate-buffered osmium tetroxide (1%). Subsequent preparation for electron microscopy was as described previously[17]. *a*, Cytoplasmic microtubules with 13 protofilaments in part of a cytopharyngeal microtubule ribbon. *b–d*, Spindle microtubules in micronuclei at the same late anaphase stage as Fig. 4*b* showing: *b*, a 14 protofilament microtubule; *c*, microtubules with 15 protofilaments and one with 16 (at bottom); *d*, a microtubule with 15 protofilaments (at top) and one with 16 (at bottom).

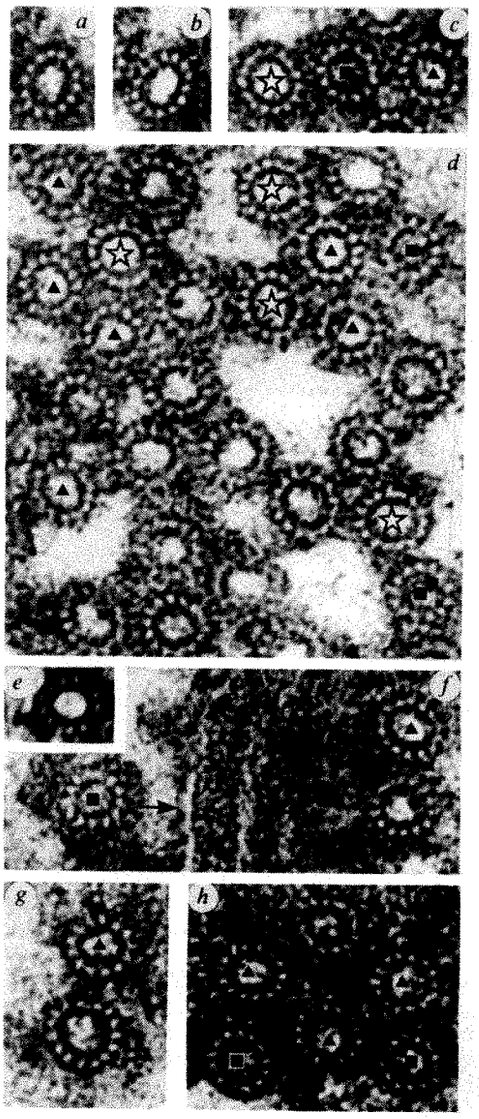


Fig. 3 Cross-sections of microtubules with 13 (■), 14 (▲) and 15 (☆) protofilaments in *N. ovalis* prepared for electron microscopy as described in Fig. 2 apart from the use of NP40 at a concentration of 0.1%. All ×370,000. *a–d*, spindle microtubules in micronuclei at late anaphase showing *a*, *b*, microtubules with 14 protofilaments, *c*, *d*, microtubules with three different protofilament values in close proximity to each other; *e*, a kinetochore microtubule with 13 protofilaments in a metaphase micronucleus; *f*, a juxtanuclear cytoplasmic microtubule (■) positioned closely alongside the envelope (arrows) and nucleoplasmic spindle of an elongating micronucleus, *g*, *h*, microtubules in dividing macronuclei. The microtubule nearest the bottom of *g* has at least 15 protofilaments.

in general (Fig. 4*a*), although there are no centrioles at spindle poles[17]. The vast majority of microtubules have 'conventional' diameters of 24 nm; in *Nyctotherus* (no data for *Paramecium*) they have 13p (Fig. 3*e*) and can be induced to disassemble by exposure to low temperature (0 °C) and antimitotic drugs[18]. However, anaphase B (chromatid separation due to spindle elongation[19]) is especially pronounced; micronuclei become dumb-bell-shaped at this stage of mitosis (Fig. 1). A compact bundle of well-aligned microtubules (Fig. 4*b*) extends along the mid-portion of a micronucleus throughout the period of rapid[20] and marked elongation. In *Paramecium* most of these microtubules have diameters, up to 32 nm, that are detectably greater than 24 nm[12,21], while in *Nycototherus* they are cold and drug stable[18] unlike those that predominated earlier. Furthermore, most of the microtubules have more than 13p. Microtubules with 15p predominate in *Paramecium* (Fig. 2*c*, *d*) and some with 16p (Fig. 2*c*, *d*), 14p (Fig. 2*b*) and 13p are also present. In *Nyctotherus* most of the microtubules have 14p (Fig. 3*a–d*) but microtubules with 15p and 13p (Fig. 3*c*, *d*) also occur.

At no stage does macronuclear 'amitosis' involve the construction of a microtubule array that can be regarded as a mitotic spindle, although events are somewhat more orderly than was once supposed[20]. Microtubules with 13–16p are present (Fig. 3*g*, *h*) in the dividing macronucleus of *Nyctotherus*. Only 13p microtubules have been detected in *Paramecium* macronuclei to date (however, few unequivocal assessments of p numbers have been possible because the microtubules are not well aligned after organisms have been made permeable and fixed).

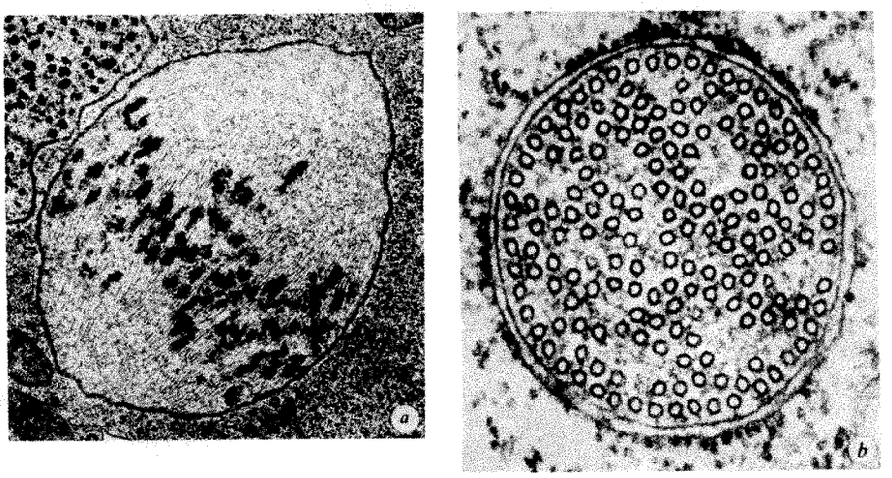


Fig. 4 *a*, Longitudinal section through a micronucleus in *P. tetraurelia* that is either approaching metaphase or has just embarked on anaphase. The clumps of dense material concentrated near the equator represent condensed chromatids. ×10,500. *b*, Cross-section of a nearly fully elongate micronuclear spindle in *P. tetraurelia*. The well-aligned spindle microtubules are surrounded by the nuclear envelope. Differences in microtubule diameters are apparent. ×70,000.

This is the first report of complete microtubules with p numbers other than 13 occurring in unicellular organisms and in association with mitosis. It also provides the first example of a situation in which most of the microtubules that assemble in normal conditions *in vivo* are composed of 14p (Fig. 3*d*) (only one 14p microtubule has previously[4] been found *in vivo*), and striking instances of variation in p number for microtubules in close spatial association (Figs 2*c*, *d* and 3*c*, *d*, *h*). The few examples, and puzzling occurrence, of complete microtubules with more (14p–16p)[4–7] or less (11p, 12p)[4,6] than 13p documented so far (from representatives of four animal phyla including a mammal[7]) suggest no immediately obvious reason why some microtubules have a p value other than 13. Variation in p number is evidently not a commonly exploited basis for the functional differentiation of microtubules. For example, the complete cytoplasmic microtubules of *Paramecium* and *Nyctotherus* are interconnected in very different ways, are structurally associated with a diversity of other cell components, and almost certainly perform a wide range of different cytoskeletal and mechanochemical functions in the various arrays in which they occur[8,13,14,20,22]—yet they are all apparently composed of 13p.

At least some of the tubulin in the 15p spindle microtubules of *Paramecium* is serologically related to some of that in the organism's 13p cytoplasmic microtubules[15]. However, some of the 'protofilaments' apparent in certain flagellar microtubules after fixation in the presence of tannic acid apparently include proteins other than tubulin[23,24]. Variation in p number may be just a crude preliminary indication of the true diversity of microtubule architecture that abounds.

There is evidence that p number is determined *in vivo* when microtubule assembly is initiated and that its specification originates from microtubule-organizing centres[25]. Is the situation in ciliate nuclei sometimes like that during *in vitro* microtubule assembly when microtubule-organizing centres and p number fidelity are both lacking? There is temporal control of the p number that predominates during micronuclear mitosis in *Nyctotherus* as reported above. Preliminary indications of positional control for nucleoplasmic microtubules with different p numbers in both organisms are emerging. Further analyses of nucleoplasmic microtubules in ciliates should be of especial value in further elucidating the spatial control and functional significance of variation in microtubule protofilament numbers.

We thank Mrs M. Seebach, Mr R. Siedel, Miss K. A. K. Hendry, Mr D. L. J. Roche and Mr J. B. Mackie for assistance.

Received 10 August; accepted 24 October 1983.

1. Amos, L. A. in *Electron Microscopy of Proteins*, Vol. 3 (ed. Harris, J. R.) 207–250 (Academic, London, 1982).
2. Tilney, L. G. *et al. J. Cell Biol.* **59**, 267–275 (1973).
3. Fujiwara, K. & Linck, R. W. *Meth. Cell Biol.* **24A**, 217–233 (1982).
4. Burton, P. R., Hinkley, R. E. & Pierson, G. B. *J. Cell Biol.* **65**, 227–233 (1975).
5. Nagano, T. & Suzuki, F. *J. Cell Biol.* **64**, 242–245 (1975).
6. Chalfie, M. & Thomson, J. N. *J. Cell Biol.* **93**, 15–23 (1982).
7. Saito, K. & Hama, K. *J. Electron Microsc. Tokyo* **31**, 278–281 (1982).
8. Jurand, A. & Selman, G. G. *The Anatomy of Paramecium aurelia* (Macmillan, London, 1969).
9. Tucker, J. B. *Nature* **232**, 387–389 (1971).
10. Lynn, D. H. *Biol. Rev.* **56**, 243–292 (1981).
11. Corliss, J. O. *The Ciliated Protozoa: Characterization, Classification and Guide to the Literature*, 2nd edn (Pergamon, New York, 1979).
12. Cohen, J., Beisson, J. & Tucker, J. B. *J. Cell Sci.* **44**, 153–167 (1980).
13. Eichenlaub-Ritter, U. in *Microtubules in Microorganisms* (eds Cappuccinelli, P. & Morris, N. R.) 351–375 (Dekker, New York, 1982).
14. Eichenlaub-Ritter, U. & Ruthmann, A. *Differentiation* **24**, 97–110 (1983).
15. Cohen, J., Adoutte, A., Grandchamp, S., Houdebine, L. M. & Beisson, *Biol. Cell* **44**, 35–44 (1982).
16. Sundararaman, V. & Hanson, E. D. *Genet. Res.* **27**, 205–211 (1976).
17. Eichenlaub-Ritter, U. & Ruthmann, A. *Chromosoma* **84**, 701–716 (1982).
18. Eichenlaub-Ritter, U. & Ruthmann, A. *Chromosoma* **85**, 687–706 (1982).
19. Inoué, S. & Ritter, H. in *Molecules and Cell Movement* (eds Inoué, S. & Stephens, R. E.) 3–30 (Raven, New York, 1975).
20. Tucker, J. B., Beisson, J., Roche, D. L. J. & Cohen, J. *J. Cell Sci.* **44**, 135–151 (1980).
21. Tucker, J. B. in *Microtubules* (eds Roberts, K. & Hyams, J. S.) 315–357 (Academic, London, 1979).
22. Plattner, H., Westphal, C. & Tiggemann, R. *J. Cell Biol.* **92**, 368–377 (1982).
23. Linck, R. W. & Langevin, G. L. *J. Cell Sci.* **58**, 1–22 (1982).
24. Linck, R. W. *Ann. N. Y. Acad. Sci.* **383**, 98–121 (1982).
25. Scheele, R. B., Bergen, L. G. & Borisy, G. G. *J. molec. Biol.* **154**, 485–500 (1982).
26. Duerr, A., Pallas, D. & Solomon, F. *Cell* **24**, 203–211 (1981).

Weaver mutation has differential effects on the dopamine-containing innervation of the limbic and nonlimbic striatum

Suzanne Roffler-Tarlov & Ann M. Graybiel

Departments of Neurology and Anatomy, Tufts University School of Medicine, Boston, Massachusetts 02111, USA and Department of Psychology and Brain Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Ungerstedt[1] observed that the dopamine-containing innervation of the forebrain can be divided into two parts: a nigrostriatal system, originating mainly in the pars compacta of the substantia nigra and innervating the caudoputamen; and a mesolimbic system arising mainly in the ventral tegmental area and innervating the nucleus accumbens and olfactory tubercle. This classification has since been modified and extended with the discovery of the mesocortical dopamine system[2,3]. The original distinction between nigrostriatal and mesolimbic systems nevertheless was pivotal in suggesting that the basal ganglia are related to limbic as well as to sensorimotor functions, and remains of interest because dopaminergic mechanisms may be implicated not only in the aetiology of sensorimotor impairments such as those of Parkinson's disease[4], but also in neuropsychiatric disorders such as schizophrenia[5–7]. The striatal targets of the mesolimbic and nigrostriatal systems are now known to be distinct also in terms of forebrain connections, despite some overlap of fibre projections[18,19]. The nucleus accumbens–olfactory tubercle region and abutting caudoputamen (together called the 'ventral' or 'limbic' striatum) are characteristically related to limbic parts of the

forebrain, whereas the large remainder of the caudoputamen (the 'dorsal' or 'non-limbic' striatum) is most closely related to sensorimotor regions[19,20,34]. We report here evidence that the mesolimbic and nigrostriatal systems are differentially affected in the mutant mouse *weaver*, and in particular that dopamine is severely depleted in the dorsal striatum of weaver but relatively spared in the ventral striatum. We conclude that dopamine-containing fibre systems innervating the limbic and non-limbic striatum can be influenced separately in genetic disease and that genetic control, whether direct or indirect, may be exerted at the single-gene level.

Weaver (*wv*) an autosomal recessive mutation in the mouse, has been intensively studied because of its effects on the cerebellum. The weaver disease is expressed in the cerebellum early in postnatal life when most granule cells die in their proliferative zone after failing to migrate to the internal granular layer[8]. Quite recently it has been discovered that the weaver mouse also suffers a severe deficiency of dopamine in the forebrain[9,10]. This finding prompted the present study, in which we sought to determine the specific extent and location of this neurotransmitter defect in the weaver striatum. Biochemical and anatomical observations were made on homozygous weaver animals (*wv*/*wv*) and heterozygous littermate controls (+/*wv*) of both sexes obtained by mating C57BL6/CBA (+/*wv*) heterozygotes purchased from the Jackson Laboratories (Bar Harbor, Maine). Offspring were taken for study at 1–9 months of age. Homozygous weaver animals were identified behaviourally by their severe ataxia, hypotonia, and fine tremor[8]. Heterozygote littermates have no such obvious behavioural abnormalities but do have moderate cerebellar atrophy and were identified by retrospective examination of the cerebellum[11,12]. Homozygous (+/+) littermates have not yet been examined.

For the biochemistry, whole-region dissections of the caudoputamen, nucleus accumbens, olfactory tubercle and midbrain were made from 420-μm-thick transverse slices cut from 17 weaver and 17 control brains hardened *in vivo* by transcardial perfusion with ice-cold phosphate-buffered saline (0.1 M PO_4, 0.9% saline) under conditions of barbiturate (Nembutal) anaesthesia[13] (see Fig. 1). Whole-retina samples were also taken from some animals, so that the values for dopamine in the basal ganglia could be compared with those of the dopamine-containing amacrine cell population in the retina. Catecholamines were extracted from the isolated brain regions and retinas by homogenization in 0.1 M $HClO_4$. The dopamine in the supernatant was isolated using alumina mini-columns and was measured by electrochemical detection following separation by high performance liquid chromatography[14]. The protein content of the precipitate was determined by the method of Lowry *et al.*[15].

For the anatomy, 30 μm-thick sections were cut in a cryostat from frozen unfixed brains (13 weavers, 14 littermate controls), thaw-mounted onto slides and processed by de la Torre's glyoxylic acid method[16] for the visualization of catecholamine-induced fluorescence. Sections were viewed and photographed under fluorescent illumination with the aid of a Leitz Ploemopak.

Table 1 summarizes the biochemical findings for all mice studied. In the controls, the concentrations of endogenous dopamine extracted from the caudoputamen, nucleus accumbens and olfactory tubercle were similar, though values for the caudoputamen were slightly higher than those for the ventral subdivisions. Dopamine levels for all three striatal districts were, as expected[17], high (>200 pmol per mg protein) relative to values for the midbrain and retina (~7 pmol per mg protein). Measurements of samples from the weaver mice confirmed that there are reduced levels of dopamine in the striatum of this mutant[10] and further demonstrated that the defect is neither shared by all dopaminergic neurons in the central nervous system nor equivalent in all regions that are affected. First, the dopamine content of the weaver retinas was indistinguishable from that of control retinas, suggesting that the dopamine-containing amacrine cells are normal in these mutants. Second, there was a remarkable non-uniformity in the depletion of dopamine in the weaver striatum, with by far the largest reduction in dopamine levels appearing in the dorsal striatum.

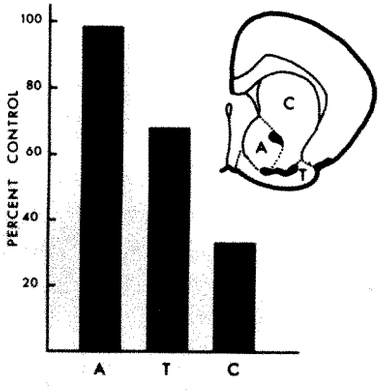


Fig. 1 The bar graph summarizes measurements, expressed as % of littermate control values, of the concentrations of endogenous dopamine extracted from the nucleus accumbens (A), olfactory tubercle (T) and caudoputamen (C) of weaver mice. Thirty-four mice (17 weaver, 17 control) were used. All brain samples were obtained from mice anaesthetized with sodium pentobarbital (Nembutal, 40 mg per kg) and perfused through the heart with chilled phosphate-buffered saline[13]. The cold-hardened brains were removed and placed on the stage of a tissue sectioner, were covered with agar and were cut into a series of 420-μm-thick transverse slices. The face of each slice was viewed through a dissecting microscope with indirect illumination from below so that nuclei and fibre tracts could be distinguished by their differential translucency. Whole-region dissections of the caudoputamen, nucleus accumbens and olfactory tubercle were made by removing the structure from each level at which it appeared with fine cold blades. The inset to the right of the bar graph shows the borders followed in removing the three striatal districts from a representative slice. Samples containing the caudoputamen, nucleus accumbens and olfactory tubercle were homogenized in 0.1 M perchloric acid. After centrifugation, the dopamine in the supernatant was isolated on alumina mini-columns, separated by high performance liquid chromatography and measured electrochemically[14]. The protein content of the precipitate was determined by the method of Lowry *et al.*[15]. Fourteen of the weaver-littermate pairs were between 1 and 4 months of age. Three pairs were 6–9 months old. As no differences between the young and older animals were found, values for all ages are shown together. See Table 1 for values expressed as pmol dopamine per mg protein.

Figure 1 summarizes the differential loss of dopamine in the striatum and shows an example of the boundaries followed in separating the three striatal subdivisions studied. The most striking contrast was between the concentrations of dopamine in the caudoputamen and in the nucleus accumbens: there was a 67% loss of dopamine in the weaver caudoputamen relative to control values, but virtually complete sparing of dopamine in the weaver nucleus accumbens. The olfactory tubercle showed an intermediate effect (32% loss). This differential pattern of biochemical abnormality was already established in the 1 month-old weavers and appeared unchanged in the oldest animals studied (9 months). As shown in Table 1, the dopamine concentration in the weaver midbrain was also abnormal, being reduced 31% relative to the control values at all ages sampled in the 1 to 9 month interval.

Measurements of protein made in weaver and in control mice (Table 1) point to a slight but consistent atrophy of the weaver caudoputamen, where protein content was reduced by 15%. By contrast, the protein concentrations measured for the nucleus accumbens and olfactory tubercle, and for the midbrain, were normal, indicating that the volumes of these regions were unaffected in the mutants. These results suggest that the striatal abnormality in the weaver mouse includes a component of tissue loss and that this, like the defect in dopamine, is expressed most strongly in the dorsal striatum.

The neurotransmitter defect in the weaver striatum was vividly demonstrated in the histofluorescence sections prepared

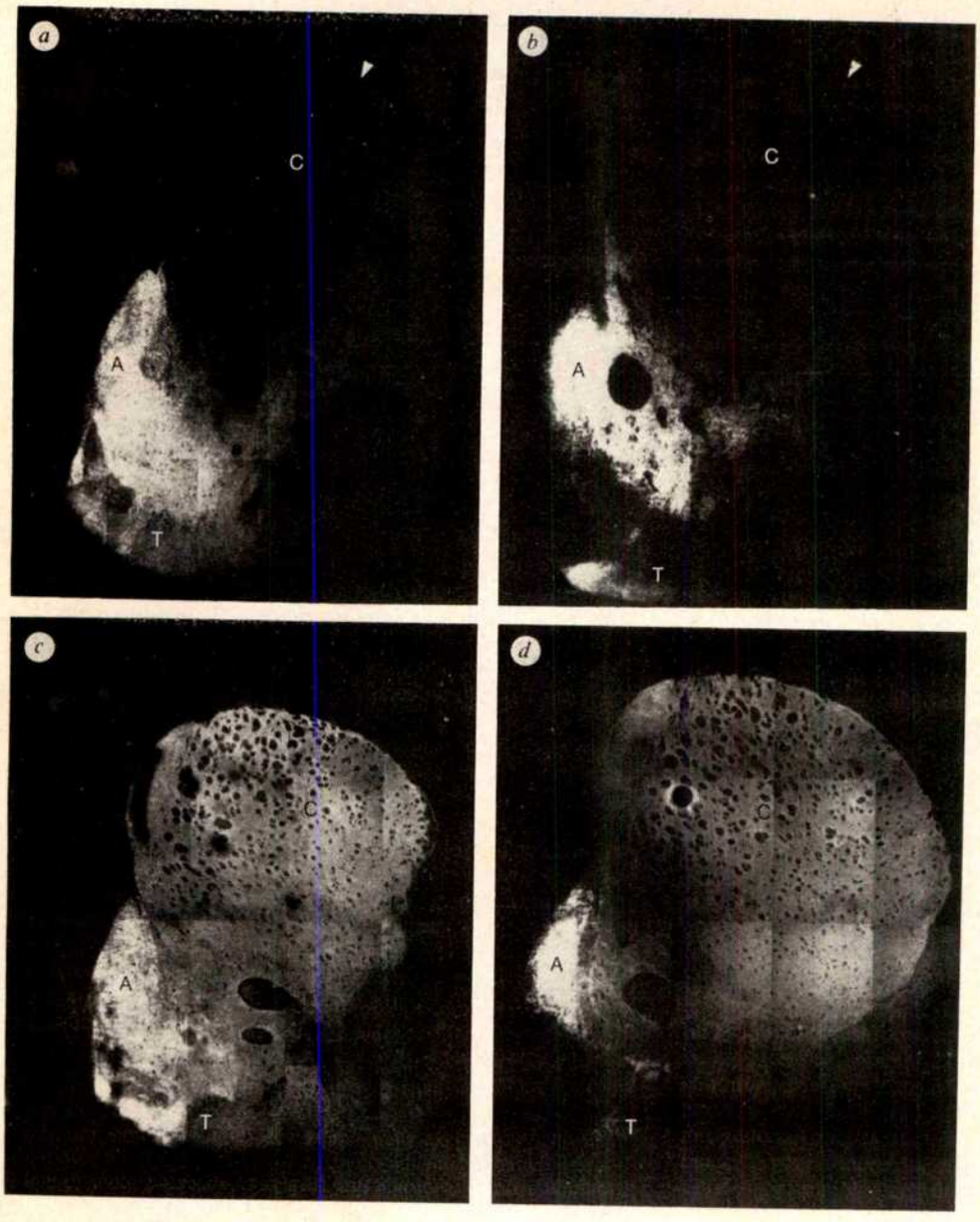


Fig. 2 Montage-photographs illustrating catecholamine-induced fluorescence (white) in the striatal complex of a 1-month-old homozygous weaver mutant mouse (*a*, *b*) and its heterozygous littermate control (*c*, *d*). Rostral cross-sections (A and C) are at midlevels of the nucleus accumbens (A). Caudal cross-sections (B and D) are near caudal tip of nucleus accumbens and olfactory tubercle (T). The sections from the control brain show the bright fluorescence distinguishing the entire striatal complex from surrounding tissue and the brilliant fluorescence in nucleus accumbens and medial olfactory tubercle. By contrast, in the sections from the weaver mutant, fluorescence is very weak in the caudoputamen (C) but appears normal in the nucleus accumbens and probably also the medial part of the olfactory tubercle. Note residual fluorescence in most ventral caudoputamen, which is considered together with the nucleus accumbens and olfactory tubercle as part of the ventral striatum.

for the anatomical experiments. This is evident in the montage-photographs of Fig. 2, which illustrate transverse sections through two levels of the striatum in a 1 month-old weaver mouse and in its littermate control. In the control brain (Fig. 2*c* and *d*) the catecholamine-induced fluorescence is brilliant in the nucleus accumbens and medial olfactory tubercle and bright throughout the rest of the striatum. As a result, the non-dopaminergic fibre bundles piercing the striatal tissue stand out as dark against the neuropil. The intensity of fluorescence varies somewhat within the caudoputamen and markedly within the nucleus accumbens and olfactory tubercle. Some of this heterogeneity almost certainly reflects local differences in innervation density or type, especially in the accumbens and olfactory tubercle. The details of the patterning varied from section to section.

In the weaver brain (Fig. 2*a*, *b*) the entire dorsal part of the caudoputamen is darkened. The fluorescence is not completely missing in this region, because there still is a discriminable difference in brightness between striatal and non-striatal tissue (see arrows). Even so, compared to that in the littermate control, the fluorescence clearly is very weak dorsally and hardly visible dorsolaterally. By contrast, in the most ventral part of the caudoputamen, below the depth-level of the anterior commissure, the intensity of fluorescence approaches control levels, and both the nucleus accumbens and the medial part of the olfactory tubercle show a brilliant apparently normal fluorescence. In the nucleus accumbens of the weaver, the degree of heterogeneity in the distribution of fluorescence resembles that in the control (contrast Fig. 2*a*, *c*). The relative brightness in at least the medial part of the olfactory tubercle also seems similar.

A similar loss of fluorescence in the caudoputamen and sparing of fluorescence in the nucleus accumbens and medial olfactory tubercle was observed in all the weaver brains examined and in each, a gradient of loss was evident in the caudoputamen, the least dimming of fluorescence being ventral. The patterns in the olfactory tubercle were more variable, for although the medial part of the tubercle always seemed to be spared, fluorescence laterally was more difficult to see in some weaver brains than in others. Variability may partly have been associated with edge artefacts produced at the time of sectioning.

To a remarkable degree, the topography of dopamine loss in the weaver striatum respects the subdivisions of the striatum drawn on the basis of its nigrostriatal and mesolimbic inputs and represented in the terms dorsal (non-limbic) and ventral (limbic) striatum. It is particularly striking that in the weaver mouse, the region of remaining fluorescence exceeds the nucleus accumbens and medial olfactory tubercle to include the ventral part of the caudoputamen, just as the mesolimbic system (as

Table 1 Content of dopamine and protein in three striatal regions, midbrain and retina of weaver and littermate control mice

Region	Genotype	Dopamine (pmol per mg protein)	Dopamine % Control	Protein (mg)	Protein % Control
Striatum					
Caudoputamen	*+/wv*	272 ± 18 (21)		1.63 ± 0.02	
	wv/wv	89 ± 4 (23)	33*	1.39 ± 0.03	85*
N. accumbens	*+/wv*	242 ± 13 (26)		0.104 ± 0.005	
	wv/wv	238 ± 11 (25)	98	0.112 ± 0.006	108
Olfactory tubercle	*+/wv*	228 ± 10 (6)		0.162 ± 0.006	
	wv/wv	156 ± 10 (8)	68*	0.155 ± 0.008	96
Midbrain	*+/wv*	7.3 ± 0.4 (11)		5.36 ± 0.34	
	wv/wv	5.1 ± 0.3 (10)	69*	5.00 ± 0.26	93
Retina	*+/wv*	7.0 ± 0.63 (11)		0.328 ± 0.009	
	wv/wv	7.9 ± 0.53 (10)	113	0.343 ± 0.013	105

Extractions and measurements of dopamine and protein were made following whole-region dissections of striatum and midbrain as described for Fig. 1. The retina was dissected with the aid of a microscope after removing the eye and bissecting the retina with sharp blades inserted into the optic stalk. A total of 17 control and 17 weaver mice were used but not all regions were examined in each animal. For the three divisions of striatum, dopamine content was measured separately in the right and left hemispheres. There were no left-right differences, thus dopamine and protein values from right and left are combined in the table. Animals ranged in age from 1 to 9 months with most being under 4 months of age. All regions were examined in both young and old animals. No differences were detected between younger and older animals, and thus data from all animals were pooled. Values shown are mean ± standard error of the mean. The numbers in parentheses represent numbers of samples. Statistical comparisons were conducted with Student's two tailed t-test. $^*P < 0.001$.

charted in the rat) projects to the ventral caudoputamen in addition to the accumbens-tubercle proper[18–21]. Although a detailed mapping is still needed, we conclude that the weaver disease affects dopamine levels mostly in the dorsal striatum and spares dopamine mostly in the ventral striatum. This suggestion is strengthened by our preliminary observations on the distribution of tyrosine hydroxylase-like immunoreactivity in the weavers, which also shows a sharp depletion dorsally (manuscript in preparation).

These findings strongly suggest that the nigrostriatal and mesolimbic dopamine systems are differentially vulnerable in genetic disorders. Just how the weaver mutation affects the dopamine-containing neurons in the midbrain, however, is not yet clear. In the cerebellum, the extensive death of granule cells in the early postnatal period is thought to result from a failure of granule cells to migrate from their proliferative zone[22]. Most evidence shows the migratory defect to be a consequence of a direct action of the weaver gene on the granule cells[23–26] but the possibility of a direct effect on the Bergmann glial guide fibres has not been excluded[24,25]. Schmidt *et al.*[10] have suggested that the dopamine defect in weaver is also developmental because striatal dopamine levels are nearly normal in neonatal weavers but fail to increase with age. These authors describe cell loss in the adult weaver substantia nigra pars compacta, but report normal concentrations of dopamine in the brainstem. Our own work shows that the concentration of dopamine in the weaver midbrain is decreased by about one-third (31%) relative to control values (Table 1). In the anatomical material, fluorescing neurones can be detected in each of the midbrain dopaminergic cell groups, including the pars compacta of the substantia nigra, but results from a quantitative study, now under way, will be needed to determine whether a particular subpopulation of dopaminergic neurones is missing in the weaver. Given the pattern of striatal loss, an abnormality of neurones in the substantia nigra's pars compacta would clearly be most likely.

Plausible alternatives to cell loss include not only metabolic defects but also abnormality of certain axon-collateral systems, as has been suggested for the mutant mouse tottering[27]. Any of these factors could reflect a primary action of the weaver gene on midbrain neurons and glia (for example, on cells migrating at a particular time) or could represent effects secondary to changes in the forebrain (for example, in response to specific defects in target cells or receptors). The possibility of a differential loss of dopamine receptors in the dorsal striatum deserves special emphasis because Boehme and Ciaranello[28] have shown that strain differences exist in striatal dopamine receptor binding in inbred mice, and that there is independent genetic control of receptors in the olfactory tubercle and more dorsal striatum. A study of the distribution of dopamine receptors in weaver is clearly needed. Strain differences in the numbers of dopamine-containing neurones in the midbrain and in corresponding differences in midbrain tyrosine hydroxylase activity[29] have also been reported, but apparently the effects are similar in the substantia nigra and ventral tegmental area[30].

A general implication of the present findings is that genetic control of dopaminergic systems in the forebrain may be exerted separately on subunits defined in functional[31], pharmacological[32], and anatomical[1] terms. Of the parts of the dopamine-recipient forebrain so far analysed in weaver, the nucleus accumbens is singled out as being fully spared a dopamine deficit, the dorsolateral caudoputamen as being most severely affected. No hereditary disease in the human has yet been shown to affect different parts of the striatal dopamine mechanism with such selectivity. The findings in weaver point to the potential for such dissociations, however, and this is of interest given the suspected differential contributions of dopaminergic fibres projecting to the dorsal and ventral striatum in modulating the organization of movement patterns and their motivational contexts[31]. In this regard it is striking that in Huntington's disease, an autosomal dominant disorder in which the striatum atrophies, neurones in the nucleus accumbens are spared relative to those in the caudoputamen[33] just as the content of dopamine and of protein in the weaver nucleus accumbens is spared relative to that in the weaver caudoputamen. The weaver disorder might thus hold clues to general patterns of tissue and neurochemical loss produced in the striatum in inherited extrapyramidal diseases.

We thank Dr William Shucart and the Department of Neurosurgery at Tufts New England Medical Center and the Wills Foundation for support to initiate this study. We are grateful for support from the Lena Marcus Fund, the Edith C. Blum Foundation, NIH 2S07-RR05598 and NIH EYO 2866 and thank Ms Dianne Sahagian and Mr Alfred Giuffrida for technical support and Mr Henry Hall for photography.

Received 25 July; accepted 28 September 1983.

1. Ungerstedt, U., *Acta physiol. scand.* **82,** Suppl. 367, 1–48 (1971).
2. Thierry, A. M., Blanc, G., Sobel, A., Stinus, L. & Glowinski, J. *Science* **182,** 499–501 (1973).
3. Lindvall, O., Björklund, A., Moore, R. Y. & Stenevi, U. *Brain Res.* **81,** 325–331 (1974).
4. Hornykiewicz, O. *Wiener Klinische Wochenschrift* **75,** 309–312 (1963).
5. Stevens, J. R. *Arch. Gen. Psychiatry* **29,** 177–189 (1973).
6. Iversen, L. L. *Science* **199,** 1084–1089 (1975).
7. Mackay, A. V. P., Iversen, L. L., Rossor, M., Spokes, E., Bird, E., Arregui, A., Creese, I. & Snyder, S. H. *Arch. Gen. Psychiatry* **39,** 991–997 (1982).
8. Sidman, R. L. in *Physiological and Biochemical Aspects of Nervous Integration* (ed. Carlson, F. D.) 164–193 (Prentice-Hall, Inc., Englewood Cliffs, N.J., 1968).
9. Lane, J. D., Nadi, N. S., McBride, W. J., Aprison, M. H. & Kusano, K. *J. Neurochem.* **29,** 349–350 (1977).

10. Schmidt, M. J., Sawyer, B. D., Perry, K. W., Fuller, R. W., Foreman, M. M. & Ghetti, B. *J. Neurosci.* **2**, 376–380 (1982).
11. Rakic, P. & Sidman, R. L. *J. of Comp. Neurol.* **152**, 103–132 (1973).
12. Roffler-Tarlov, S. & Turey, M. *Brain Res.* **247**, 65–73 (1982).
13. Zigmond, R. & Ben-Ari, Y. *J. Neurochem.* **26**, 1285–1287 (1976).
14. Moyer, T. P. & Jiang, N.-S. *J. Chromatography* **153**, 365–372 (1978).
15. Lowry, O. H., Rosebrough, N. I., Farr, A. L. & Randall, R. J. *J. Biol. Chem.* **193**, 265–275 (1951).
16. de la Torre, J. C. *J. Neurosci. Methods* **3**, 1–5 (1980).
17. Versteeg, D. H. G., Van der Gugten, De Jong, W. & Palkovits, M. *Brain Res.* **113**, 563–574 (1976).
18. Fallon, J. H. & Moore, R. Y. *J. Comp. Neurol.* **180**, 545–580 (1978).
19. Kelley, A. E., Domesick, V. B. & Nauta, W. J. H. *Neuroscience* **7**, 615–630 (1982).
20. Heimer, L. & Wilson, R. D. in *Golgi Centennial Symposium* (ed. Santini, M.) 177–193 (Raven Press, New York, 1975).
21. Beckstead, R. M., Domesick, V. B. & Nauta, W. J. H. *Brain Res.* **175**, 191–217 (1979).
22. Rezai, Z. & Yoon, C. H. *Develop. Biol.* **29**, 17–26 (1972).
23. Sotelo, C. & Changeaux, J.-P. *Brain Res.* **77**, 484–491 (1974).
24. Goldowitz, D. & Mullen, R. J. *J. Neurosci.* **2**, 1474–1485 (1982).
25. Hatten, M. E., Liem, R. K. H., Shelanski, M. L. & Mason, C. A. *Society for Neuroscience* Abstract, 63.21 (1982).
26. Willinger, M., Margolis, D. M. & Sidman, R. L. *J. Supramol. Struc. and Cell. Biochem.* **17**, 79–86 (1981).
27. Levitt, P. & Noebels, J. L. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4630–4634 (1981).
28. Boehme, R. E. & Ciaranello, R. D. *Brain Res.* **266**, 51–65 (1983).
29. Ross, R. A., Judd, A. B., Pickel, V. M., Joh, T. M. & Reis, D. J. *Nature* **264**, 654–656 (1976).
30. Baker, H., Joh, T. H. & Reis, D. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4369–4373 (1980).
31. Iversen, S. D. & Fray, P. J. in *The Neural Basis of Behavior* 229–269 (Spectrum Publications, Inc., 1982).
32. Fuxe, K., Fredholm, B. B., Agnati, L. F. & Corrodi, H. *Brain Res.* **146**, 295–311 (1978).
33. von Sattel, J.-P., Ferrante, R. J. & Richardson, E. P. *J. Neuropath. and Exp. Neurol.*, submitted.
34. Graybiel, A. M. & Ragsdale, C. W. in *Development and Chemical Specificity of Neurons* (eds Cuénod, M., Kreutzberg, G. W. & Bloom, F. E.) 239–283 (Elsevier, Amsterdam, 1979).

Adrenal chromaffin cells form functional cholinergic synapses in culture

M. Ogawa*, T. Ishikawa† & A. Irimajiri

Department of Physiology and †Department of Anatomy, Kochi Medical School, Nankoku, Kochi 781-51, Japan

Adrenomedullary cells and autonomic ganglion cells originate from the neural crest. Both cell types synthesize, store and release catecholamines; however, their structural and functional properties are distinctly different. Aloe and Levi-Montalcini[1] have shown *in vivo* that when the adrenal medulla is exposed to exogenous nerve growth factor (NGF) most cells differentiate into neuronal cells substantially similar to sympathetic neurones. Experiments *in vitro*[2–7] have also shown that neonatal as well as adult adrenal chromaffin cells and their neoplastic correlate (PC12 cells) undergo neurone-like morphologic differentiation in response to NGF. From these morphological and biochemical studies alone, however, it remains uncertain whether the functional neuronal transformation is also accompanied. We report here that the adrenal chromaffin cells in culture can differentiate into neuronal cells having functional synapses which were found to be cholinergic in nature. Furthermore, the type of synaptic vesicles in the newly formed synapses was apparently dependent upon K^+ levels in the culture medium.

Adrenal glands were removed aseptically from 8- to 10-day-old Wistar strain rats of either sex. Medullae were carefully freed from capsular and cortical tissue by using a pair of knives and/or fine tungsten needles under a dissecting microscope, and then incubated with dispase (1,000 U per ml culture medium; Godo Shusei) for 1 h at 36 °C. After washing by centrifugation and resuspension, the cells were seeded onto 35 mm Falcon dishes containing a control culture medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum). Cultures were maintained at 37 °C in a water-saturated atmosphere of 95% air and 5% CO_2; the medium was changed every 2 to 3 days. A fraction of 2.5S NGF, prepared from the submandibular glands of adult male mice[8], was added at a concentration of 10–100 ng per ml of medium throughout the culture period. Glass micropipettes filled with 4 M potassium acetate (electrode resistance, 20–60 MΩ) were used for both recording membrane potential and passing current by means of a high input-impedance amplifier with the bridge circuit.

As regards purity of the cell populations thus prepared, an exhaustive, phase-microscopic surveillance was routinely made at least twice (on days 3 and 6 of culture) for every preparation of medullary cells; this was possible because of the relatively low density of the cells plated ($1–3\times10^3$ cells per dish). The surveillance revealed that the maximum contamination by non-chromaffin, polygonal, neuronal cells having large nuclei with conspicuous nucleoli was 0.3% (at most 3 neuronal cells per dish), a result in good agreement with Unsicker *et al.*[2], who reported a value of 0.25% (~50/20,000) for the neuronal contamination. This value, together with histochemical evidence (Fig. 1*B*, *C*), ensures the present study from the possible risk of selectively impaling the originally neuronal cells. This latter risk could also be evaded by an omission of NGF from the early stage of cell culture because under that condition neuronal contaminants, if any, would not have survived[9]. The rat adrenal chromaffin cells, as shown with human chromaffin cells[4], survived more than 20 days in culture without NGF. Cultures to which NGF was added during the second week contained approximately the same number and percentage of process-forming cells as cultures in which NGF was added at the beginning of the culture period. The only difference was that cholinergic synaptic interactions were first detected around 20 days (see below).

Shortly after the individual or small groups of cells had settled down onto the dish surface, the initially dispersed chromaffin cells showed a tendency to adhere to each other to form numerous large cell clusters, and fine unmyelinated processes concomitantly began to grow out from the cell soma. Such processes continued to grow and the final dense networks of neurites were usually formed after 2 weeks in culture (Fig. 1*A*). Fluorescence cytochemistry similar to that used by Unsicker *et al.*[2,3], revealed that almost all of the clustered cells at this stage still retained the chromaffin nature as judged from their strong catecholamine fluorescence (Fig. 1*B*, *C*). Around the end of 2 weeks or later, examination under the electron microscope (Fig. 1*D*) revealed the formation of some synaptic structures or varicosities that contained abundant clustered small clear vesicles (40–50 nm in diameter) and a few large dense-cored vesicles (~90 nm). The former vesicles are similar in size to vesicles usually observed in the synapses of cultured sympathetic neurones[10,11]. Note also the occurrence of intermediate organelles in cells at this time (Fig. 1*E*). There are now pleomorphic dense-cored vesicles. These may reflect the transition from a typical chromaffin granule inclusion to the neuronal synaptic organelles, including round electron-lucent and dense-cored vesicles.

Synaptic interaction between the chromaffin cells was tested by impaling, with microelectrodes under visual control, pairs of cells randomly selected from two neighbouring clusters. As shown in Fig. 2*A*, intracellular stimulation of cell 2 (c2) elicited an excitatory postsynaptic potential (e.p.s.p.) in cell 1 (c1) with a delay of 3.7 ms, which, in turn, occasionally triggered an action potential. When examined in a pair of nearest clusters of 4 weeks culture, such evoked e.p.s.ps were recorded from 36 pairs of cells out of 50, including 17 pairs which exhibited 'reciprocal' synaptic interaction. This observation also supports our contention that the events described here are associated with the chromaffin cells but not with the neuronal contaminants. The following observations indicate that the synaptic transmission involved was chemically mediated: (1) a delay of a few milliseconds between firing in the presynaptic cell and depolarization in the postsynaptic cell, (2) no passive transfer of hyperpolarizing

*Present address: Physiology Section, The Biological Sciences Group, The University of Connecticut, Storrs, Connecticut 06268, USA.

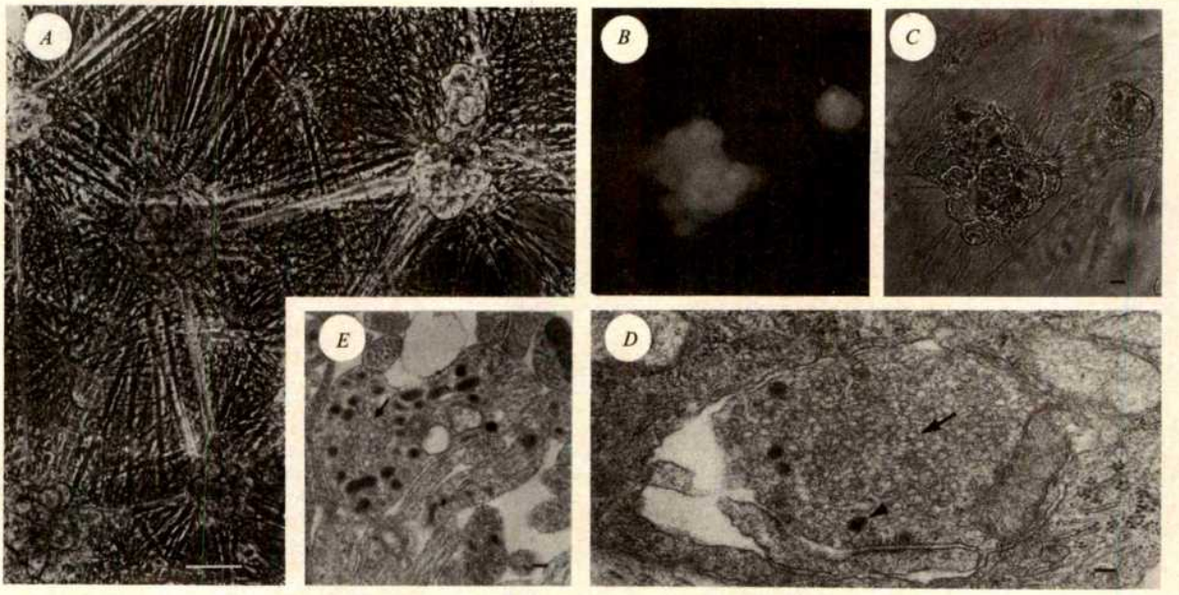


Fig. 1 ***A***, Phase-contrast micrograph of chromaffin cells cultured with NGF for 30 days. The cells, immediately after dissociation, assumed a round shape with no stumps of processes. In 1 week of culture, many of the isolated chromaffin cells tended to gather into numerous clusters, from which fine fibrous processes extended. In 2 to 3 weeks, contaminating fibroblasts increased to confluency, while the clustered chromaffin cells built up a complex network of straight fibres interconnecting the clusters. In this micrograph, fibroblastic proliferation was substantially suppressed by treatment with cytosine arabinoside (10 μM) over days 21 and 22 after plating. Scale bar, 100 μm. ***B***, Glyoxylic acid-induced catecholamine fluorescence[14] of the clustered cells after 2 weeks in culture. The same cells can be identified in *C*. In some cases catecholamine-specific fluorescence, though weaker than that of the cell soma, was also detected from neurite extensions (not shown). ***C***, Light micrograph of the field in *B*. Scale bar, 10 μm. ***D***, Electron micrograph of a synaptic structure formed between the cultured chromaffin cells 35 days after plating. Note the synaptic membrane thickening of increased density and the accumulation of small clear vesicles (arrow); a few large dense-core vesicles having an electron-lucent halo around the core (arrowhead) are also present. The specimen was fixed in phosphate buffered 2% glutaraldehyde (*p*H 7.2) followed by buffered 1% osmium tetroxide, dehydrated through graded ethanol, and then embedded in Spurr's epoxy resin[15]. Scale bar, 100 nm. ***E***, Electron micrograph of an intermediate cell type of chromaffin cells cultured for 2 weeks. Small clear vesicles (arrow) and pleomorphic dense-cored vesicles are present. Preparation method similar to *D*. Scale bar, 100 nm.

or subthreshold depolarizing pulses between the test cells, (3) the amplitude of e.p.s.ps varied from one presynaptic stimulus to another, (4) the amplitude of e.p.s.ps was sensitive to the level of postsynaptic membrane potential, increasing with hyperpolarization, and (5) the amplitude of e.p.s.ps increased with an increase in the extracellular Ca^{2+} concentration.

In an attempt to identify the transmitter type responsible for the chemical transmission, we applied several cholinergic and adrenergic blocking agents over the cells that demonstrated synaptic interaction. As shown in Fig. 2, the e.p.s.ps were reversibly affected by (+)tubocurarine (TC), a cholinergic blocker, and also by hexamethonium (data not shown). In contrast, none of these synaptic responses was affected by phentolamine, an α-adrenergic blocker, even at 0.1 mM. The chromaffin cells usually showed spontaneous synaptic potentials after 2 weeks in culture (Fig. 3). The cell illustrated in Fig. 3*A* exhibited spontaneous e.p.s.ps, summation of which gave rise to action potentials. The time course of these responses was similar to that observed in the evoked synaptic responses. These spontaneous e.p.s.ps were also abolished by the cholinergic blocking agent (Fig. 3*B*).

The above data collectively indicate that the adrenal chromaffin cells can develop several neuronal characteristics and form cholinergic synapses between them. This finding raises the possibility that the cultured chromaffin cells may also be plastic with respect to the transmitter choice as reported for the sympathetic ganglion cells, in which the culture environment is known to determine the neurotransmitter selection (see ref. 12 for review). In fact, it has been shown that elevated K^+ concentrations in culture medium prevent the conversion of the superior cervical ganglion cells from an adrenergic to a cholinergic phenotype[13]. When we cultured the chromaffin cells in an NGF-containing high K^+ medium, which was made by diluting Dulbecco's minimal essential medium (DMEM) with 0.2 M KCl

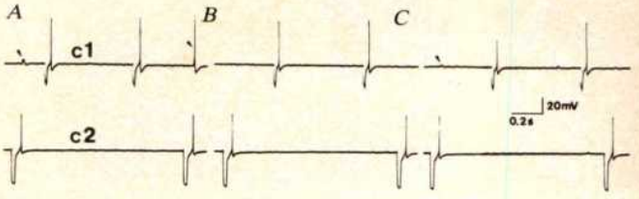


Fig. 2 Intracellular recording of membrane potentials and e.p.s.ps from the chromaffin cells demonstrating chemical synaptic transmission after 32 days culture. Test cells 1 and 2 (c1 and c2), separately chosen from a pair of neighbouring clusters, were stimulated asynchronously by anodal break excitation. Traces *A*, *B* and *C* are all from the same paired cells. For electrophysiological experiments, the culture dish was placed on an inverted phase-microscope stage thermostated at 33–35 °C by circulating water. The test dish was perfused, at about 25 ml h^{-1}, with a prewarmed control solution of Eagle's medium containing 5 mM HEPES (*p*H 7.4). *A*, Anode-break excitation in c2 evoked sub- or suprathreshold e.p.s.p in c1 (arrowheads) with a delay of 3.7 ms; however, firings of c1 had no such effect on c2, indicating that a synapse mechanism was involved. *B*, Perfusion with TC (25 μM) abolished the synaptic response. No change was observed in the anode-break action potentials or in the resting levels of both cells. *C*, After 10 min perfusion with the control solution the cells resumed synaptic activity.

to yield ~20 mM K^+, the cells showed a progressive elongation of processes and formed dense neurite networks such as shown in Fig. 1*A*. Spontaneous or evoked synaptic activity, however, was detected in none of more than 100 cells examined in over 25 culture sets of the high K^+ medium (Fig. 3*C*). On the other hand, the resting potentials of these cells were in the normal range, and the cells were able to produce action potentials

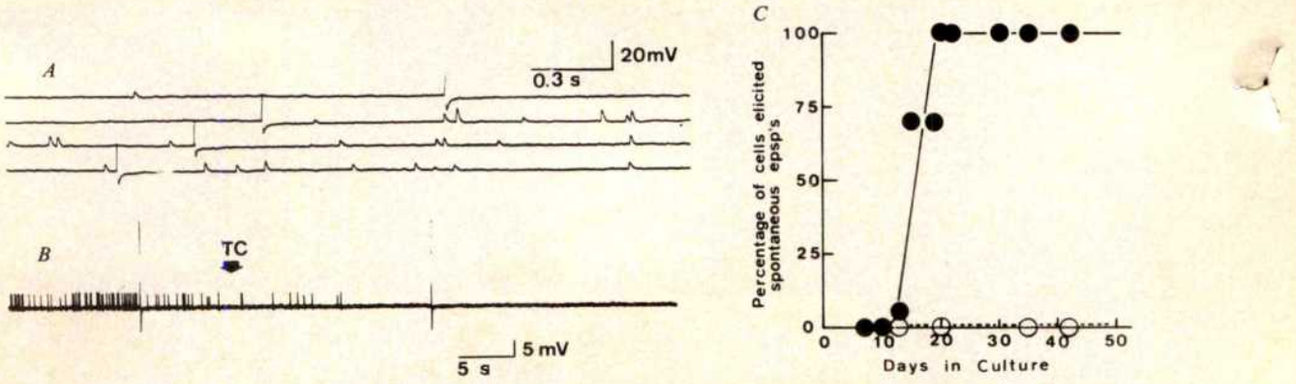
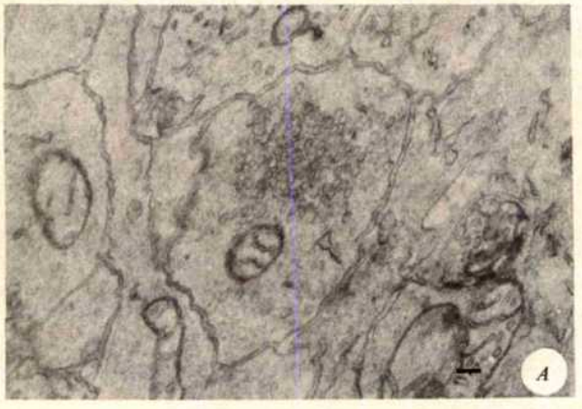


Fig. 3 *A*, Spontaneous synaptic activity recorded from a chromaffin cell cultured for 35 days. All traces are from one continuous record. *B*, Spontaneous e.p.s.ps were also sensitive to TC (25 μM). *C*, Development of spontaneous e.p.s.ps from cells cultured in normal K^+ medium (●) and no such occurrence of e.p.s.ps in high K^+ cultures (○). In each case, 20 cells randomly sampled from 10 different clusters were impaled by a microelectrode with its tip held inside the respective test cells for at least 5 min. Each point represents the percentage of successful detection of spontaneous e.p.s.ps. Test medium was Eagle's minimal essential medium containing 5.3 mM K^+. Test cells were all from the same preparation.

Sister cultures with the normal K^+ medium (DMEM diluted with 0.2 M NaCl instead of 0.2 M KCl) showed the same pattern of synaptic activities as in the control culture. The loss of synaptic activity in the cells cultured in the high K^+ medium is not due to the absence of sensitivity to acetylcholine (ACh), since the responses to ACh iontophoresis (as high as 400 mV nC^{-1}) were indistinguishable from those observed in the control cells. The chromaffin cells, cultured in the high or the normal K^+ medium, were not responsive to adrenaline and noradrenaline.

To determine whether the high K^+ condition modulates the transmitter choice towards the enhanced adrenergic selection, the population of synaptic vesicles was examined ultrastructurally using the $KMnO_4$-fixation procedure, a well known technique to identify the adrenergic vesicles[10,11]. As shown in Fig. 4*B*, most of the synaptic vesicles found in the high K^+ culture were granular, a fact indicative of adrenergic vesicles. In the normal K^+ cultures, however, synapses and varicosities contained over 60% clear vesicles (Fig. 4*A*). Such an observation implies that, in the high K^+ medium, the chromaffin cells may form predominantly adrenergic synapses, and yet the adrenergic synaptic activity failed to be detected electrophysiologically because of a lack of postsynaptic receptors for catecholamines.

Finally, our observation that the adrenal chromaffin cells from neonatal rats can be transformed into neuronal cells equipped with the functional synapses of cholinergic or adrenergic nature depending on the culture condition, supports the view that the precursors of the adrenal and the sympathetic neuronal cells are pluripotential at a certain stage in development and then differentiate into their respective phenotypes under the control of some environmental factors.



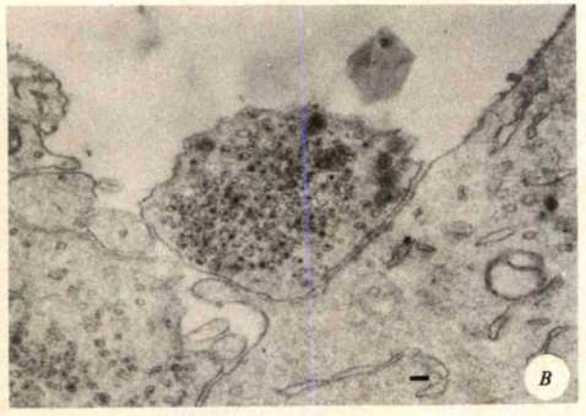


Fig. 4 Electron micrographs ($KMnO_4$ fixation) of synaptic vesicles contained in the chromaffin cells that were cultured with NGF for 30 days in: *A*, normal K^+ medium, and *B*, high K^+ medium. The cells were preincubated for 30 min at 37 °C with 10^{-5} M noradrenaline freshly dissolved in DMEM, washed in Ringer solution, and then fixed in $KMnO_4$ (3%, in Ringer) at 4 °C for 1 h. After washing the cells were stained *en bloc* by uranyl acetate (1%, in 0.1 M nabic acid buffer, *p*H 5.2) at 4 °C for 1–2 h. Dehydration and embedding were processed as in legend to Fig. 1*D*. Scale bar 100 nm.

We thank Professors M. Kuno, G. Pilar and J. Tuttle for helpful discussion and critical reading of the manuscript and Professor A. Inouye for his continual encouragement. This work was supported, in part, by grants 56570032 and 57213028 to M.O. from the Ministry of Education, Science and Culture of Japan.

Received 18 October 1982; accepted 28 September 1983.

1. Aloe, L. & Levi-Montalcini, R. *Proc. natn. Acad. Sci. U.S.A.* **76,** 1246–1250 (1979).
2. Unsicker, K., Krisch, B., Otten, U. & Thoenen, H. *Proc. natn. Acad. Sci. U.S.A.* **75,** 3498–3502 (1978).
3. Unsicker, K. & Chamley, J. H. *Cell Tissue Res.* **177,** 247–268 (1976).
4. Tischler, A. S., De Lellis, R. A., Biales, B., Nunnemacher, G., Garraba, V. & Wolfe, H. J. *Lab. Invest.* **43,** 399–409 (1980).
5. Naujoks, K. W., Korshing, S., Rohrer, H. & Thoenen, H. *Devl Biol.* **92,** 365–379 (1982).
6. Greene, L. A. & Tischler, A. S. *Proc. natn. Acad. Sci. U.S.A.* **73,** 2424–2428 (1977).
7. Doupe, A. J., Patterson, P. H. & Landis, S. C. *Soc. Neurosci. Abstr.* **8,** 257 (1982).
8. Bocchini, V. & Angeletti, P. V. *Proc. natn. Acad. Sci. U.S.A.* **64,** 787–794 (1969).
9. Johnson, M. I., Ross, C. D., Meyers, M., Spitznagel, E. L. & Bunge, R. P. *J. Cell Biol.* **84,** 680–691 (1980).
10. Landis, S. C. *Devl Biol.* **77,** 349–361 (1980).
11. Chun, L. L. Y. & Patterson, P. H. *J. Cell Biol.* **75,** 694–704 (1977).
12. Patterson, P. H. *A. Rev. Neurosci.* **1,** 1–17 (1978).
13. Walicke, P. A., Campenot, R. B. & Patterson, P. H. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5767–5771 (1977).
14. De la Torre, J. C. & Surgeon, J. W. *Histochemistry* **49,** 81–93 (1976).
15. Spurr, A. R. *J. ultrastruct. Res.* **26,** 31–43 (1969).

Slow transmission of neural activity in hippocampal area CA1 in absence of active chemical synapses

A. Konnerth, U. Heinemann & Y. Yaari*

Department of Neurophysiology, Max-Planck-Institut für Psychiatrie, Am Klopferspitz 18A, 8033 Planegg-Martinsried, FRG
* Department of Physiology, Hebrew University Medical School, PO Box 1172, Jerusalem 91010, Israel

Transmission of neural activity in the mammalian cortex involves the operation of chemical synapses[1,2]. Here we report that activity of hippocampal pyramidal cells (HPC) *in vitro* can spread through the neural aggregate even when chemical synaptic transmission is blocked by lowering the external Ca^{2+} concentration ($[Ca^{2+}]_o$). In these conditions focal stimulation of the HPC body layer (stratum pyramidale) at area CA1 evokes a localized repetitive neural response which, when large enough, spreads along this layer in both transverse directions. It was recently reported for similar conditions[3,4] that the activated HPC often discharge in synchrony. However, the spread of the neural response does not depend upon the occurrence of synchronized HPC activity, and therefore represents a distinct phenomenon.

Several lines of evidence suggest that K^+ accumulation in the extracellular space is responsible for the transmission of neural activity through area CA1 in conditions of blocked chemical synapses. (1) The mean velocity of spread was 1.7 mm s^{-1}, which probably is too slow to be mediated by electrical coupling or ephaptic interactions, but is compatible with K^+ redistribution by a spatial buffer mechanism[5]. (2) A rise in $[K^+]_o$ precedes and accompanies the spread of the neural response. (3) Focal applications of K^+ at the CA1 stratum pyramidale can elicit similar neural responses. (4) The spread is accelerated or slowed by increases or decreases in the baseline level of $[K^+]_o$, respectively. Such K^+-mediated transmission of neural activity may have an important role in the spread of epileptiform discharges, which does not necessarily conform to anatomical pathways[6], and which involves synchronous neural firing[7] and large decreases and increases in $[Ca^{2+}]_o$ and $[K^+]_o$[8,9], respectively.

Experiments were performed on 44 rat hippocampal slices, prepared and maintained in a perfused chamber using previously described techniques[10,11]. Changes in $[Ca^{2+}]_o$ and $[K^+]_o$ were monitored with ion-selective microelectrodes (ISM)[8]. Field potentials were recorded with either the reference barrel of the ISM or with single NaCl-filled microelectrodes. Measurements were usually obtained simultaneously from 2–4 adjacent sites (0.02–1 mm apart) in the stratum pyramidale.

To block chemical synaptic transmission, Ca^{2+} was omitted from the physiological solution (in mM: 124 NaCl, 5 KCl, 1.25 $NaHPO_4$, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose) perfusing the slices. This resulted in a slow decrease of $[Ca^{2+}]_o$ to levels of $1–5 \times 10^{-5}$ M[11]. During the decay of $[Ca^{2+}]_o$, the synaptically evoked responses of CA1 HPC, induced by stimulating the commissural/Schaffer collateral afferent fibres in the stratum radiatum, initially became repetitive and then gradually diminished, until they were totally blocked[11,12]. In these conditions, however, single-shock stimuli applied at the stratum pyramidale (Fig. 1*A*) evoked prolonged (1–5 s), large (1–7 mV) negative potential shifts (NPS) (Fig. 1*B*), which were maximal in amplitude at the stratum pyramidale and smaller when recorded from the dendritic layers (strata radiatum and oriens). Recordings with K^+-selective ISM showed that each NPS is accompanied by a transient increase (1–7 mM above baseline levels) in $[K^+]_o$ (Fig. 2), which was also maximal at the stratum pyramidale. These findings suggest that the NPS reflect the depolarization of neurones and glia induced by K^+ released

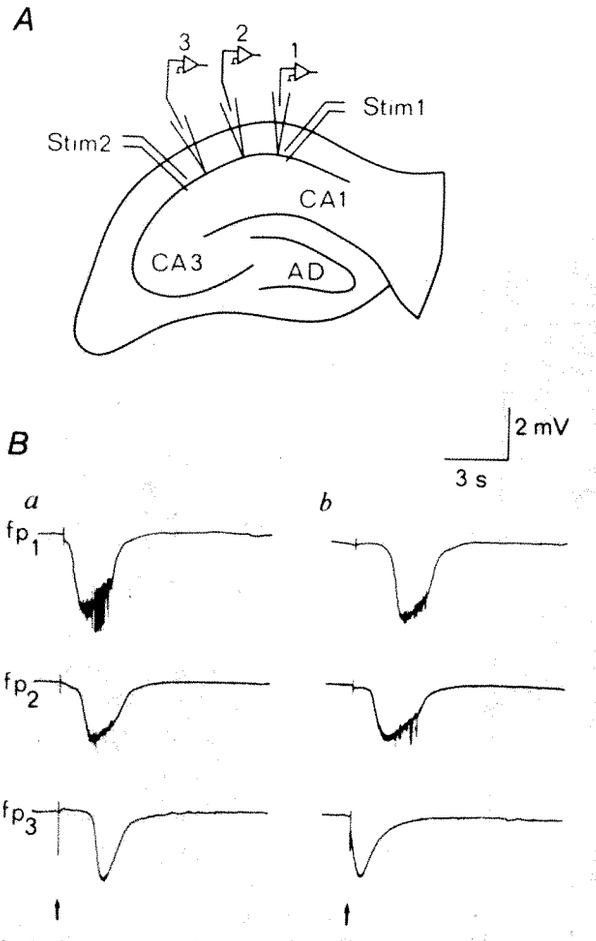


Fig. 1 Transmission of evoked neural activity along hippocampal area CA1 after blocking chemical synaptic transmission by lowering $[Ca^{2+}]_o$. ***A***, The experimental arrangement. Recording microelectrodes (1–3), filled with 100 mM NaCl, were positioned 200 μm apart at the CA1 stratum pyramidale. Stimulating electrodes (Stim1, Stim2) were placed 20–50 μm from the ends of the microelectrode array. AD, area dentata. ***B***, Simultaneous recordings by the three microelectrodes (1–3) of the field potentials (fp_1, fp_2 and fp_3, respectively), evoked in response to single-shock stimuli (70 μs, 40 nA) delivered by Stim1 (*a*) or Stim2 (*b*). Arrows indicate the stimulation artefacts. The responses, composed of a negative potential shift on top of which population spikes can be observed at two recording sites (fp1, fp2), appeared about 25 min after changing from the standard solution to perfusing solution lacking Ca^{2+}. The simultaneous recordings demonstrate an increase in latency of the NPS with the distance between the recording and stimulating sites, thus suggesting a spread of neural activity along the stratum pyramidale.

from activated neurones[3,11,13]. Indeed, extracellular single unit recordings revealed increased firing of HPC during the NPS. Furthermore, at approximately 60% of the slices, the evoked NPS were associated with bursts of population spikes (Fig. 1*B*, upper traces), indicating that during the NPS, the repetitive firing of individual HPC often became synchronized[3,4]. Similar responses were also observed in slices perfused with a 0.2 mM Ca^{2+}-containing solution, in which the synaptically evoked field potentials were also completely blocked. These responses were promptly abolished by reintroducing the standard perfusing solution, whereas the normal synaptically evoked field potentials reappeared. With prolonged (>1 h) perfusion in low-Ca^{2+} solutions, NPS also appeared spontaneously in most of the slices, as has been recently described[3,11].

Once initiated by lowering $[Ca^{2+}]_o$, the responses described above could be evoked by stimulation anywhere along the CA1 stratum pyramidale. When the recording microelectrode was placed near (20–50 μm) the site of stimulation, the response had a brief delay of several milliseconds. This delay increased

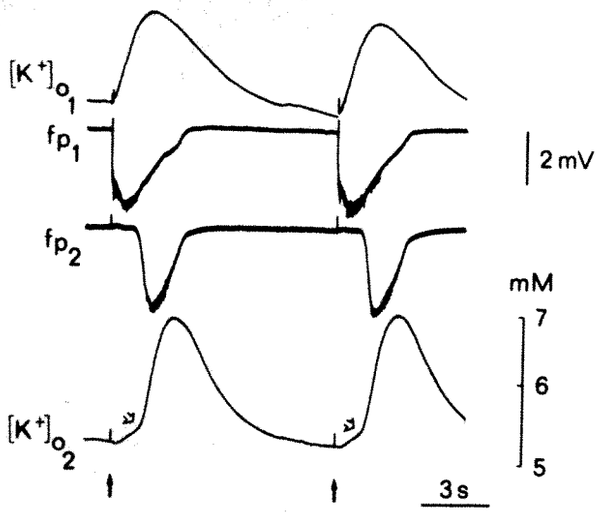


Fig. 2 Changes in $[K^+]_o$ which accompany the spread of neural activity along area CA1, in a slice with blocked chemical synaptic transmission. The experimental arrangement and procedure were basically as described in Fig. 1 legend, except that recording microelectrodes 1 and 2 are double-barrelled K^+-ISM[13], placed 300 μm apart. The K^+-selective barrel was filled with valynomycine exchanger and 100 mM KCl, and had a minimum response of 45 mV to a 10-fold change in $[K^+]_o$; the reference barrel contained 124 mM NaCl. The responses to two consecutive single-shock stimuli (filled arrows indicate stimulus artefacts) delivered by Stim1 are shown. Simultaneous recordings of the field potentials (fp_1, fp_2) and the changes in $[K^+]_o$ ($[K^+]_{o_1}$, $[K^+]_{o_2}$) by ISM 1 and 2, respectively, suggest a spread of neural activity along the stratum pyramidale away from the stimulation site. Note that ISM 1 monitors a monophasic K^+ transient, whereas ISM 2 shows a biphasic increase (transition between the two phases marked by open arrows).

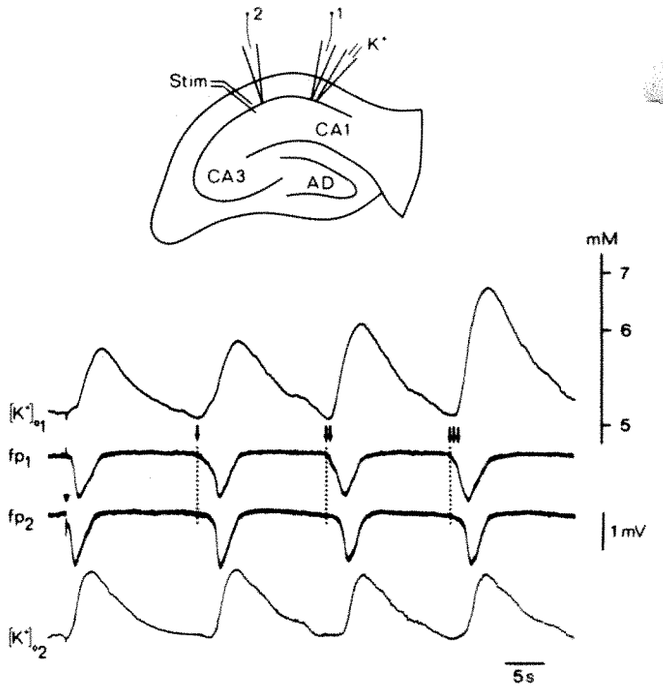


Fig. 3 Spreading NPS evoked at CA1 stratum pyramidale by direct electrical stimulation and K^+ pressure application, in a slice with blocked chemical synaptic transmission. Upper part illustrates experimental arrangement of stimulating electrode (Stim), recording K^+-ISM (1 and 2) and K^+ pressure ejection micropipette (K^+), which were all positioned in the CA1 stratum pyramidale. ISM 1 and 2 were 500 μm apart. The K^+ ejection micropipette, 50 μm from ISM 1, contained 100 mM KCl and had a tip diameter of 1 μm. KCl was applied by pressure pulses of 1 bar and 200 ms. Repetitive KCl pulses were delivered with an interpulse interval of 100 ms. The lower part demonstrates simultaneous recordings of the field potentials (fp_1, fp_2) and the changes in $[K^+]_o$ ($[K^+]_{o_1}$, $[K^+]_{o_2}$) in response to a single-shock stimulation (marked by triangle) and to 1, 2 and 3 pulses of KCl (marked with arrows and dotted lines). Note the spread of the response from its site of initiation towards the distant ISM, and the similarity between the NPS evoked by electrical stimulation and by K^+ application. Similar ejections of NaCl (from a micropipette filled with 100 mM NaCl) were ineffective in eliciting a response.

with the distance between the sites of stimulation and recording. With simultaneous recording from 2–4 separated sites along this layer, the response appeared as a wave of activity, which could be transmitted from the site of stimulation towards either end of area CA1 (Fig. 1*B*). The velocity of this spread varied in different slices from 0.44 to 4.2 mm s^{-1} (1.7 ± 1.21 mm s^{-1}, mean ± s.d., $n = 11$), but in any one slice was the same in both directions. When stimuli were delivered simultaneously at both ends of area CA1 no summation of the response occurred at a recording site in between. Furthermore, several seconds following stimulation at one end of area CA1, a stimulus delivered to the other end was ineffective in eliciting a spreading response. These findings suggest that regardless of its direction of spread, the same neural population participates in the response.

In the absence of normal chemical synaptic function other factors must mediate the spread of neural activity in area CA1. Possible mechanisms are electrical coupling[14,15], ephaptic (electrical field) effects[4,16] and accumulation of K^+ in the extracellular space[11,17,18]. Whether CA1 HPC are electrically coupled is still a matter of controversy[19–21], whereas evidence for the existence of ephaptic interactions has recently been reported[4]. Either of these mechanisms, however, is probably too fast to account simply for our findings. If activity spreads across the densely packed CA1 HPC population from cell to cell, each HPC body being about 20 μm in diameter[22], transmission across two adjacent HPC at a mean velocity of 1.7 mm s^{-1} would require ~24 ms. In contrast, where electrical coupling has been demonstrated in the hippocampus by paired intracellular recordings, transmission of an action potential across two coupled neurones occurs within 1–2 ms[14,15]. Ephaptic interactions among HPC also tend to synchronize their firing within a similarly brief period[4]. Therefore, cell-to-cell transmission of activity via these mechanisms would be expected to spread at a velocity of 20–40 mm s^{-1}, which is at least one order of magnitude higher than what we have observed. It is possible, however, that electrical coupling and ephaptic interactions influence the activated HPC to fire in synchrony, thereby giving rise to the frequently observed population spikes (Fig. 1*B*). These mechanisms have been suggested recently to account for the synchronization of CA1 HPC firing during short afterdischarges[4] or during spontaneous field bursts[3], which occur in solutions with low $[Ca^{2+}]$. Clearly, the fact that the NPS also spread in slices which did not exhibit synchronous population spikes, suggests that synchronization and spread are distinct phenomena, which may differ in their underlying mechanisms.

The observed slow spread of neural activity compares well with the time course of redistribution of $[K^+]_o$ transients through the glial 'spatial buffer' mechanism[5,23,24]. Therefore, an alternative possible mechanism is that K^+ ions, released by active neurones, spread in the extracellular space[13,18,23], and depolarize to threshold neighbouring and perhaps remote quiescent neurones. This action would result in a relatively slow progressive spread of the 'discharge zone'. It has been shown in different preparations that the release of K^+ ions from active neurones can alter the excitability of neighbouring cells[17,25,26], and the fact that HPC CA1 neurones are tightly packed[27] may allow for an effective 'K^+-coupling' mechanism. Indeed, in approximately 80% of our recordings, performed in 27 different slices, the $[K^+]_o$ increases associated with the spreading wave of activity, consisted of two distinct phases (Fig. 2). The first phase was slower and preceded the appearance of the NPS at the recording site; therefore, it may reflect the redistribution of K^+ from an adjacent active zone[24]. The second phase consisted of

a much steeper and larger increase in $[K^+]_0$, and was correlated in time with the appearance of the NPS and the population spikes at the recording site. It is probably due to the local release of K^+ from the bursting neurones nearby. Thus, when the recording ISM was close to the stimulating electrodes, only a monophasic steep increase in $[K^+]_0$ was observed (Fig. 2).

To test the 'K^+-coupling' hypothesis we examined whether similar spreading responses can be elicited by the local release of exogenous K^+. In accordance with this hypothesis, we found that pressure ejection of KCl, but not NaCl, through the tip of a micropipette at the CA1 stratum pyramidale, could elicit typical NPS, similar to the responses evoked by direct electrical stimulation (Fig. 3). These responses also spread from their site of initiation through area CA1 (Fig. 3). Increases in the amount of locally applied K^+ hastened the appearance of the NPS, but did not significantly alter their amplitude (Fig. 3), indicating their all-or-none nature. Accordingly, reducing below a critical level the amount of applied K^+, produced only a local response which did not develop into a full NPS, and did not spread to the remote recording ISM (not shown). As would be predicted from a 'K^+-coupling' mechanism, we also found that elevating the $[K^+]$ in the perfusing solution by 1–2 mM, markedly accelerated the spread of the NPS, whereas lowering it by 1–2 mM had the opposite effect.

In sharp contrast to the responsiveness of CA1 HPC, we were consistently unable to evoke NPS and population spikes by focal stimulation at area CA3 in similar conditions. Furthermore, neural responses evoked at area CA1 always spread towards area CA3, but failed to invade it. A similar difference between the two hippocampal areas has been noted recently in their ability to generate spontaneous NPS in low-Ca^{2+} solutions[3,11]. The reason for these differences is unknown, but it may be relevant that CA1 HPC are more densely packed than CA3 HPC[28], for the efficacy of both K^+-coupling and ephaptic interactions should increase with neuronal density.

The question arises as to whether nonsynaptic transmission of neural activity could occur in the intact brain. At physiological $[Ca^{2+}]_0$, the spread of neural discharge through a cortical structure would be limited by powerful lateral and recurrent inhibitory synaptic mechanisms[2,29]. However, intense cortical activity, observed most dramatically in the hippocampus and neocortex during an epileptic seizure, is accompanied by large decreases in $[Ca^{2+}]_0$ and increases in $[K^+]_0$[8,9,13,30,31]. Indeed, in the baboon neocortex, reduction in $[Ca^{2+}]_0$ to levels below 0.1 mM during an ictal episode have now been found (S. Comtes, U.H., J. Louvel, C. Menini and R. Pumain, in preparation). Such ionic changes are expected to markedly reduce the efficacy of Ca^{2+}-dependent synaptic inhibition[31] and augment HPC membranal excitability[32], thereby facilitating nonsynaptic excitatory interactions among these neurones. The close contiguity of neuronal membranes in hippocampal area CA1[27] would make it a favourable substrate for such interactions, and may explain why seizure discharges in this structure do not seem to spread along anatomical paths by necessity[6], and perhaps why the hippocampus is peculiarly susceptible to epilepsy[33].

This work was supported by DFG grants He 1128/2-2 and He 1128/2-3. Y.Y. is a Batsheva de Rothschild Scholar and was supported by an ETP grant and by the Meller Endowment Fund. We thank Professor H. D. Lux for helpful discussions and critical reading of the manuscript.

Received 15 April; accepted 17 October 1983.

1. Krnjevic, K. *Physiol. Rev.* **54,** 418–540 (1974).
2. Shepherd, G. M. *The Synaptic Organization of the Brain* (Oxford University, New York, 1974).
3. Jefferys, J. G. R. & Haas, H. L. *Nature* **300,** 448–450 (1982).
4. Taylor, C. P. & Dudek, F. F. *Science* **218,** 810–812 (1982).
5. Heinemann, U., Neuhaus, S. & Dietzel, I. in *Cerebral Blood Flow, Metabolism and Epilepsy* (eds Baldy-Moulinier, M. & Meldrum, B. S.) (John Libbey, London, in the press).
6. Kreindler, A. *Prog. Brain Res.* **19,** 87–133 (1965).
7. Prince, D. A. *A. Rev. Neurosci.* **1,** 395–415 (1978).
8. Heinemann, U., Lux, H. D. & Gutnick, M. J. *Expl Brain Res.* **27,** 237–243 (1977).
9. Heinemann, U., Konnerth, A., Louvel, G., Lux, H. D. & Pumain, R. in *Physiology and Pharmacology of Epileptogenic Phenomena* (eds Klee, M. R., Lux, H. D. & Speckmann, E. J.) 29–35 (Raven, New York, 1982).
10. Skrede, K. K. & Westgaard, R. H. *Brain Res.* **35,** 589–593 (1971).
11. Yaari, Y., Konnerth, A. & Heinemann, U. *Expl Brain Res.* **51,** 153–156 (1983).
12. Konnerth, A. & Heinemann, U. *Brain Res.* **270,** 185–189 (1983).
13. Heinemann, U. & Lux, H. D. *Brain Res.* **120,** 231–249 (1977).
14. MacVicar, B. A. & Dudek, F. A. *Science* **213,** 782–785 (1981).
15. MacVicar, B. A. & Dudek, F. A. *J. Neurophysiol.* **47,** 579–592 (1982).
16. Green, J. D. & Petsche, H. *Electroenceph. clin. Neurophysiol.* **13,** 837–846 (1961).
17. Yarom, Y., Grossman, Y., Gutnick, M. J. & Spira, M. F. *J. Neurophysiol.* **48,** 1089–1097 (1982).
18. Hounsgaard, J. & Nicholson, C. *J. Physiol., Lond.* **340,** 359–388 (1983).
19. Knowles, W. D., Funch, P. G. & Schwartzkroin, P. A. *Neuroscience* **7,** 1713–1722 (1982).
20. Andrew, R. D., Taylor, C. P., Snow, R. W. & Dudek, F. E. *Brain Res. Bull.* **8,** 211–222 (1982).
21. Taylor, C. P. & Dudek, F. E. *Brain Res.* **235,** 351–357 (1982).
22. Lorento De No, R. *J. Psychol. Neurol.* **45,** 113–177 (1934).
23. Dietzel, I., Heinemann, U., Hofmeier, G. & Lux, H. D. *Expl Brain Res.* **46,** 73–84 (1980).
24. Gardner-Medwin, A. R. & Nicholson, C. *J. Physiol., Lond.* **335,** 375–392 (1983).
25. Kocsis, J. D., Malenka, R. C. & Waxman, S. G. *J. Physiol., Lond.* **334,** 225–244 (1983).
26. Katz, B. & Miledi, R. *Proc. R. Soc.* **B216,** 497–507 (1982).
27. Green, J. D. & Maxwell, D. S. *Electroenceph. clin. Neurophysiol.* **13,** 837–846 (1961).
28. Vinogradova, O. S. in *The Hippocampus* Vol. 2 (eds Isaacson, R. L. & Pribram, K. H.) 3–69 (Plenum, London, 1975).
29. Spencer, W. A. & Kandel, E. R. in *Basic Mechanisms of the Epilepsies* (eds Jasper, H. H., Ward, A. A. & Pope, A.) 575–603 (Little Brown, Boston, 1969).
30. Dietzel, I., Heinemann, U., Hofmeier, G. & Lux, H. D. *Expl Brain Res.* **46,** 73–84 (1982).
31. Krnjevic, K., Morris, M. E. & Reiffenstein, R. J. *Can. J. Physiol. Pharmac.* **58,** 579–583 (1980).
32. Frankenhaeuser, B. & Hodgkin, A. L. *J. Physiol., Lond.* **137,** 218–244 (1957).
33. Green, J. D. & Shimamoto, T. *Archs Neurol. Psychiat., Lond.* **70,** 687–702 (1953).

Are baclofen-sensitive $GABA_B$ receptors present on primary afferent terminals of the spinal cord?

G. W. Price, G. P. Wilkin*, M. J. Turnbull† & N. G. Bowery‡

Department of Pharmacology, St Thomas's Hospital Medical School, London SE1 7EH, UK
* Department of Biochemistry, Imperial College, London SW7 2AZ, UK
† ICI Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

The site of action of the antispastic drug baclofen has long been considered to reside in the spinal cord[1,2] although supraspinal effects have also been reported[3–6]. This β-chlorophenyl derivative of the neurotransmitter γ-aminobutyric acid (GABA) depresses both monosynaptic and polysynaptic transmission in the cord[7] possibly through a decrease in transmitter release rather than by any antagonism at postsynaptic receptors[7–10]. Recently, baclofen has been shown to be a selective ligand for a bicuculline-insensitive GABA receptor ($GABA_B$) site[11,12] that occurs widely in the mammalian central nervous system including the spinal cord[13]. The apparent importance of the cord in the therapeutic effects of this drug prompted us to ask whether they involve $GABA_B$ site activation. As an initial step we have located these receptors by autoradiography, comparing them with classical $GABA_A$ sites. We report here that $GABA_B$ sites, unlike $GABA_A$ sites, are present in high concentrations in laminae I, II, III and IV of the dorsal horn and that after the neonatal administration of capsaicin this binding is reduced by 40–50%.

The distribution of $GABA_A$ and $GABA_B$ binding sites was determined in 10-μm sections of rat spinal cord. Prepared sections were placed on glass slides and allowed to dry before storage at −20 °C for periods up to 2 weeks. For the binding procedure the sections were thawed, washed and dried prior to incubation with ^{3}H-GABA-containing solution at 20 °C for 20 min. The incubation solution used for detection of $GABA_A$ sites was Tris-HCl buffer solution (50 mM *p*H 7.4) containing 100 μM (±) baclofen. For $GABA_B$ sites Tris-HCl buffer solution was used without baclofen but containing $CaCl_2$ (2.5 mM) and isoguvacine (40 μM) to suppress any binding to $GABA_A$ sites[13,14]. ^{3}H-GABA (50 nM) was used as the receptor ligand

‡ To whom reprint requests should be addressed.

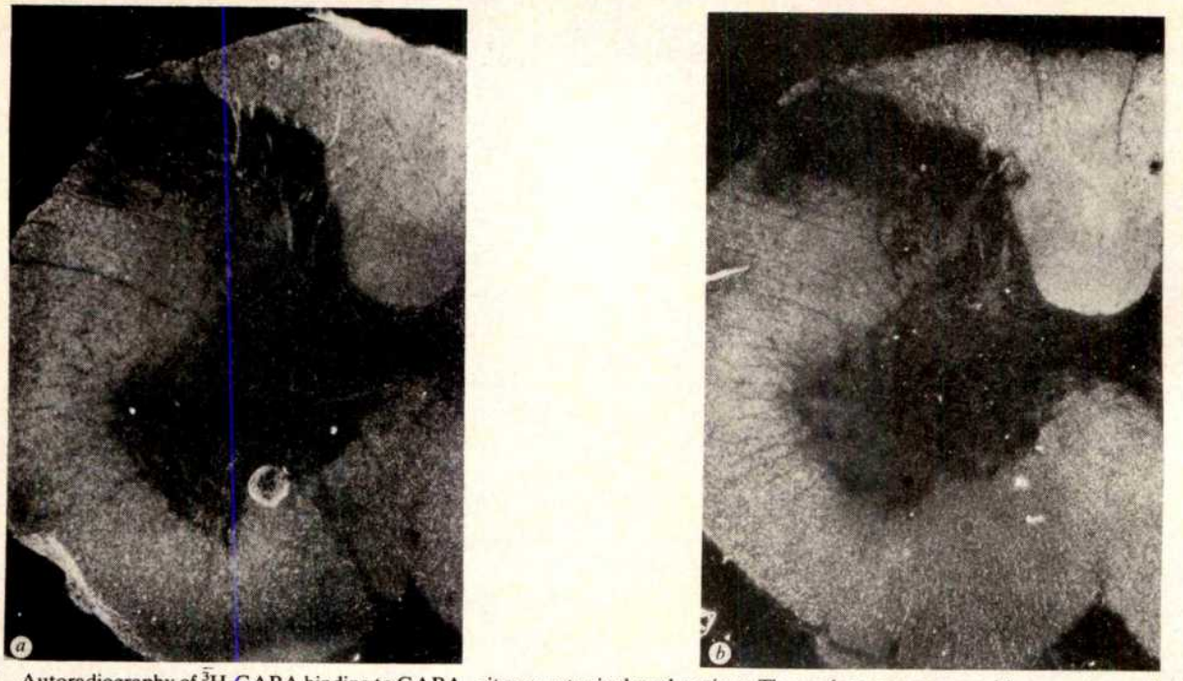


Fig. 1 Autoradiography of ^{3}H-GABA binding to $GABA_A$ sites on rat spinal cord sections. The sections were prepared from a rat anaesthetized with pentobarbitone before intracardiac perfusion with 200 ml 0.1% paraformaldehyde in 0.01 M phosphate-buffered saline (*p*H 7.4). A segment of cervical cord was removed and immersed in isopentane in liquid nitrogen. Transverse sections (10 μm) were mounted on glass slides and allowed to dry at ambient temperature for 1 h before storing at −20 °C. For the binding procedure the sections were allowed to thaw and dry for 45 min before immersion in Tris-HCl solution (50 mM *p*H 7.4) containing sucrose (190 mM) for 60 min. After this the sections were dried before covering with 100 μl of the Tris solution containing ^{3}H-GABA (60 Ci mmol^{-1}, 50 nM) and (±)baclofen (100 μM) without (*a*, total binding) or with (*b*, nonspecific binding) unlabelled isoguvacine (100 μM). This was left in contact for 20 min after which the solution was aspirated off and the sections briefly rinsed twice in large volumes of Tris solution. The sections were allowed to dry and coverslips previously dipped in Ilford K5 emulsion (emulsion:water 1:1.5) were attached to the slides with Loctite glue and placed in a light-tight box containing silica-gel for 16 days at +4 °C. The emulsion was developed at 16 °C in Kodak D19 developer (3 min) and fixed in 25% sodium thiosulphate solution. After several washes the slides were dried overnight and the coverslips fixed permanently in position with DPX mounting medium. Sections were viewed under dark-field optics with a Reichart Polywar microscope. Note in *a* the even density of grains throughout the grey matter indicating the presence of $GABA_A$ sites in all laminae.

throughout these studies and was therefore present in both incubation solutions at the same concentration. After incubation, the sections were briefly rinsed twice before being air dried. The distribution of ^{3}H-GABA binding sites was then investigated using the dry-mounting autoradiographic technique previously described[13,15]. Each slide was placed in close apposition to a coverslip coated with Ilford K5 photographic emulsion for 14–21 days. After developing, the grains per unit area were counted under dark-field optics. To facilitate comparison between the distributions of $GABA_A$ and $GABA_B$ sites, adjacent sections from the same cord were routinely incubated in appropriate conditions.

$GABA_A$-binding sites were evenly distributed throughout the grey matter of the spinal cord (Fig. 1), although there were slightly more in the dorsal horn than in the ventral regions. In contrast, the distribution of $GABA_B$-binding sites was not uniform (Fig. 2). Although $GABA_B$ sites could be detected throughout the grey matter, a clear band of high grain density was evident in the dorsal horn. This band was located mainly in laminae II and III (Fig. 2) but considerable numbers of grains were also evident in laminae I and IV and in the dorsal aspects of lamina X around the central canal.

A comparative analysis of the laminae grain densities indicated that there were more $GABA_B$ sites in the dorsal laminae than in the ventral laminae whereas $GABA_A$ sites showed very little fluctuation (Fig. 3).

Many studies of peripheral and central nervous tissue show that $GABA_B$ sites can occur presynaptically[11,16–21] although a

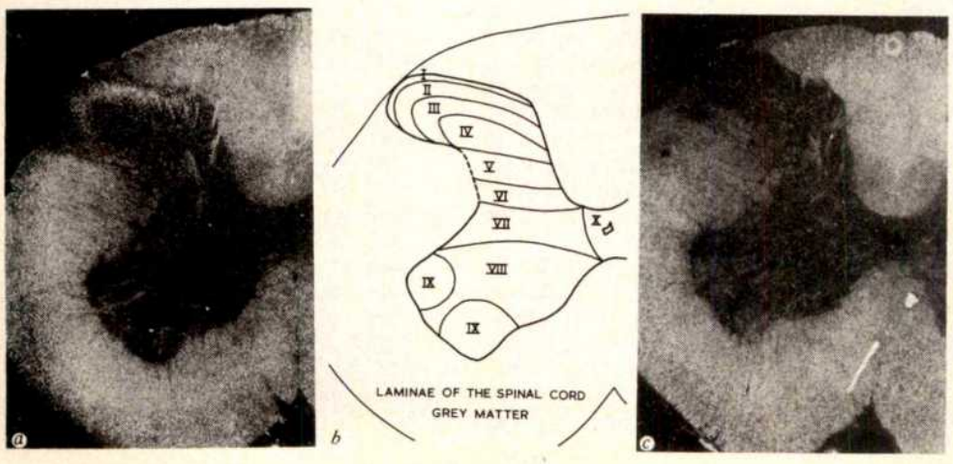


Fig. 2 Autoradiography of ^{3}H-GABA binding to $GABA_B$ sites in rat spinal cord. Sections of spinal cord were prepared and treated as described for Fig. 1 except that the washing and incubating solutions contained 2.5 mM $CaCl_2$ and the incubating solutions contained 40 μM isoguvacine. $CaCl_2$ facilitates binding to $GABA_B$ sites while isoguvacine suppresses all binding to $GABA_A$ sites[12]. (±)Baclofen (100 μM) was absent in *a* but present in *c* to obtain the background (non-specific) binding. *b*, Diagram of the separation between the laminae. In *a* grains corresponding to $GABA_B$ sites appear right across the grey matter but the distribution is uneven. A dense band of grains is apparent in laminae II and III.

Table 1 Distribution of $GABA_B$ sites in the dorsal horn of the rat spinal cord after neonatal administration of capsaicin

Lamina	Control (specific grains per 1,000 μm^2)	Capsaicin-treated	% Reduction
I	49.7 ± 5.4	29.8 ± 3.6	40.0
II_{outer}	60.2 ± 5.6	32.6 ± 3.1	45.8
II_{inner}	64.7 ± 6.2	32.8 ± 5.0	49.3
III	57.0 ± 8.6	26.8 ± 2.2	53.0
IV	40.7 ± 8.0	24.7 ± 2.6	39.3

Mean values ± s.e.m. Data obtained from average density in each lamina of six sections from capsaicin-treated animals and control littermates injected with vehicle alone.

postsynaptic location is possible[22]. The question arises therefore are $GABA_B$ sites in the dorsal horn pre- or postsynaptic to the afferent fibre inputs? In all systems so far studied which show a presynaptic location, the activation of $GABA_B$ sites reduces the evoked release of neurotransmitter. Such an action on terminals in the dorsal horn might well explain how baclofen acts as an analgesic[23–25] and suppresses at least part of the symptoms of morphine withdrawal[26–28].

Autoradiographic studies at the light microscope level in normal tissue cannot distinguish between pre- and postsynaptic locations. However, by selective elimination of the neural components, a distinction might become evident. It has been shown, for example, that after dorsal rhizotomy, opiate receptor binding decreases by 40% whilst leaving the binding of ^3H-neurotensin intact[29]. It is, therefore, unlikely that neurotensin binding sites are located presynaptically whereas there may be a population of opiate sites on the afferent terminal. Of course, this latter conclusion discounts any possible postsynaptic changes which may arise from presynaptic degeneration. Nevertheless, we have used this approach in an attempt to locate the $GABA_B$ site. Selective degeneration of sensory afferent fibres was produced by the neonatal administration of capsaicin[30] following the procedure adopted by Faulkner and Growcott[31]. Three to four months after injection, transverse sections of cord were prepared. Sections were also prepared from littermates injected with vehicle alone. The binding of ^3H-GABA was then performed as described above and the distribution of binding sites determined by autoradiography (Table 1). Capsaicin treatment reduced the number of $GABA_B$ binding sites in substantia gelatinosa by 40–50% when compared with control littermates. Immunocytochemical analysis of the sections for the presence of substance P by the peroxidase–anti-peroxidase (PAP) technique[32] indicated that the capsaicin treatment had produced a marked reduction in the peptide content of the gelatinosa when compared with littermates injected with vehicle alone. Similarly, a reduction in the fluoride-resistant acid phosphatase (FRAP) activity[33] was also noted. Both of these systems are considered to be markers for primary afferent terminals[34,35]. Thus, taken together, these data confirm that at least some of the $GABA_B$ sites detected in sections of normal spinal cord are present on primary afferent terminals. Whether the remaining sites are located presynaptically on descending pathways or on postsynaptic elements remains unclear.

The distribution of $GABA_B$ sites in the cord corresponds closely with that of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD), a marker for GABA-containing synaptic terminals[36,37]. The location of this enzyme in the dorsal horn agrees with a presynaptic function for GABA[36,37]. Thus it is possible that the GABA released at these axo-axonic contacts is impinging on $GABA_B$ receptors. Alternatively, as pointed out by Palacios *et al.*[38], bicuculline-sensitive $GABA_A$ receptors may receive this innervation. These authors have described the location of ^3H-muscimol-binding sites in spinal cord incubated in conditions which would facilitate binding to $GABA_A$ sites, but only a low concentration of sites could be discerned. Singer and Placheta[39], using membrane pellets prepared from rat dorsal horn, have shown that neonatal capsaicin treatment can reduce the number of ^3H-muscimol-binding sites

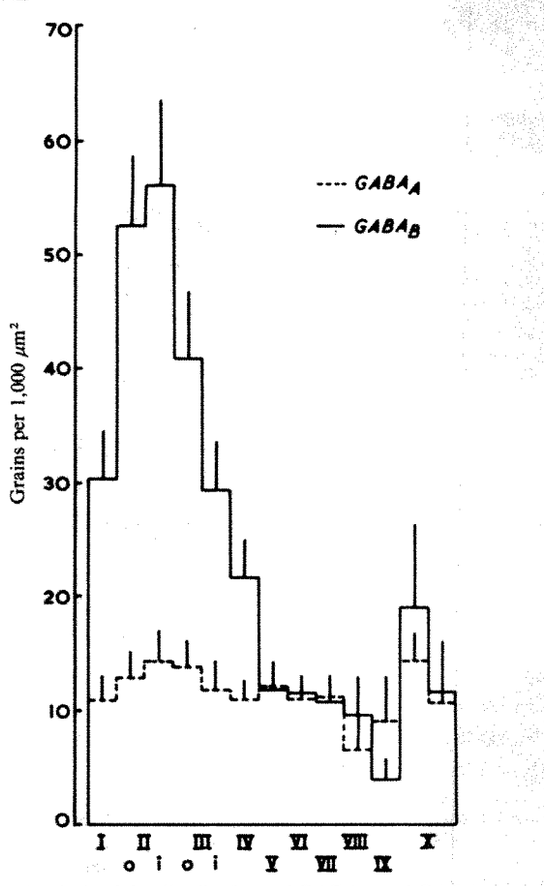


Fig. 3 Analysis of the distributions of $GABA_A$ and $GABA_B$ sites in rat spinal cord sections. Transverse sections of spinal cord were prepared and incubated with ^3H-GABA for $GABA_A$ or $GABA_B$ site detection as described in the legends to Figs 1 and 2. Autoradiograms were subsequently prepared as described in Fig. 1. Grain densities in the individual laminae were determined by counting three areas in each lamina or lamina region (inner, i; or outer, o) from the spinal cords of six rats. Measurements were made after ascertaining that grain accumulation was linear with time. Mean values ± s.e.m. (vertical lines) are shown. The solid outline represents $GABA_B$ site concentrations whereas the broken outline represents $GABA_A$ site concentrations.

by 20–30%. This would support the long-established view that bicuculline-sensitive GABA receptors ($GABA_A$ sites) are present on primary afferent terminals. However, since the concentration of $GABA_B$ sites is much higher they may have a greater role in GABA-mediated axo-axonic transmission in the spinal cord.

G.W.P. is a SERC-CASE award recipient in collaboration with ICI Pharmaceuticals Division. Financial support from the MRC is gratefully acknowledged. We thank Dr M. Hanley and Dr F. Schon for performing the substance P and FRAP analyses respectively, Dr P. Krogsgaard-Larsen for supplies of isoguvacine, CIBA-Geigy Ltd for (±)baclofen, and Mrs J. Andrews for typing the manuscript.

Received 30 June; accepted 14 October 1983.

1. Bein, H. J. in *Spasticity—a topical survey* (ed. Birkmayer, W.) (Hans Huber, Vienna, 1972).
2. Fehr, H. U. & Bein, H. J. *J. int. Med. Res.* **2**, 36 (1974).
3. Saelens, J. K., Welch, J., Robson, R. D. & Roffman, M. *Fedn Proc.* **36**, 395 (1977).
4. Davies, J. & Watkins, J. C. *Brain Res.* **70**, 501–505 (1974).
5. Olpe, H. R., Koella, W. P., Wolf, P. & Haas, H. L. *Brain Res.* **134**, 577–580 (1977).
6. Lanthorn, T. H. & Cotman, C. W. *Brain Res.* **225**, 171–178 (1981).
7. Davies, J. *Br. J. Pharmac.* **72**, 373–384 (1981).
8. Fox, S., Krnjevic, K., Morris, M. E., Puil, E. & Werman, R. *Neuroscience* **3**, 495–515 (1978).
9. Davidoff, R. A. & Sears, E. S. *Neurology* **24**, 957–963 (1974).
10. Henry, J. L. *Neuropharmacology* **21**, 1085–1093 (1982).
11. Bowery, N. G. *et al. Eur. J. Pharmac.* **71**, 53–70 (1981).
12. Hill, D. R. & Bowery, N. G. *Nature* **290**, 149–152 (1981).
13. Wilkin, G. P., Hudson, A. L., Hill, D. R. & Bowery, N. G. *Nature* **294**, 584–587 (1981).
14. Bowery, N. G., Hill, D. R. & Hudson, A. L. *Br. J. Pharmac.* **78**, 191–206 (1983).
15. Young, W. S. & Kuhar, M. J. *Brain Res.* **179**, 255–270 (1979).
16. Bowery, N. G. *et al. Nature* **283**, 92–94 (1980).
17. Muhyaddin, M., Roberts, P. J. & Woodruff, G. N. *Br. J. Pharmac.* **77**, 163–168 (1982).

18. Hughes, P. R., Morgan, P. F. & Stone, T. W. *Br. J. Pharmac.* **77**, 691–695 (1982).
19. Cain, C. R. & Simmonds, M. A. *Neuropharmacology* **21**, 371–373 (1982).
20. Kilpatrick, G. J., Muhyaddin, M. S., Roberts, P. J. & Woodruff, G. N. *Br. J. Pharmac.* **78**, Suppl. 6P (1983).
21. Fillenz, M. & Fung, S. C. *J. Physiol.*, **339**, 390 (1983).
22. Bowery, N. G., Price, G. W., Turnbull, M. J. & Wilkin, G. P. *Br. J. Pharmac.* **79**, Suppl. 189P (1983).
23. Cutting, D. A. & Jordan, C. C. *Br. J. Pharmac.* **54**, 171–179 (1975).
24. Levy, R. A. & Proudfit, H. K. *J. Pharm. exp. Ther.* **202**, 437–445 (1977).
25. Cutting, D. A. & Jordan, C. C. *Scot. med. J.* **25**, S17–S22 (1980).
26. Brodie, M. E. & McQueen, E. G. *Proc. Univ. Otago med. Sch.* **53**, 40–41 (1975).
27. Ladewig, D. *Neurol. Psychiat. Prax. (D)* **10** (1980).
28. Bowery, N. G. *Trends pharmac. Sci.* **3**, 400–403 (1982).
29. Ninkovic, M., Hunt, S. P. & Kelly, J. S. *Brain Res.* **230**, 111–119 (1981).
30. Jancso, G., Kiraly, E. & Jancso-Gabor, A. *Nature* **270**, 741–743 (1977).
31. Faulkner, D. C. & Growcott, J. W. *J. pharm. Pharmac.* **32**, 656–657 (1980).
32. Sternberger, L. A. in *Immunocytochemistry*, 104–169 (Wiley, New York, 1979).
33. Disbrey, B. D. & Rack, J. H. *Histological Laboratory Methods* (Livingstone, Edinburgh, 1970).
34. Gamse, R., Holzer, P. & Lembeck, F. *Br. J. Pharmac.* **68**, 207–213 (1980).
35. Nagy, J. I., Hunt, S. P., Iversen, L. L. & Emson, P. C. *Neuroscience* **6**, 1923–1924 (1981).
36. McLaughlin, B. J., Barber, R., Saito, K., Roberts, E. & Wu, J-Y. *J. comp. Neurol.* **164**, 305–322 (1975).
37. Barber, R. P., Vaughn, J. E., Saito, K., McLaughlin, B. J. & Roberts, E. *Brain Res.* **141**, 35–55 (1978).
38. Palacios, J. M., Wamsley, J. K. & Kuhar, M. J. *Brain Res.* **222**, 285–307 (1981).
39. Singer, E. & Placheta, P. *Brain Res.* **202**, 484–487 (1980).

Bonding of molecular oxygen to T state human haemoglobin

Andrzej Brzozowski*, Zygmunt Derewenda*, Eleanor Dodson†, Guy Dodson†, Mieczysław Grabowski*, Robert Liddington†, Tadeusz Skarżyński* & David Vallely§

* Department of Crystallography, Institute of Chemistry, University of Lodz, 91–416 Lodz, Nowotki 16, Poland
† Department of Chemistry, University of York, Heslington, York YO1 5DD, UK

Haemoglobin (Hb) is the tetrameric protein molecule that in vertebrate blood transports oxygen from the lungs to the tissues. This function depends on four subunits in the molecule binding cooperatively so that their affinity for oxygen increases as the level of oxygenation increases. X-ray analysis has shown that deoxyhaemoglobin, which has a low oxygen affinity, and oxyhaemoglobin, which has a high oxygen affinity, differ principally in their subunit or quaternary structures[1], referred to as the T and R states, respectively. As it switches from the T state to the R state during oxygenation, Hb increases its oxygen affinity. However, the structural pathway between deoxy- and oxyhaemoglobin is not known, principally because there has been no accurate structural knowledge of the intermediate states. We report here the crystal structure of T state human Hb in which the α chains are oxygenated and the β subunits are oxygen-free. In this crystal the Hb appears to be in an intermediate state between the unliganded T state and the liganded R state. There is also evidence that the Hb molecule operates by loading and unloading the β haems and thus the α-oxy,β-deoxy Hb crystal may represent a physiologically important state[2].

Perutz has proposed[3] that oxygen's binding to Fe(II) in deoxyhaemoglobin alters the stereochemistry at the haem which destabilizes the T state quaternary structure, favouring its switch to the R state quaternary organization with its higher oxygen affinity. The crystal structures of deoxyhaemoglobin[4] and oxyhaemoglobin[5] have been determined with considerable accuracy and consequently there is a detailed atomic description for these two structures[6]. The partially oxygenated T-state Hb crystal has parallels with the crystalline intermediate produced by Anderson[7] and agrees with the α subunits in the T structure having the higher oxygen affinity because the binding of oxygen to the β haems is hindered by $(CH_3)_\gamma$ of the distal valine[3,8]. The crystals were grown from polyethylene glycol (PEG), following the procedure of Ward and his colleagues[9], except that air was allowed entry into the crystallization vessels and the crystals were transferred to more concentrated solutions of PEG before X-ray study[10]. A number of crystal forms were produced with these conditions which were identifiable from their X-ray diffraction patterns[11]. They are all similar to the crystal form reported by Ward *et al.*[9], containing one complete molecule in the asymmetric unit. Preliminary studies at 3.5 Å resolution on two of the more reproducible forms showed that one was apparently unliganded while the other appeared to contain liganded α haems (unpublished work).

Table 1 Stereochemical features of the haem in various molecules

Molecule	Fe–O (Å)	Fe–Ô–O (°)	O–O (Å)	Fe–Nε (His F8) (Å)	(Å)	Fe–Ct
T state Hb(PEG)						
α_1-oxy	1.81	160 ± 5	1.23	2.23†	2.10§	0.2*
α_2-oxy	1.81	160 ± 5	1.24	2.50†	2.39§	0.2*
β_1-deoxy	—	—		2.21†	2.39§	0.3*
β_2-deoxy	—	—		2.20†	2.14§	0.3*
T state Hb[5]						
α-deoxy				2.00‡		0.60
β-deoxy						
R state Hb[4]						
α-oxy	1.67	153	1.22	1.94	1.94	0.13
β-oxy	1.83	158	1.24	2.06	2.06	0.08
Mb[14]						
deoxy	—	—	—	2.22	2.22	0.42
oxy	1.83	115	1.18	2.07	2.07	0.18

Fe–Ct, the distance from the haem plane to the Fe(II).
* The haem plane is defined by the least squares plane through the four pyrrole nitrogens.
† These values (e.s.d. 0.1–0.2 Å) were obtained after six cycles of refinement with Fe–Nε restraints removed. The Fe–Nε distance in the initial set of coordinates lay between 2.08–2.11 Å, essentially the distance to which it had been restrained.
‡ Diamond refinement at 2.5 Å (ref. 5).
§ Refinement values obtained with the Jack–Levitt procedure[15]; weak restraints were applied to the Fe–Nε distance.

A 2.1 Å dataset was subsequently collected at Laboratoire pour l'Utilisation de Rayonments Electromagnetique (LURE) on a partially liganded crystal. The crystal structure was refined from the human deoxyhaemoglobin coordinates[6], positioned correctly in the PEG Hb cell by molecular replacement calculations[11]. The initial agreement factor $R(=\sum||F_o|-|F_c||/\sum|F_o|)$ was 0.52; on refinement, this has been reduced to 0.21.

The refinement of the crystal structure was carried out using the fast Fourier least squares procedure of Agarwal[12]. At a resolution of 2.1 Å the calculated shifts are inaccurate and the correct peptide geometry was imposed[13] on the atomic coordinates after each shift calculation.

Refinement of the well-ordered atoms is essentially complete with the estimated error in their atomic positions being between 0.1 and 0.2 Å. The further lengthy crystallographic refinement needed on some of the poorly ordered residues, and in improveing and extending the descriptions of the solvent structure, will be published elsewhere. It will not alter materially the picture we have of the stereochemical features at the haem groups presented here.

The bond distances and angles at the Fe(II) which are most liable to be affected by ligation unfortunately presented a particular difficulty since they are not known with the same accuracy as those in peptides. The effect of different restraints of geometries on the refinement of the groups about the haem was explored. A parallel refinement was also carried out with the Jack-Levitt procedure which was used in the oxyhaemoglobin study[15]. The coordinate sets finally arrived at agreed satisfactorily within their standard deviations, the well-defined atoms in the haems differing by less than 0.18 Å. There is nonetheless evidence from the refinement calculations and from the electron density (Figs 1 and 2 and Table 1) that the Fe–Nε (His F8) distance in the α_2 subunit is rather longer than the normal values, which raises the possibility that the Fe is in the high-spin state.

The electron density at the α haems and the β haems is illustrated in Figs 1 and 2. The oxygen peak is well defined and



Fig. 1 Stereo view of the electron density at and surrounding the α haems calculated with $2F_o - F_c$ coefficients and excluding the haem and proximal and distal histidines from the phasing. The atoms in the haems and proximal and distal histidines are shown: *a*, $\alpha 1$; *b*, $\alpha 2$.

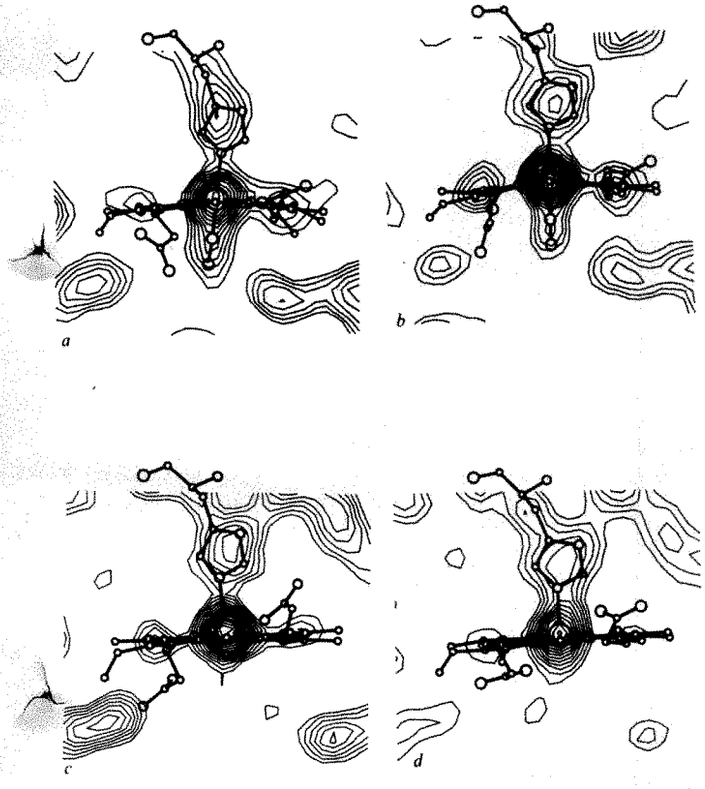


Fig. 2 The haem and proximal histidine viewed edge-on. A section of the electron density, calculated as for Fig. 1, through the Fe and proximal histidine (and oxygen in the case of the α haems) is shown *a*, $\alpha 1$; *b*, $\alpha 2$; *c*, $\beta 1$; *d*, $\beta 2$.

its height suggests that it is fully occupied in the crystal. Calculations on the oxygen's occupancy confirm this.

Two notable features in the partially oxygenated molecule are, first, that the arrival of the oxygens at the α haems has produced only limited movement from the T state. Second, there is a marked folding of the haems in both α subunits. (Refinement of high salt deoxyhaemoglobin at 1.7 Å (G. Fermi, personal communication) and PEG deoxyhaemoglobin at 2.1 Å (unpublished results) reveals that the α haems in these molecules are buckled in an identical fashion to that observed in the partially oxygenated form.) This stereochemistry lifts the Fe(II) about 0.2 Å out of the plane defined by the four pyrrol nitrogens; note that the Fe(II) is at, or very near to, the apex of the pyramid formed by the planes of the four pyrrole rings. In contrast, the β haems are essentially flat and the 5 coordinate Fe(II) is about 0.3 Å out of the plane (Fig. 2).

This indicates that the globin is restraining the haem and the oxygen from assuming a more favourable stereochemistry, thereby reducing the stability of the T state relative to the R state. The particularly close interaction between the haemoglobin molecules in these crystals undoubtedly serves to stabilize the T state quaternary structure and is evidently preventing the switch to the R state and inhibiting further ligation of the β haems.

Detailed analysis of the structural changes associated with oxygen's binding can only be made when the fully deoxygenated molecule's crystal structure is refined and can be compared to the liganded molecule. Then the significance of the Fe–Nε distances and of the different oxygen interactions in the two α subunits can be better assessed. Comparison is being extended to the structures of the deoxy Hb and the fully liganded T state Hb aquomet and azide species. In this way we hope to elucidate how the steric effects in the Fe coordination and in the haem's geometry lead to the structural movements in the globin that destabilize the T state, thus favouring the R state and generating increased oxygen affinity.

We thank Drs Roger Fourme, Philip Evans, Simon Phillips, Boaz Shaanan and Max Perutz for help and support, Dr Yves Mauguen for data collection and LURE for facilities. This study has been supported by EMBO, the MRC, the Royal Society, the SERC and the Polish Ministry of Science and Higher Education (Contract RIII, 13.1.1).

Received 13 June; accepted 24 October 1983.

1. Baldwin, J. & Chothia, C. *J. molec. Biol.* **129,** 175–220 (1979).
2. Weber, G. *Nature* **300,** 603–606 (1982).
3. Perutz, M. F. *Nature* **237,** 495–499 (1977).
4. Fermi, G. *J. molec. Biol.* **97,** 237–256 (1975).
5. Shaanan, B. *Nature* **296,** 683–684 (1982).
6. Fermi, G. & Perutz, M. F., in Haemoglobin and Myoglobin (eds Phillips, D. C. & Richards, F. M.) (Clarendon, Oxford, 1981).
7. Anderson, L. *J. molec. Biol.* **94,** 33–49 (1975).
8. Viggiano, G. & Ho. C. *Proc. natn. Acad. Sci. U.S.A.* **76,** 3673–3677 (1979).
9. Ward, K. B., Wishner, B. C., Lattman, E. E. & Love, W. E. *J. molec. Biol.* **98,** 161–171 (1975).
10. Grabowski, M. J. *et al. Biochem. J.* **171,** 277–279 (1978).
11. Derewenda, Z. S., Dodson, E. J., Dodson, G. G. & Brzozowski, A. M., *Acta crystallogr.*, **A37,** 407–413 (1981).
12. Agarwal, R. C., *Acta crystallogr.*, **A34,** 791–809 (1978).
13. Dodson, E. J., Isaacs, N. W. & Rollett, J. R. S., *Acta crystallogr.* **A34,** 931–935 (1978).
14. Phillips, S. E. V. *J. molec. Biol.* **142,** 531–554 (1980).
15. Jack, A. & Levitt, M. *Acta crystallogr.* **A34,** 931–935 (1978).

Ferrara β^0 thalassaemia caused by the β^{39} nonsense mutation

Mario Pirastu*, Laura del Senno†, Francesco Conconi†, Calogero Vullo‡ & Yuet Wai Kan*

* Howard Hughes Medical Institute Laboratory and Division of Medical Genetics and Molecular Hematology, Department of Medicine, University of California, San Francisco, California 94143, USA
† Centro Studi Biochimici Morbo di Cooley, Universitá degli Studi di Ferrara, Ferrara, Italy
‡ Divisione Pediatrica, Ospedale Sant'Anna, Ferrara, Italy

Ferrara type of β^0 thalassaemia has two unusual features: first, normal β-globin chain synthesis is inducible either in cell-free systems prepared from patients' reticulocytes by adding supernatant factors from non-thalassaemic reticulocyte lysates[1] or in heterologous cell-free translation of thalassaemic mRNA[2]; second, β-globin synthesis is inducible in patients *in vivo* after blood transfusion[3,4]. We now describe a molecular lesion of the β-globin gene that is common to nine cases of Ferrara β^0 thalassaemia but cannot be reconciled with the inducible response.

DNA from nine patients with this disorder was analysed by the synthetic deoxyoligonucleotide method[5,6]. Two nonadecamers were used as hybridization probes, one homologous to the normal β-globin gene sequences at the region corresponding to β^{39}, and the other to the same region of a β^0-thalassaemia gene caused by a β^{39} (Gln, CAG→TAG) nonsense mutation[7]. In well-defined hybridization and washing conditions, each probe hybridizes only to its homologous sequence. The DNA sequences from 17 chromosomes of the nine patients hybridized with the β^0-thalassaemia probe (Fig. 1), indicating that the primary lesion responsible for Ferrara β^0 thalassaemia is the β^{39} nonsense mutation. The nature of the mutation in the remaining chromosome has not been defined. The β^{39} nonsense mutation is widespread throughout the Mediterranean region. It has been detected in other parts of Italy, Greece, Turkey, Morocco, Lebanon and Spain (M.P. and Y.W.K., unpublished data).

We cannot reconcile our present findings with the previously reported results. One possible explanation for the *in vitro* induction of β-globin synthesis is that suppressor tRNA was present in the supernatant factors used. It has been demonstrated previously that the amber termination codon in β^0 thalassaemia can be suppressed with an amber suppressor tRNA[8–10]. However, although a tRNA with a UGA suppressor has been described in rabbit reticulocytes[11], no amber suppressor has yet been detected in humans. Furthermore, the suppressor tRNA theory cannot explain the *in vivo* transfusion results. We have examined transfusion records of 100 Sardinian β^0 thalassaemia patients with the same β^{39} nonsense mutation and did not observe induction of β-globin synthesis at any time following transfusion. The Ferrara patients should be reinvestigated in light of the present findings.

This research was supported in part by grants from the NIH (AM 16666), UNICO National Inc., and the CNR (Progretto Finalizzato Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie and 82.0119.83). Y.W.K. is an Investigator of the Howard Hughes Medical Institute.

Received 1 September; accepted 18 October 1983.

1. Conconi, F., Rowley, P. T., del Senno, L., Pontremoli, S. & Volpato, S. *Nature new Biol.* **238,** 83–87 (1972).
2. Conconi, F. *et al. Ann. N.Y. Acad. Sci.* **334,** 120–131 (1980).
3. Conconi, F. *et al. Nature* **254,** 256–259 (1975).
4. Ottolenghi, S. *et al. Nature* **266,** 231–234 (1977).
5. Wallace, R. B., Schold, M., Johnson, M. J., Dembek, P. & Itakura, K. *Nucleic Acids Res.* **9,** 3647–3656 (1981).
6. Conner, B. J., Reyes, A. A., Morin, C., Itakura, K. & Wallace, R. B. *Proc. natn. Acad. Sci. U.S.A.* **80,** 278–282 (1983).
7. Pirastu, M. *et al. New Engl. J. Med.* **309,** 284–287 (1983).
8. Chang, J. C., Temple, G. F., Trecartin, R. F. & Kan, Y. W. *Nature* **281,** 602–603 (1979).
9. Trecartin, R. F. *et al. J. clin. Invest.* **68,** 1012–1017 (1981).
10. Temple, G. F., Dozy, A. M., Roy, K. L. & Kan, Y. W. *Nature* **296,** 537–540 (1982).
11. Geller, A. I. & Rich, A. *Nature* **283,** 41–46 (1980).

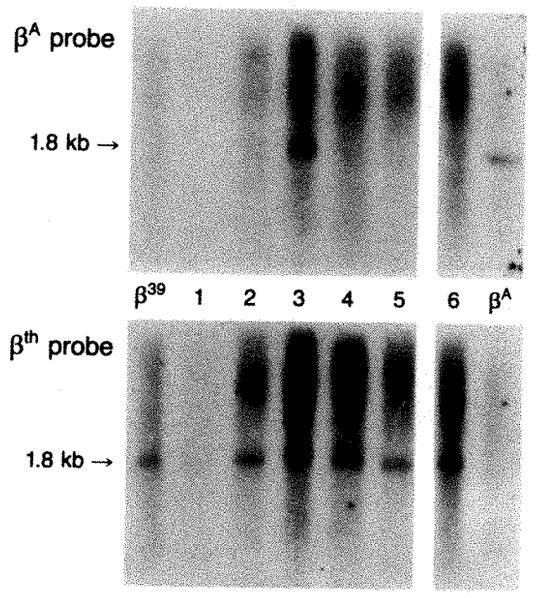


Fig. 1 Autoradiographs of gels containing DNA from Ferrara patients, hybridized with synthetic deoxyoligonucleotide probes. The method used has been described in detail[7]. Briefly, DNA was digested with *Bam*HI, electrophoresed on agarose gels, and hybridized with two ^{32}P-labelled nonadecamer probes. The β^A probe has the sequence 5′ CCTTGGACCCAGAGGTTCT 3′ and is homologous to the coding strand of the normal β-globin gene at the position corresponding to amino acid numbers 35–42. The β^{th} probe has the sequence 5′ AGAACCTCTAGGTCCAAGG 3′ and is homologous to the noncoding strand of the β-thalassaemia gene with the β^{39} (Gln, CAG→TAG) nonsense mutation at the same amino acid position. Results of samples obtained from six of the nine Ferrara β^0 thalassaemia patients (1–6) are compared with DNA from a Sardinian with the same β^{39} nonsense mutation (β^{39}) and with DNA from a non-thalassaemic individual (β^A). Patient 3 is the only double heterozygote for the β^{39} nonsense mutation and another undefined β-thalassaemia lesion. All other patients were homozygous for the β^{39} nonsense mutation.

Most κ immunoglobulin mRNA in human lymphocytes is homologous to a small family of germ-line V genes

David L. Bentley

Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104, USA

The mammalian immune system produces 10^6–10^8 different antibody-combining sites[1]. It has been established that three factors contribute to the diversity of immunoglobulin variable regions: multiple gene segments in the germ line, somatic recombination of these segments and somatic mutation[2]. However, the relative importance of these components in generating the antibody repertoire is unknown. One way to assess the importance of the germ-line component relative to the somatic components would be to determine the number of germ-line *V* genes expressed. Here, I report that a family of about 25 human germ-line *V* genes encodes over 50% of κ mRNA in spleen or peripheral blood lymphocytes. This observation agrees with gene-counting experiments which indicated that the total number of V_κ genes in the human genome is quite small, about 50 or less[3]. Such a small number of germ-line V_κ sequences implies that somatic mutation is the major source of human κ-chain diversity.

κ-Chains constitute about two-thirds of light chains in human serum[4]. Over 180 peptide sequences of human κ variable (*V*) regions have been determined and can be placed into four subgroups (V_κ1–4)[5,6]. The present study investigated the expression of V_κ genes by hybridization of cloned human genes to lymphocyte mRNA. A constant region (C_κ) probe will hybridize to all κ mRNAs while a V_κ probe will only detect the subset of mRNAs encoded by genes which cross-hybridize with that particular sequence. Thus, the amount of hybridization of a V_κ probe relative to a C_κ probe corresponds to the fraction of mRNA encoded by a given set of cross-hybridizing V_κ genes[7]. The number of homologous genes in the germ line can be estimated from hybridization of the V_κ probe to genomic DNA. In this way, it is possible to quantitate the contribution of a given number of germ-line V_κ genes to the pool of κ light chains expressed by an individual.

Two cloned human V_κ genes from subgroups 1 and 3 were used as hybridization probes. The DNA sequences of these genes are shown in Fig. 1. The V_κ1 sequence is from an unrearranged gene, *HK101*, isolated from fetal liver DNA[8]. The V_κ3 sequence is from two overlapping cDNA clones made from the NG9/9.1 hybridoma which is the fusion product of a mouse plasmacytoma and a human spleen cell[9]. The NG9 V region is one amino acid longer than the HK101 V region. The two sequences show 61% amino acid homology and 72% nucleotide homology. In the framework coding regions, the DNA sequences are 77% homologous and in complementarity determining regions they are 63% homologous.

The spectrum of V_κ genes that cross-hybridizes with each of these two cloned genes in a genomic Southern blot is shown in Fig. 2*a*. It is clear that most of the hybridizing restriction fragments are detected by both the V_κ1 and V_κ3 probes, confirming a previous experiment with other probes[3]. This family of genes is also likely to contain V_κ4 sequences which are more closely related to V_κ1 and V_κ3 than these two are to one another. The group of restriction fragments identified by the V_κ1 and V_κ3 sequences contains about 25 V_κ genes including at least one pseudogene[8]. This number is based on several lines of evidence, including counting hybridization bands in Southern blots[3], exhaustive cloning and sequencing of V_κ genes from one band[10], probe excess solution hybridization[11] and counting the frequency of hybridizing clones in a phage λ library[11]. The expression of these V_κ genes was analysed by hybridization of the same two probes to lymphocyte mRNA.

Initially κ-chain sequences in spleen poly(A)$^+$ RNA were characterized by Northern blot hybridization to C_κ- and V_κ1-specific restriction fragments. In both cases a doublet about 1,100 bases long was seen (Fig. 2*b*). A similar result was obtained when total spleen RNA was analysed (data not shown). The size of the hybridizing RNA is that expected for κ mRNA and it shows that genuine mRNAs from rearranged *V* and *C* genes and not abberrant transcripts are being observed. Densitometry of the Northern blot autoradiogram showed that the intensity of the signal with the V_κ1 probe was 75% of that with the C_κ probe, implying that a substantial proportion of κ mRNAs hybridize with this variable region sequence.

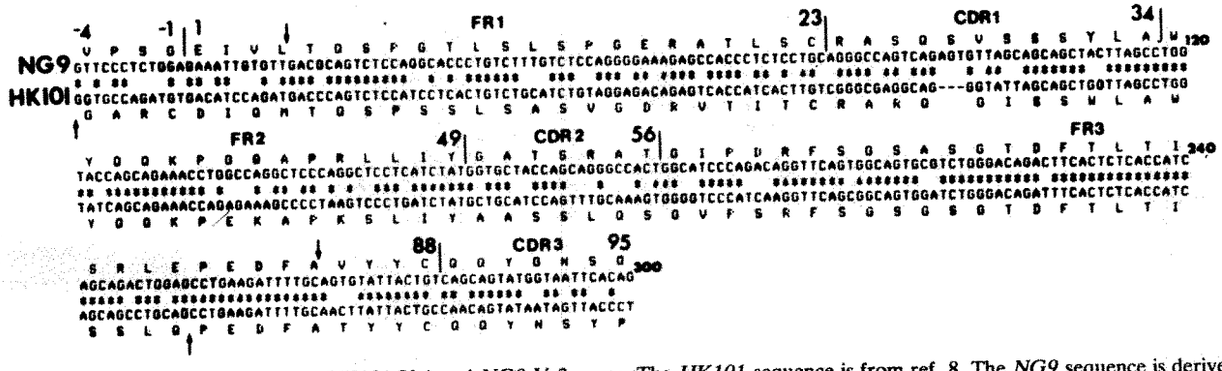


Fig. 1 DNA sequence comparison of *HK101* V_κ1 and *NG9* V_κ3 genes. The *HK101* sequence is from ref. 8. The *NG9* sequence is derived from two cDNA clones that extend from codon 59 into the *J* region (*NG9*/V_κ3)[3] and from codon 4 to codon 84 (*NG9*/3). The amino acid translation is given in one-letter code and the numbering is according to ref. 5. The *NG9* sequence confirms the positions of N-terminal leucine residues reported in ref. 9. The framework (FR) and complementarity determining regions (CDR) are indicated. The *NG9* *V* region has an extra residue at position 27A relative to the *HK101*/*V* region. Nucleotide identity is shown with asterisks. Arrows mark the ends of fragments used to probe spleen RNA.

Methods: Construction and sequencing of the *NG9*/3 cDNA clone. cDNA synthesis was primed on 2 μg of poly(A)$^+$ RNA (a gift of C. Milstein) with 0.5 ng of a ^{32}P-labelled single-stranded 123-base fragment from the 5′ end of the *C* region. The synthesis of the primer was by the method used for preparation of prime-cut probes described in Fig. 3 legend. Primer was annealed to RNA in 20 mM PIPES *p*H 6.4, 1 mM EDTA, 0.5 M KCl in a 15-μl reaction for 6 h at 58 °C. The annealing reaction was diluted 10-fold into a reverse transcriptase reaction and incubated at 42 °C for 1 h. Full-length cDNA was isolated from a denaturing gel and used for second strand synthesis by the Klenow fragment of polymerase I. The hairpin was cleaved with S_1 nuclease. The ends were repaired with T4 DNA polymerase and then blunt-end ligated into the *Hin*dII site of mp7. The *NG9*/3 clone was selected by *in situ* plaque hybridization with the *NG9*/V_κ3 insert. The *NG9*/3 insert was obtained in both orientations in M13 vectors and was completely sequenced on both strands by the chain termination method[22].

For more rigorous quantitative analysis, total RNA from spleen or peripheral blood lymphocytes was hybridized in solution to saturation with an excess of a single-stranded, uniformly ^{32}P-labelled restriction fragment containing $V_\kappa 1$, $V_\kappa 3$ or C_κ sequences. RNA–DNA hybrids were detected by resistance to the single-strand-specific exonuclease VII (exoVII). An exonuclease was chosen to preserve hybrids with internal mismatches. The endonuclease S_1 reduced the yield of full-length resistant hybrids with the V_κ probes by fourfold relative to exoVII. The restriction fragment probes had irrelevant vector or intron sequences at the ends, so the shorter nuclease-resistant RNA–DNA hybrids could be resolved on a gel. Figure 3 shows an example of the results. Arrows indicate the exoVII-resistant fragments corresponding to full-length protection of the immunoglobulin-coding sequences. The results of several experiments with RNA from two spleens are summarized in Table 1. The data were quantitated by counting the radioactivity in bands excised from gels. After correcting for the different numbers of ^{32}P-labelled nucleotides in the nuclease-resistant fragments, the relative amounts of hybridization of the V_κ and C_κ probes was calculated. In both spleens over 20% of κ mRNA was detected by each of the two V_κ probes.

It was important to establish whether or not the $V_\kappa 1$ and $V_\kappa 3$ genes cross-hybridize with the same mRNAs in the nuclease-resistance assay. In a control experiment, the *HK101* $V_\kappa 1$ sequence did not form an exoVII-resistant hybrid with $V_\kappa 3$ mRNA from the NG9 hybridoma (data not shown), showing that these two V_κ probes cannot cross-react with identical subsets of the κ mRNA population. When a mixture of the two probes ($V1+3$) was hybridized to RNA in a 5:1 DNA:RNA molar ratio of each, the amount of hybridization equalled the sum of that observed with each probe alone in a 10:1 molar ratio ($V1+V3$; see Fig. 3, Table 1). This result shows that the hybridizations proceeded to completion and that the two cloned V_κ genes react with completely non-overlapping subsets of κ mRNA. These experiments contrast with filter hybridizations in which $V_\kappa 1$ and $V_\kappa 3$ sequences readily cross-react. The reasons for the greater specificity of the RNA–DNA solution hybridization experiments are that the hybrids are formed in more stringent conditions and then subjected to nuclease digestion. It is most likely the $V_\kappa 1$ and $V_\kappa 3$ genes only form exoVII-resistant hybrids with mRNAs from their respective subgroups.

The family of germ-line genes in Fig. 2*a* includes subgroups 1 and 3 sequences and their expression is therefore given by the sum of the $V_\kappa 1$ and $V_\kappa 3$ components of spleen mRNA. In spleen *a*, at least 61% and in spleen *b*, at least 53% of κ mRNA is homologous to these 25 germ-line *V* genes (Table 1). These are minimum values because it is unlikely that the two V_κ probes would form full-length exoVII-resistant hybrids with every transcript homologous to this family of germ-line genes. The exoVII protection experiments therefore substantiate the value of 75% determined by Northern blot hybridization (Fig. 2*b*). The large amount of $V_\kappa 1$ and $V_\kappa 3$ mRNA in spleen was also observed in peripheral blood lymphocytes (PBLs) from another donor. Over

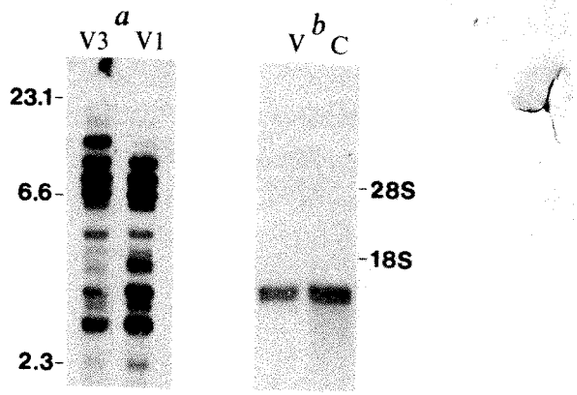


Fig. 2 *a*, Southern blot hybridization of $V_\kappa 1$ and $V_\kappa 3$ probes to human DNA. The author's peripheral blood DNA (10 μg) digested with *Bgl*II was electrophoresed in a 1% agarose gel and transferred to nitrocellulose. Adjacent tracks from the gel were hybridized to $V_\kappa 1$- and $V_\kappa 3$-specific restriction fragments labelled by nick translation or filling-in after exonuclease digestion with T4 DNA polymerase. The probes were the 558-bp *Pst*I fragment from *HK101*/80[3] which extends from 130 bases upstream of the gene to codon 79 and the 266-bp insert from *NG9*/3 excised with *Bam*HI. Hybridization was according to ref. 23 in 5×SSC, 50% formamide at 42 °C for 24 h. The blots were washed at 65 °C in 2×SSC, 0.1% SDS for 30 min and then in 50 mM Tris-HCl, *p*H 7.6, 10 mM sodium pyrophosphate, 2 mM Na_2EDTA, 1× Denhardt's solution, 0.05% SDS, 0.05% sarkosyl for 15 min. *b*, Northern blot hybridization of V_κ and C_κ probes to human spleen RNA. Poly(A)$^+$ RNA (2 μg) from spleen *b* was electrophoresed in a 1.2% formaldehyde agarose gel and blotted onto nitrocellulose[24]. Adjacent tracks were hybridized to nick-translated restriction fragments (about 1×10^8 d.p.m. per μg, 1×10^6 d.p.m. ml^{-1}) in conditions described in Fig. 2*a*. The V_κ probe was the same fragment of the *HK101* gene used in *a*. The C_κ probe was the 469-bp insert from the clone M13/C_κ6 excised with *Bam*HI[10]. The fragment extends downstream from codon 115 in the constant region. The positions of *Xenopus laevis* ribosomal RNA markers are shown.

60% of κ mRNA from PBLs hybridized to a mixture of $V_\kappa 1$ and $V_\kappa 3$ probes (Fig. 3). The diversity of the V_κ mRNA sequences detected is not known, although this important question could be answered by sequencing lymphocyte cDNA clones. What is clear from these experiments is that the majority of κ mRNA in human lymphocytes is encoded by about 25 germ-line *V* genes.

The frequencies of $V_\kappa 1$ and $V_\kappa 3$ mRNAs in spleen correspond with the frequencies of these subgroups in serum κ chains of various individuals and in the panel of sequenced myeloma proteins. The amount of subgroup 3 among serum κ-chains has been measured by serological means[12], by N-terminal sequencing of pooled light chains[13] and by isolation of a subgroup-specific peptide[14]. These studies gave average values of 21–31%. Thirty-four per cent (62/182) of sequenced myeloma chains

Table 1 Amounts of hybridization of V_κ and C_κ probes to spleen RNA

	C	*V1*	*V1/C*	*V3*	*V3/C*	*V1+V3/C*	*V1+V3/V1+3*
Spleen *a*	2.54±0.14 (3)	1.00±0.20 (6)	39%	0.55±0.03 (5)	22%	61%	100%±14% (5)
Spleen *b*	0.81±0.03 (3)	0.19±0.04 (3)	23%	0.24±0.03 (3)	30%	53%	101%±27% (3)

Values are given in pmol X 10^{-4} of probe hybridized per μg of total RNA. Means and standard deviations from at least three independent experiments are given. The number of determinations is given in parentheses. Gel slices were counted with 63% efficiency in 0.2 ml soluene (Packard) plus 2 ml Omnifluor (NEN). Actual values were between 200 and 1,200 c.p.m. Background from an equal area of gel immediately above the bands was substracted. Background values were ≤35% of total counts. The amount of probe hybridized was calculated from the number of T residues in each exoVII-resistant fragment, and the specific activity of [α-^{32}P]dTTP in the probe synthesis reaction (see Fig. 3, Methods). The number of Ts in the *V1*, *V3* and *C* fragments were 60, 52 and 37, respectively. The RNA preparation from spleen *b* was degraded relative to that from spleen *a* and this explains the lower amounts of hybridization observed. $V1+V3/V1+3$ is the ratio of the sum of the amounts of hybridization of the $V_\kappa 1$ and $V_\kappa 3$ probes alone (0.008 pmol of each per hybridization) to the amount of hybridization of a mixture of the $V_\kappa 1$ and $V_\kappa 3$ probes (0.004 pmol of each per hybridization).

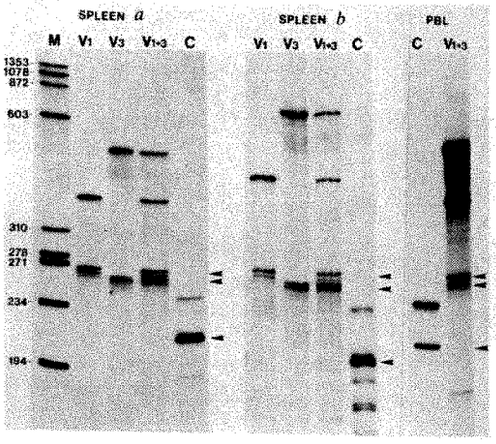


Fig. 3 Analysis of spleen and peripheral blood lymphocyte (PBL) RNA with $V_\kappa 1$, $V_\kappa 3$ and C_κ probes by exoVII protection. For each track total RNA (spleen, 3 μg; PBLs, 1 μg) prepared according to ref. 27 was hybridized to a single-stranded uniformly labelled probe, the hybrids digested with exoVII and electrophoresed on a 5% sequencing gel. Two spleens, *a* and *b*, were obtained from transplant donors. PBLs from a third individual were purified by density gradient sedimentation on Lymphoprep (Pharmacia). The tracks are labelled with the relevant probes *V*1, *V*3, *C* or *V*1 + *V*3 which was an equimolar mixture of the two *V* probes. The fragments corresponding to full-length protection of immunoglobulin sequences in the probe are arrowed. Some undigested probe is evident in each track. The larger number of less than full-length resistant fragments seen in spleen *b* is due to partial degradation of this RNA sample as judged from ethidium bromide-stained agarose gels. DNA size markers are indicated for spleen *a*. The three RNA samples shown were analysed on separate gels.

Methods: Preparation of single-stranded probes. The 'prime-cut' probes complementary to mRNA were made by primed synthesis on a single-stranded M13 template followed by cutting with a restriction enzyme. The fragment extending from the primer to the restriction site was isolated from the longer M13 template strand on a sequencing gel. In a typical reaction 1 μg of template (0.4 pmol) was annealed to 5 ng of universal 17mer sequencing primer[25] in 5 μl by heating for 30 s to 80 °C and incubating at room temperature for 20 min in 20 mM Tris-HCl *p*H 8.5, 100 mM NaCl, 20 mM $MgCl_2$, 2 mM dithiothreitol[22]. The annealing mix was diluted twofold by addition of three unlabelled nucleotide triphosphates to 100 μM and one [α-^{32}P]-labelled triphosphate at 52.5 μM with a specific activity of 37 Ci mmol^{-1}. Klenow fragment was added to 250 U ml^{-1} and the reaction incubated at room temperature for 15 min. The reaction was terminated by heating to 65 °C for 5 min and then 5–10 units of restriction enzyme was added and the mix incubated at 37 °C for 1 h. The digestion was terminated by adding Na_2EDTA to 10 mM and SDS to 0.2% and an equal volume of 98% formamide, 10 mM Na_2EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue. It was boiled for 5 min and loaded on a thin denaturing sequencing gel. Single-stranded fragments were eluted from gel slices by soaking overnight at 37 °C in 0.2 ml of 0.5 M ammonium acetate, 10 mM magnesium acetate, 0.1 mM EDTA, 0.1% SDS[26]. The typical yield was 0.1 pmol or 15 ng of a 450-base fragment. Prime-cut probes synthesized in this way have an average specific activity of 6×10^7 d.p.m. per μg and are stable for several weeks. ***C*$_\kappa$ probe:** The template M13/*C*$_\kappa$6 contains a 469-base fragment starting at codon 115 and extending 170 bases into the 3′-untranslated region. This fragment was inserted into the *Hin*dII site of *mp7*. The probe was prepared by cutting with *Sac*I which cleaves between codons 201 and 202. It contained 208 bases of C_κ sequence and 35 bases of M13 sequence at the 5′ end. ***V*$_\kappa$*1* probe:** The template M13 101/320 contains a 326-base *Rsa*I–*Pst*I fragment of the *HK101* gene inserted into the *Sma*I–*Pst*I site of *mp8*. This fragment contains 77 bases of the intron and extends to codon 79. The probe was prepared by cutting with *Eco*RI which cleaves in the M13 polylinker seven bases from the end of the insert. It contained 249 bases of immunoglobulin-coding sequences and 112 bases of M13 and intron sequences at the 5′ and 3′ ends. ***V*$_\kappa$*3* probe:** The template M13 *NG9*/10 is a subclone of *NG9*/3 which contains a 243-base fragment extending from the *Hin*dII site at codon 4 to codon 84 cloned into the *Sma*I–*Bam*HI site of *mp8*. The probe was prepared by cutting with *Pvu*II which cleaves the M13 vector 183 bases from the end of the insert. It contained 243 bases of immunoglobulin-coding sequence and 232 bases of M13 sequence at the 5′ and 3′ ends. **Hybridization and exoVII digestion:** 3 μg of unfractionated spleen RNA or 1 μg of PBL RNA was hybridized to 0.008 pmol (a 10-fold molar excess over κ mRNA in spleen) of prime-cut probe (0.5–2 ng) in 5 μl of 20 mM Tris-HCl *p*H 8.0, 50 mM NaCl, 10 mM Na_2EDTA at 67 °C for 24 h in a sealed capillary. When a mixture of the two V_κ probes (*V*1 + 3) was used, 0.004 pmol of each was added. The hybrids were digested in 100 μl of the same solution containing 4 U ml^{-1} of exoVII (BRL) for 1 h at 37 °C. The reaction was terminated by ethanol precipitation and the products prepared for electrophoresis on a sequencing gel.

are subgroup 3[5]. In agreement with these values, the *NG9* $V_\kappa 3$ DNA probe hybridized to 22% and 30% of spleen RNA in two individuals. Subgroup 1 constituted 28% and 25% of serum κ chains in two studies[13,14] while 44% (81/182) of myeloma chains belong to this subgroup[5]. These values agree with frequencies of 39% and 23% obtained by hybridization of the *HK101* $V_\kappa 1$ gene to spleen RNAs. In short, the data show that spleen mRNA is a faithful representation of the κ-chain repertoire at least insofar as the contribution of the different subgroups is concerned. The agreement of the $V_\kappa 1$ and $V_\kappa 3$ frequencies determined by protein analysis and by DNA–RNA hybridization, suggests that the two cloned genes hybridize with nearly all the expressed subgroups 1 and 3 *V* region sequences. This conclusion would not be valid if each of the *V* probes by chance hybridized to transcripts from subsets of $V_\kappa 1$ and $V_\kappa 3$ genes that were expressed to a disproportionately high extent so as to coincide fortuitously with the protein data. Barring this highly unlikely possibility, the results show that close to all subgroup 1 and subgroup 3 *V*-region sequences are encoded in the germ-line by only about 25 genes.

The quantitation of V_κ sequences in lymphocyte RNA agrees with previous gene-counting experiments which showed that four quite distantly related human and mouse V_κ probes all hybridized to the same small family of *V* genes in human DNA[3]. Based on the extent of cross-hybridization observed among human V_κ sequences, it was argued that the 25 germ-line V_κ genes detected, constituted a large proportion of the total V_κ gene pool. It has now been demonstrated that this family of genes encodes over 50% of κ mRNA in human lymphocytes. Together, these data suggest that the total number of V_κ genes in the human germ line is 50 or fewer. Cory *et al.*[15] estimated that the most probable number of V_κ genes in the mouse was 90, based on statistical analysis of Southern blots hybridized to 10 different V_κ sequences. This result is consistent with the fact that the $V_\kappa 21$ group encoded by six to eight germ-line genes[16] comprises 8% of serum light chains[17]. Thus, the number of V_κ genes in the mouse germ line may also be quite small although greater than in the human germ line. Although the overall contribution of somatic mutation to the murine antibody repertoire is unknown, several examples of somatic mutation of individual *V* genes have been documented (see, for example, refs 18–20).

Comparison of the number of germ-line V_κ genes with the number of κ chains expressed by an individual allows one to assess the relative importance of the germ-line and somatic components of diversity. The number of different κ variable regions expressed in an individual is not known but it may be approximated by the repertoire of myeloma chains in the human population. Because of polymorphism, the myeloma model gives a maximum estimate for the repertoire of an individual. Quattrocchi *et al.*[21] analysed 52 myeloma proteins by peptide mapping and found that no two were identical. Also, 36 κ *V* regions of myeloma chains have been fully or almost fully sequenced[5] and no two have identical amino acid sequences up to position 95. These observations imply that in addition to the contribution of *V*–*J* joining, the number of V_κ gene-encoded sequences (amino acids 1–95) among myeloma chains is[21] at least 1,000. Direct estimates of the number of different antibodies expressed by individual mice predict a repertoire of several thousand κ-chains[1] and it is hard to see why the human repertoire should be very much smaller. It is likely, therefore, that the repertoire of V_κ gene-encoded sequences in an individual human is at least

several hundred. The small number of germ-line V_κ sequences apparently cannot account for the repertoire without extensive diversification by somatic mutation.

I thank the MRC Laboratory of Molecular Biology, T. Rabbitts, M. Bretscher and H. Weintraub for facilities, C. Milstein, M. Wickens, M. Dunaway and M. Groudine for helpful suggestions, H. Devitt for typing and particularly S. Satchwell for DNA sequencing. D.L.B. is a Beit Memorial Fellow.

Received 3 August; accepted 6 October 1983.

1. Sigal, N. & Klinman, N. *Adv. Immun.* **26,** 255–337 (1978).
2. Tonegawa, S. *Nature* **302,** 575–581 (1983).
3. Bentley, D. & Rabbitts, T. *Cell* **24,** 613–623 (1981).
4. Milstein, C. *Nature* **205,** 1171–1173 (1965).
5. Kabat, E., Wu, T., Bilofsky, H., Reid-Miller, M. & Perry, H. *Sequences of Proteins of Immunological Interest* (US Dept of Health & Human Services, Washington DC, 1983).
6. Milstein, C. & Pink, J. *Prog. Biophys. molec. Biol.* **21,** 209–263 (1970).
7. Zeelon, E., Bothwell, A., Kantor, F. & Schechter, I. *Nucleic Acids Res.* **9,** 3809–3819 (1981).
8. Bentley, D. & Rabbitts, T. *Nature* **288,** 730–733 (1980).
9. Galfre, G. & Milstein, C. *Immunology* **45,** 125–128 (1982).
10. Bentley, D. & Rabbitts, T. *Cell* **32,** 181–189 (1983).
11. Bentley, D. thesis, Cambridge Univ. (1982).
12. Solomon, A. & McLaughlin, C. *J. exp. Med.* **130,** 1295–1311 (1969).
13. Grant, J. & Hood, L. *Immunochemistry* **8,** 63–79 (1971).
14. Milstein, C., Milstein, C. & Feinstein, A. *Nature* **221,** 151–154 (1969).
15. Cory, S., Tyler, B. & Adams, J. *J. molec. appl. Genet.* **7,** 103–116 (1981).
16. Valbuena, O., Marcu, K., Weigert, M. & Perry, R. *Nature* **276,** 780–784 (1978).
17. Julius, M., McKean, D., Potter, M. & Weigert, M. *Molec. Immun.* **18,** 11–17 (1981).
18. Weigert, M., Cesari, I., Yonkovich, S. & Cohn, M. *Nature* **228,** 1045–1047 (1970).
19. Bernard, O., Hozumi, N. & Tonegawa, S. *Cell* **15,** 1133–1144 (1978).
20. Selsing, E. & Storb, U. *Cell* **25,** 47–58 (1981).
21. Quattrocchi, R., Cioli, D. & Baglioni, C. *J. exp. Med.* **130,** 401–415 (1969).
22. Sanger, F., Nicklen, S. & Coulson, A. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
23. Wahl, G., Stern, M. & Stark, G. *Proc. natn. Acad. Sci. U.S.A.* **76,** 3683–3687 (1979).
24. Thomas, P. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5201–5205 (1980).
25. Duckworth, M. *et al. Nucleic Acids Res.* **9,** 1691–1706 (1981).
26. Maxam, A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74,** 560–564 (1977).
27. Auffray, C. & Rougeon, F. *Eur. J. Biochem.* **107,** 303–314 (1980).

A lymphocyte-specific enhancer in the mouse immunoglobulin κ gene

Didier Picard & Walter Schaffner

Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, 8093 Zürich, Switzerland

During differentiation of lymphocytes into antibody-producing cells, an immunoglobulin κ variable-region gene is transcriptionally activated by rearrangement linking it to a κ constant (C_κ) region gene which is already transcribed prior to somatic rearrangement[1,2]. The presence of a transcriptional enhancer element within the large intron of the κ light-chain gene has been postulated to explain this mode of activation, supported by evidence of a chromatin region which is preferentially accessible to DNase I[3–5] and restriction enzymes[5]. This DNA region contains a segment of about 130 base pairs (bp) which is strongly conserved between mouse, rabbit and man[6]. Moreover, no transcripts are detectable from a κ gene, which is truncated within the large intron[7]. Recently, a lymphocyte-specific enhancer has been identified downstream of the joining region in immunoglobulin heavy-chain genes[8–10]. We now show direct evidence for a functionally similar enhancer within the large κ gene intron of the mouse. It is, however, less active than the heavy-chain gene enhancer. In contrast, no enhancer was found to be associated with a cloned λI light-chain gene.

Several DNA fragments from both the κ light-chain gene[11] and the λI light-chain gene[12,13] from the mouse were subcloned into a plasmid carrying the rabbit β-globin gene (Fig. 1). First we took the 2.8-kilobase (kb) *Hin*dIII–*Bam*HI fragment, which contains the 3′ half of the large intron (IVS2) and the entire constant coding region including 3′-flanking sequences of the κ gene, and inserted it downstream of the β-globin gene to yield the recombinant pβκD (Fig. 1*a*). The 7.4-kb *Eco*RI–*Eco*RI segment with the somatically rearranged λ-chain gene was subcloned as *Eco*RI–*Kpn*I fragments to give the recombinants pβλU and pβλ (Fig. 1*b*).

To study the transient expression of the β-globin gene these recombinants were transfected into mouse myeloma cells (cell line X63Ag8) by the DEAE-dextran technique. As a positive control the recombinant pTAR7 was transfected in parallel. This clone contains the immunoglobulin heavy-chain gene enhancer inserted downstream of the rabbit β-globin gene[8]. The cells were collected 40 h after transfection and the cytoplasmic RNA was analysed by a S_1 nuclease mapping experiment (Fig. 2*a*). Both pβκD, containing the κ gene fragment, and pTAR7 give rise to correctly initiated β-globin gene transcripts, although the level of transcription from pβκD is considerably lower (Fig. 2*b*). The finding of transcripts from pβκD is consistent with a recent report showing that deletion of the 2.8-kb *Hin*dIII–*Bam*HI fragment abolishes expression of the κ gene when this truncated gene is introduced into myeloma cells[7]. The recombinants with the λ gene fragments (pβλU and pβλ) gave globin gene transcripts neither in the κ chain-producing myeloma line X63Ag8 (Fig. 2*b*) nor in the myeloma line J558 (data not shown), which produces λI chains. Interestingly, even transcription of the intact λ gene transfected into myeloma cells is only detectable when the gene is linked to the simian virus 40 (SV40) or heavy-chain gene enhancer (unpublished results). This parallels the expression of the λ gene alone or linked to the SV40 enhancer on transfection into human HeLa cells[14].

To narrow down the region of the κ gene responsible for the enhancing effect, we subcloned DNA fragments from the DNase I hypersensitive region found in the large κ gene intron[3–5] (Figs 1*a*, 3*a*). These subfragments do not include the constant coding region, polyadenylation site or 3′-flanking sequences. The clone pβκD1 contains the 266-bp *Alu*I–*Hae*III fragment inserted behind the rabbit β-globin gene. In clone pβκD11 the κ gene fragment extends 209 bp further to the *Alu*I site and thus includes the entire segment of about 130 bp from the large κ gene intron (Fig. 3*b*), which is strongly conserved between mouse, rabbit and man ('κ intron conserved region')[6]. In clones pβκD2 and pβκD22 the inserts are in opposite orientation. These recombinants were also transfected into myeloma cells, and the cytoplasmic RNA was analysed by S_1 mapping. This experiment shows enhancement of β-globin gene transcription by the 475-bp κ gene fragment in either orientation (Fig. 2*c*, lanes 2, 3), whereas removal of 209 bp from the *Hae*III to the *Alu*I site abolishes the effect (Fig. 2*d*). The deletion removes about 60% of the conserved segment (Fig. 3*b*). Note that the 475-bp fragment still retains the full enhancement activity as compared with the 2.8-kb fragment in pβκD (Fig. 2*c*).

It is interesting to consider a few features of the enhancing DNA sequence (Fig. 3*b*). As DNase I hypersensitivity sites are known to be closely associated with the enhancer elements of polyoma virus[15] and SV40 [16], it is noteworthy that the inactive 266-bp κ gene intron segment retains only part of the chromatin region accessible to nucleolytic enzyme cleavage. The stretches of sequence homology[4,6] between the inactive 266-bp segment and the SV40 72-bp repeat or the polyoma enhancer segment are apparently not sufficient for enhancement. It remains to be determined whether the κ intron conserved region contains all of the enhancer activity. The κ gene enhancing element also cannot act in *trans* because co-transfection of a globin gene recombinant and the κ gene fragment subcloned in pUC8 does not yield detectable globin gene transcripts (Fig. 2*c*, lane 4). This is corroborated by the finding that the κ gene enhancer fragment does not have long open reading frames and is not detectably transcribed into an independent RNA[6]. We also conducted a transient expression assay with mouse fibroblast 3T6 cells. In contrast to a globin gene recombinant with the SV40 enhancer, pβκD22 did not give globin gene transcripts (data not shown). This indicates that the κ gene enhancer is lymphocyte specific, as was shown for the heavy-chain gene enhancer[8].

The κ gene enhancer shares common features with the previously characterized viral[17–19] and immunoglobulin heavy-

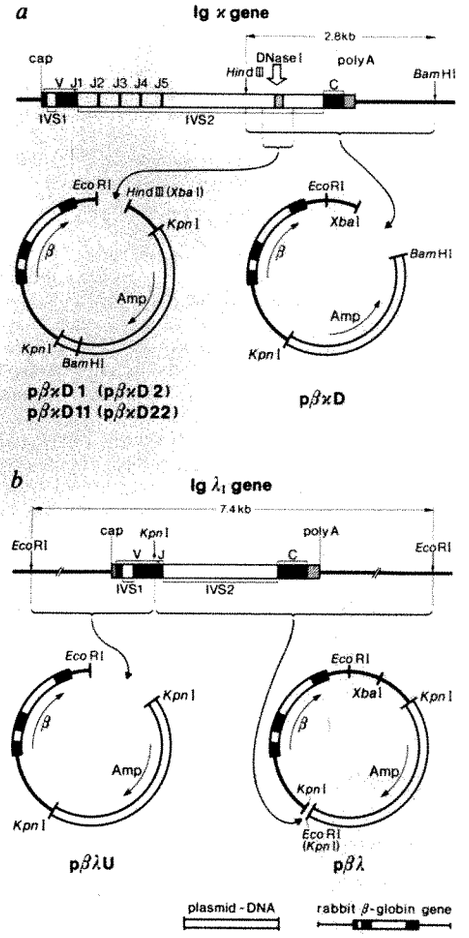


Fig. 1 Schematic representation of recombinant DNAs used. *a*, Diagram of the κ light-chain gene from the mouse myeloma MOPC-41[11,25]. The open arrow indicates the centre of the DNase I hypersensitivity site within IVS2[3–5] positioned according to ref. 4. The stippled box represents the κ intron conserved region (ref. 6, see also Fig. 3). Scheme of the strategy applied to subclone κ gene fragments into the rabbit haemoglobin β1 gene[26] recombinants pβGX and pβG- (ref. 8). pβκD: 2.8-kb *Hin*dIII–*Bam*HI κ gene fragment cloned into pβG- by fusing *Hin*dIII and *Xba*I sites. pβκD1/pβκD11: the small κ gene fragments indicated in Fig. 3 were first subcloned in either orientation into the polylinker of plasmid pUC8[27] and thereafter excised as *Eco*RI–*Hin*dIII fragments to be inserted into pβGX. Clones pβκD1 and pβκD11 thus contain the 266-bp *Alu*I–*Hae*III and the 475-bp *Alu*I–*Alu*I intron fragments (see also Fig. 3), respectively. pβκD2/pβκD22: reversed orientation of 266-bp and 475-bp intron fragments, respectively. *b*, Diagram of the mouse λI light-chain gene[12,13]. pβλU/pβλ: the two fragments generated by a partial *Kpn*I digestion of the 7.4-kb *Eco*RI fragment were inserted into the rabbit β-globin gene recombinant pβG[8]. V, J and C, variable, joining and constant regions of immunoglobulin genes. IVS1 and IVS2, intervening sequences 1 and 2.

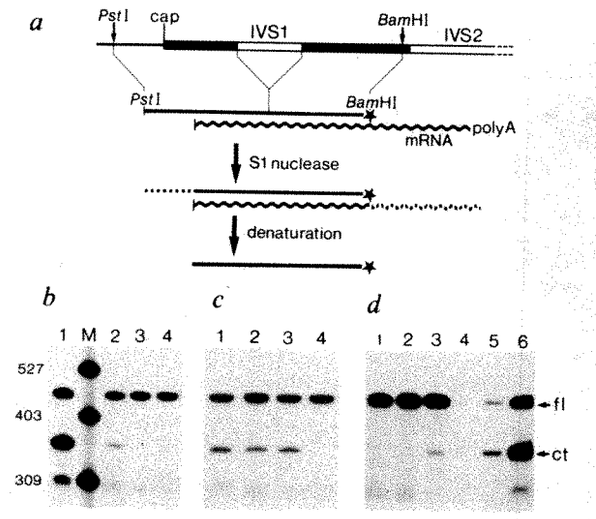


Fig. 2 Enhanced β-globin gene transcription. Tissue culture cell lines were grown and transfected as described earlier[8]. Total cytoplasmic RNA was extracted from the myeloma cells X63Ag8 40–42 h after transfection and incubated with RNase-free DNase[14] to remove residual input plasmid DNA. The recombinant pTAR7 used as a positive control contains the heavy-chain gene *Xba*I enhancer fragment cloned into the *Xba*I site of the rabbit β-globin gene recombinant pβG- (ref. 8, see also Fig. 1 legend). *a*, S_1 nuclease mapping scheme[28]. A β-globin gene clone lacking the first intervening sequence (IVS1)[29] was used to prepare the radioactive probe[30]. DNA end-labelled at the *Bam*HI site was hybridized to unlabelled RNA, treated with S_1 nuclease, denatured, fractionated by gel electrophoresis and autoradiographed (see ref. 17). *b*, Hybridization to 50 μg RNA from X63Ag8 myeloma cells transfected with recombinant pTAR7 (lane 1), pβκD (lane 2), pβλ (lane 3) and pβλU (lane 4). M, end-labelled marker DNA fragments (sizes in nucleotides) of pBR322 digested with *Hpa*II. *c*, Hybridization to 70 μg RNA from X63Ag8 myeloma cells transfected with pβκD (lane 1), pβκD11 (lane 2), pβκD22 (lane 3) and a 1:1 mixture (lane 4) of the 475-bp κ gene fragment cloned into pUC8 and the β-globin gene recombinant pβGX (see Fig. 1*a* legend). *d*, Hybridization to 90 μg RNA from X63Ag8 myeloma cells transfected with recombinant pβκD1 (lane 1), pβκD2 (lane 2) and pβκD11 (lane 3). The DNA probe protected from S_1 nuclease cleavage after hybridization to 90 μg RNA from X63Ag8 cells transfected with pTAR7 was applied to gel electrophoresis in three dilutions: lane 4, 1:50; lane 5, 1:10; lane 6, remaining 80% of protected DNA. By comparing lanes 3 and 5, the relative ratio of β-globin gene transcripts from recombinants pβκD11 (κ gene enhancer) and pTAR7 (heavy-chain gene enhancer) was estimated to be about 1:20. fl, Full-length probe (453 nucleotides); ct, fragment with correct terminus, mapping 354 nucleotides upstream from the *Bam*HI site.

chain gene enhancers[8–10]. It also acts in an orientation-independent manner over a large distance and only works in *cis*. Although there are no extended sequence homologies between the large intron of κ and heavy-chain genes[6], we consider it likely the κ gene fragment acts in the same way as the heavy-chain gene enhancer[8] and not, for example, as an uncontrolled origin of DNA replication which amplifies template copy number. Both immunoglobulin gene enhancers show a remarkable cell-type specificity. However, the κ gene enhancer is only about 5% as active as the heavy-chain gene enhancer (Fig. 2*d*) in our assays. Trivial explanations such as the quality of the DNA preparation have been excluded: the same results were obtained in several S_1 nuclease mapping experiments using independent DNA preparations; neither is it due to a deficiency of the particular myeloma line used (X63Ag8). The κ gene enhancer was also comparatively weak when pTAR7 and pβκD were transfected into the λ chain-producing myeloma J558 (not shown). The same ratio of enhancer activity was observed when the two enhancers were linked to an enhancerless SV40 T-antigen gene, and gene expression in myeloma X63Ag8 and hybridoma Sp6BU6BU was measured by immunofluorescence (not shown). Furthermore, no stimulation of κ gene enhancer activity was seen on incubation of transfected myeloma cells with bacterial lipopolysaccharide (data not shown), which in certain cells of the B-lymphocyte lineage induces the DNase I hypersensitivity region in the κ gene intron and stimulates light-chain gene transcription[3,5].

It therefore appears paradoxical that both κ and heavy-chain gene mRNAs accumulate to about the same level, have a similar turnover in myeloma cells[20], and yet the genes have enhancers of different strength. It seems possible that the low activity of the κ gene enhancer relative to the heavy-chain gene enhancer is compensated by additional transcriptional regulatory elements

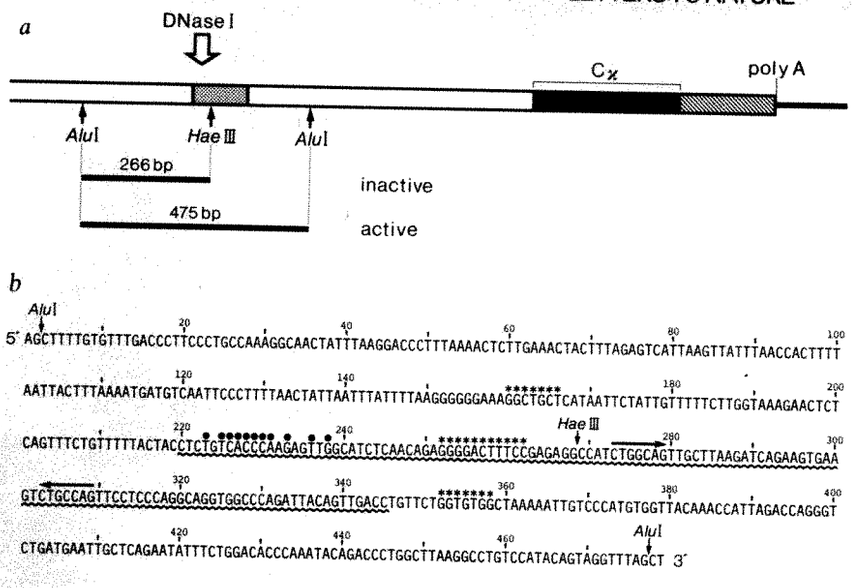


Fig. 3 κ enhancer fragments and nucleotide sequence. *a*, Enlarged schematic representation (see also Fig. 1) of κ light-chain intron (IVS2) and constant region (C_κ). Restriction sites used to generate the 266-bp and 475-bp κ enhancer fragments (black bars) are shown. Stippled box, κ intron conserved region[6]. *b*, Nucleotide sequence[5,25] of the active 475-bp κ enhancer fragment. The wavy line below the sequence represents the κ intron conserved region. Asterisks and dots above the sequence indicate sequence homologies of at least 7 bp to the SV40 (the first two blocks are mentioned in ref. 4) and polyoma virus[6] enhancer elements, respectively. Arrows indicate a 7-bp inverted repeat sequence[5]. The chromatin region, which is preferentially accessible to cleavage by DNase I[3–5] and various restriction enzymes, roughly maps to the sequence between nucleotides 180 and 420[5].

5′ to the region we have analysed. It could also mean that relative enhancer strength is only crucial in an early stage of B-cell differentiation, for example, to ensure that heavy-chain gene transcription precedes κ gene transcription. At later stages maximal transcription of both gene types could be controlled by factors acting in *trans*. In our assays, limiting factors might remain associated with the endogenous chromosomal genes. Such *trans*-acting factors have been described for 5S RNA gene transcription[21] and for 'TATA box' recognition[22], where they form 'preinitiation complexes' which are highly stable. More recently, *trans*-acting viral proteins have been described which permit efficient transcription of viral and nonviral genes even in the absence of enhancer sequences.[23]

This concept could also explain the absence of an enhancer sequence from the λ light-chain gene clone, although the latter finding may be related to the unique structure of murine λ genes[24]. As there is only one λ variable region for each of the two constant region clusters, an enhancer could also be located far upstream of the variable region.

We thank P. Leder, S. Tonegawa, S.-Y. Chung and G. Köhler for gifts of the κ light-chain gene, the λ light-chain gene, the myeloma line X63Ag8 and the myeloma line J558, respectively. We thank H. Hengartner and J. Banerji for valuable discussions, C. Southgate, G. Gilmartin, and M. L. Birnstiel for critical reading of the manuscript, and F. Ochsenbein for preparing the graphics. This work was supported by the Swiss National Research Foundation and by the Kanton of Zürich.

Received 14 September; accepted 28 October 1983.

1. Mather, E. L. & Perry, R. P. *Nucleic Acids Res.* **9**, 6855–6867 (1981).
2. Van Ness, B. G. *et al. Cell* **27**, 593–602 (1981).
3. Parslow, T. G. & Granner, D. K. *Nature* **299**, 449–451 (1982).
4. Chung, S.-Y., Folsom, V. & Wooley, J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2427–2431 (1983).
5. Parslow, T. G. & Granner, D. K. *Nucleic Acids Res.* **11**, 4775–4792 (1983).
6. Emorine, L., Kuehl, M., Weir, L., Leder, P. & Max, E. E. *Nature* **304**, 447–449 (1983).
7. Queen, C. & Baltimore, D. *Cell* **33**, 741–748 (1983).
8. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729–740 (1983).
9. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 717–728 (1983).
10. Neuberger, M. S. *EMBO J.* **2**, 1373–1378 (1983).
11. Seidman, J. G. & Leder, P. *Nature* **276**, 790–795 (1978).
12. Brack, C. & Tonegawa, S. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5652–5656 (1977).
13. Bernard, O., Hozumi, N. & Tonegawa, S. *Cell* **15**, 1133–1144 (1978).
14. Picard, D. & Schaffner, W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 417–421 (1983).
15. Herbomel, P., Saragosti, S., Blangy, D. & Yaniv, M. *Cell* **25**, 651–658 (1981).
16. Cremisi, C. *Nucleic Acids Res.* **9**, 5949–5964 (1981).
17. de Villiers, J. & Schaffner, W. *Nucleic Acids Res.* **9**, 6251–6264 (1981).
18. Banerji, J., Rusconi, S. & Schaffner, W. *Cell* **27**, 299–308 (1981).
19. Moreau, P. *et al. Nucleic Acids Res.* **9**, 6047–6068 (1981).
20. Schibler, U., Marcu, K. B. & Perry, R. P. *Cell* **15**, 1495–1509 (1978).
21. Bogenhagen, D. F., Wormington, W. M. & Brown, D. D. *Cell* **28**, 413–421 (1982).
22. Davison, B. L., Egly, J.-M., Mulvihill, E. R. & Chambon, P. *Nature* **301**, 680–686 (1983).
23. Green, M. R., Treisman, R. & Maniatis, T. *Cell* **35**, 137–148 (1983).
24. Tonegawa, S. *Nature* **302**, 575–581 (1983).
25. Max, E. E., Maizel, J. V. Jr & Leder, P. *J. biol. Chem.* **256**, 5116–5120 (1981).
26. Maniatis, T. *et al. Cell* **15**, 687–701 (1978).
27. Vieira, J. & Messing, J. *Gene* **19**, 259–268 (1982).
28. Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **7**, 1175–1193 (1979).
29. Weber, H. *et al. ICN–UCLA Symp. molec. cell. Biol.* **33**, 367–385 (1981).
30. Rusconi, S. & Schaffner, W. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5051–5055 (1981).

Conservative segregation of nucleosome core histones

I. M. Leffak

Department of Biochemistry, Wright State University, Dayton, Ohio 45435, USA

Density labelling studies have shown that nascent histones are not mixed with parental histones during the assembly of nucleosome cores[1,2]. However, experiments in other laboratories, examining histone deposition with respect to newly synthesized DNA, have been interpreted as suggesting that a substantial proportion of core histones (>15%) are randomized at each chromatin replication[3–8]. The data presented here support our previous results in showing that conservatively assembled nucleosome core histone octamers are conservatively segregated over successive cell generations. It is also shown that the nucleosome cores assembled during 1-β-D-arabinofuranosylcytosine inhibition of DNA synthesis are conservatively segregated for a minimum of five or six cell generations. These results suggest that the nonrandom assembly of nucleosome cores is not merely a coincidence of the mechanism of histone transport into the nucleus and that the conservative mode of nucleosome segregation is a fundamental feature of chromatin replication, one which is stable to modulations in chromatin packaging.

Our laboratory has previously shown that when MSB cells (a line of leukaemic chicken lymphocytes[9]) are incubated for 1 h in medium containing ^{15}N-, ^{13}C-, ^{2}H-substituted amino acids (plus tracer ^{3}H-lysine), the histone synthesized during this incubation (~5–10% of the total histones) bands with a 4–5% increase in density on denaturing isopycnic caesium formate–guanidine hydrochloride (CsFo-GuCl) gradients. By isolation of chromatin immediately after a 1-h density pulse and subsequent cross-linking of nucleosome core histone octamers, it was demonstrated that the ^{3}H-labelled octamers are conservatively assembled; that is, each octamer contains eight density-substituted histones[2].

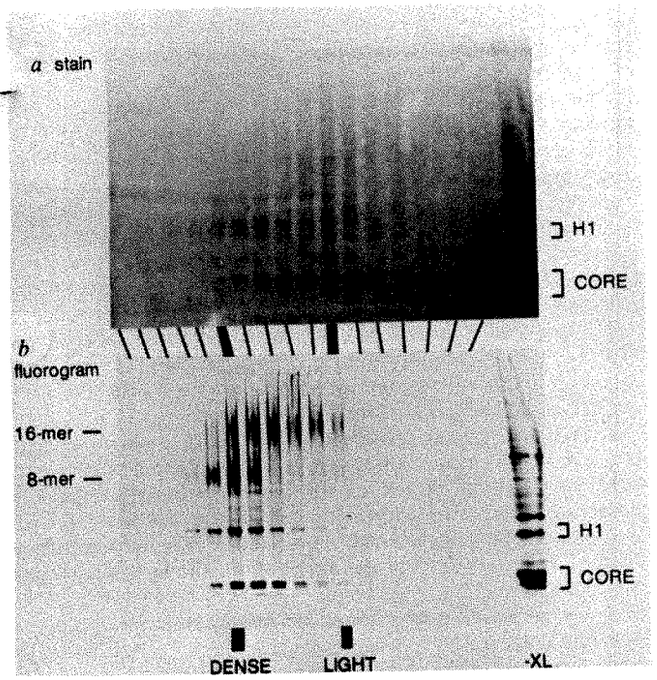


Fig. 1 Conservative segregation of nucleosome core histones. Nucleosome core octamers containing density-labelled, ^{3}H-histones were separated from unlabelled octamers on CsFo-GuCl. The gradient was analysed by SDS-PAGE. *a*, Coomassie blue stained gel. Note that bulk 8-mers and 16-mers are not readily detected due to the strongly decreased binding of Coomassie blue to chemically cross-linked histones[22]. *b*, Fluorogram. 'DENSE' ($\rho = 1.30$ g ml^{-1}) and 'LIGHT' ($\rho = 1.24$ g ml^{-1}) refer to peak positions of uncross-linked, density-substituted, ^{3}H-labelled histone and cross-linked, light, ^{14}C-labelled histone markers run in a parallel gradient. Lane marked −XL represents uncross-linked marker H1-, non-histone-depleted chromatin before centrifugation.

Methods: Exponentially growing MSB cells were washed with phosphate-buffered saline (PBS) and resuspended in 1 ml (8×10^6 cells ml^{-1}) of growth medium containing density-substituted amino acids (Merck MB1808) reconstituted to approximate RPMI-1640. After a 15-min preincubation, ^{3}H-lysine (NEN, 71 Ci mmol^{-1}, 200 μCi ml^{-1}) was added for 1 h. The cells were washed with PBS and returned to RPMI-1640 for a further 72 h of culture. Chromatin depleted in H1 and non-histone protein was isolated, an aliquot was cross-linked with dithiobissuccinimidyl propionate and the mixture of cross-linked and uncross-linked chromatin centrifuged to equilibrium on CsFo-GuCl as described previously[2]. The gradients were collected from below and fractions (65 μl) microdialysed against SDS-PAGE sample buffer[23] omitting β-mercaptoethanol at all times. Fractions were analysed by 5–22% gradient acrylamide SDS-PAGE[23]. The gel was processed for fluorography using Enhance (NEN) and exposed to preflashed[24] Kodak X-Omat AR film at −70 °C.

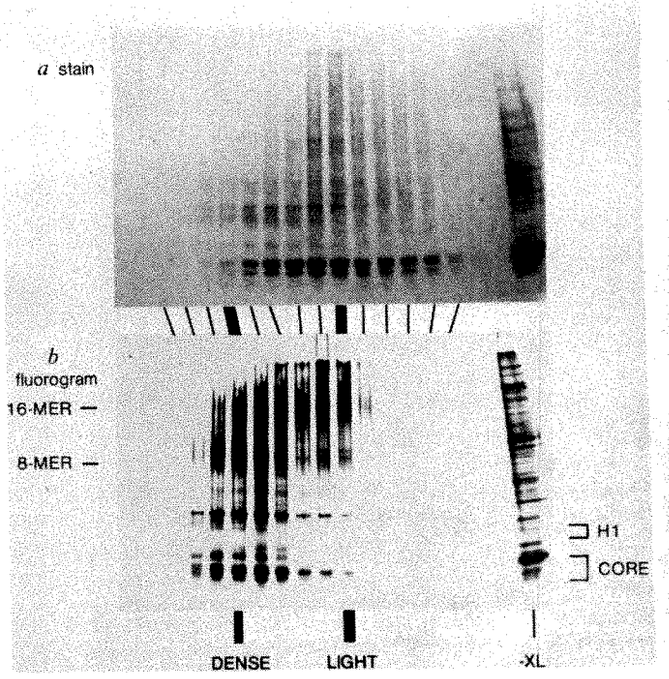


Fig. 2 Conservative segregation of nucleosome cores assembled during ara-C incubation. Octamers containing density-labelled, ^{3}H-histones synthesized during ara-C incubation were separated from unlabelled histone octamers on CsFo-GuCl. The gradient was analysed by SDS-PAGE. *a*, Stained gel; *b*, fluorogram.

Methods: An exponentially growing culture of cells was incubated for 30 min with ara-C (50 μg ml^{-1}), pelleted at low speed and resuspended in density-substituted medium containing ara-C for 30 min. ^{3}H-lysine was added to 200 μCi ml^{-1} and the incubation continued for 90 min. Cells were pelleted, washed with PBS and returned to fresh RPMI-1640 (no drug) for 72 h of culture. Depleted chromatin was isolated, cross-linked and centrifuged on a CsFo-GuCl gradient as in Fig. 1.

The conservative assembly of newly synthesized cores suggests, but does not prove, that the parental histone octamers may be inherited in a nonrandom fashion. To examine directly the stability of core histone octamers, MSB cells were pulsed for 1 h in density-substituted medium (plus tracer ^{3}H-lysine) and then washed and resuspended in their normal growth medium for an additional five or six cell generations. At this point the ^{3}H-labelled, density-substituted histone represents less than 1% of the total histone population.

Chromatin was isolated after the density labelling pulse and five to six generation chase and depleted of histone H1 and several non-histone proteins[2] so as to permit exclusive examination of the core histone octamer. The depleted chromatin was cross-linked with dithiobissuccinimidyl propionate and centrifuged to equilibrium in CsFo-GuCl. On these gradients the DNA pellets, and proteins not cross-linked to one another band independently. The gradient was fractionated and individual fractions analysed by 5–22% acrylamide gradient SDS-polyacrylamide gel electrophoresis (PAGE). The Coomassie blue stained gel of Fig. 1*a* shows the distribution of bulk (light, parental) histone in the gradient. The fluorogram of this gel (Fig. 1*b*) reveals that the histone octamers labelled with ^{3}H-lysine before the chase in unlabelled medium still appear as uniformly dense particles, as defined by the position of uncross-linked dense histones run in the same gradient. Furthermore, the density distribution of the ^{3}H-octamers assayed after the chase is the same as that before the chase[1,2]. These octamers, therefore, have been conservatively inherited.

When MSB cells are incubated in 10^{-4} M 1-β-D-arabinofuranosylcytosine (ara-C), DNA synthesis is inhibited while core histone synthesis proceeds at ~20% of control levels in 100–200-fold excess over nascent DNA[10]. In these conditions, nucleosome cores are assembled conservatively[2]; however, the structure of the chromatin synthesized during ara-C incubation is altered in that these nucleosomes adopt a shortened repeat length. On release from ara-C inhibition, the repeat length of this chromatin slowly returns to control levels[10]. This system has been used to investigate whether the nucleosome histone octamers assembled during ara-C incubation are stably maintained during drug recovery and subsequent cell growth.

MSB cells were incubated in 10^{-4} M ara-C for 2 h in the presence of density-substituted medium plus ^{3}H-lysine tracer, washed and then transferred to normal growth medium for an additional five or six cell generations. Depleted chromatin was isolated, cross-linked as described in Fig. 1 legend and centrifuged to equilibrium in CsFo-GuCl. The stained gel of Fig.

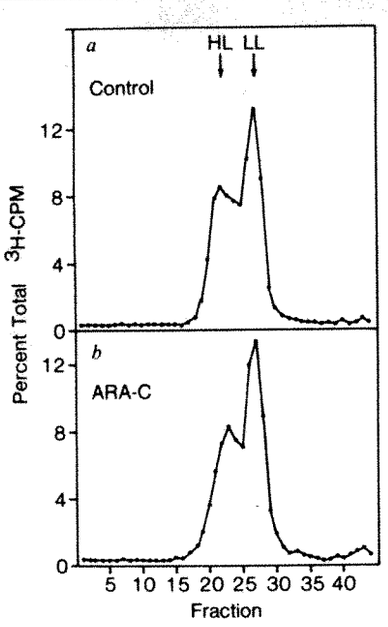


Fig. 3 Replication after ara-C incubation. The incorporation of bromodeoxyuridine by cells labelled 18 h earlier under control (*a*) or ara-C incubations (*b*) with ^{3}H-thymidine was assayed by CsCl density gradient centrifugation.

Methods: An exponentially growing cell culture was split 1 : 1 with fresh RPMI-1640 and incubated with or without ara-C (50 μg ml^{-1}) for 1 h. The cultures were concentrated to 1 ml (10^7 cells ml^{-1}) and incubated with ^{3}H-thymidine (NEN, 24 Ci mmol^{-1}) at 5 μCi ml^{-1} (control) or 200 μCi ml^{-1} (ara-C culture), for 2 h. Each culture was pelleted, washed and resuspended in warmed RPMI-1640 containing 5 μg ml^{-1} thymidine in the presence (ara-C culture) or absence (control) of ara-C for an additional hour. Cultures were pelleted and resuspended in fresh RPMI-1640 in the absence of ara-C for 18–20 h. Bromodeoxyuridine (Sigma) was added to each culture to 50 μg ml^{-1} and the cells were collected after 3 h. DNA was prepared by treatment of isolated nuclei with 10 mM Na_2EDTA, 0.2% SDS, protease K (200 μg ml^{-1}, 3 h, 37 °C), phenol extraction and ethanol precipitation. The DNA was sheared by three passages through a 26-gauge syringe needle, 50 μl mixed with 2.8 ml of 5.93 M caesium chloride and centrifuged in the Beckman SW60 Ti rotor at 30,000 r.p.m. for 48 h. Fractions were collected from below and counted in Beckman Ready-Solv MP. Arrows indicate positions of light (LL) and hybrid (HL) density markers run in parallel. (Control experiments (not shown) demonstrated that greater than 70% of the ^{3}H-thymidine in *b* was incorporated during the ara-C incubation.)

2*a* shows the banding pattern of the bulk histones while the fluorogram of Fig. 2*b* demonstrates that the ^{3}H-labelled histones synthesized during the ara-C incubation comprise uniformly dense octamers after the five to six generation chase in normal medium.

Control experiments (not shown) indicate that >85% of the ^{3}H-histone incorporation occurred during the initial ara-C incubation and that >90% of the ^{3}H-histones are retained after five or six cell doublings.

To show that MSB cells are not precluded from subsequent replications as a result of ara-C incubation, cells were incubated in the presence of ^{3}H-thymidine for 2 h with or without ara-C. After washing and resuspension in drug-free medium overnight, the cells were transferred to medium containing bromodeoxyuridine for 3 h. Figure 3 demonstrates that after the bromodeoxyuridine pulse ~30% of the total cellular DNA is found to exhibit a heavy–light density. (Similar results are obtained when the initial incubation is performed in dense amino acid medium with or without ara-C (not shown).) This is the amount of hybrid density DNA expected based on the fraction of cells in S phase during the bromodeoxyuridine pulse. Therefore, the nucleosome cores assembled during ara-C incubation are able to replicate thereafter, and the histone octamer is stably inherited.

The conservative segregation of histones is a stringent biological test of nonrandom nucleosome assembly. The present results are therefore inconsistent with recent reports that 15% or more of nascent core histones are assembled at random into chromatin[3–8]. In this type of experiment, a result suggesting random deposition or segregation of histones must be considered in the light of undiscerned artefactual randomization. A nonrandom result, however, cannot have arisen artefactually. Randomization of 15% of the core histones at each replication would result in >75% of the ^{3}H-labelled histones banding in hybrid density particles close to the light marker after the five to six generation chase in our experiments. This is clearly not the case.

The present observations agree with the novel experiments of Prior *et al.*[11] showing that exogenous histone H3 molecules taken up by *Physarum* microplasmodia are both conservatively assembled and segregated in nucleosomes.

Although the data presented in this work indicate that, in general, the associations between histones within a nucleosome core are stable during replication, these experiments might not detect an alternative segregation mechanism for a small subpopulation of histones. In this regard, it is of interest that in each of several repeats of the experiments shown in Figs 1 and 2, a small amount of hybrid density ^{3}H-octamers (≤10% of the octamer population, as assayed by densitometry) is observed near the light region of the gradient. While it is possible that a variable 1–2% of the total ^{3}H-histones segregates randomly at each replication, the conservative assembly[1,2] and segregation of the vast majority of histone suggest that it may be more likely that a larger, invariant subpopulation of the ^{3}H-histones (≈10%) segregates following a nonrandom pattern which is distinct from that of bulk histone.

These experiments do not directly address the question of the stability of the interaction between a core histone octamer and a particular DNA strand. Several reports have suggested that during replication parental histones may be nonrandomly segregated with respect to one or the other DNA strand[12–19] (but see ref. 20). The present data are not inconsistent with these observations.

The fluorogram of Fig. 1*b* indicates that after five or six cell generations there has been no mixing of new and old histones within individual octamers; however, most of the 16-mer particles band with hybrid density. In contrast, when 16-mers are examined immediately after deposition, no more than 10–20% show a hybrid density (compare Fig. 2, ref. 2). If the 16-mer particles result from the cross-linking of linearly contiguous octamer cores, the present data suggest that, as a result of repeated replication in normal medium, most dense octamers have come to be alongside light octamers. This situation could arise if individual nucleosomes segregated randomly (with respect to DNA strand) or if a domain of oligonucleosomes segregated conservatively[21] but the boundaries of such a domain were variable.

When DNA synthesis is inhibited by ara-C the deposition of newly synthesized histone octamers is accompanied by a decrease in the nucleosome repeat length[10]. The chromatin is subsequently remodelled such that the repeat length returns to normal. The results presented here show that the integrity of the octamer core is stable despite repositioning with respect to DNA sequence and modulation of chromatin packaging.

My thanks to Ms Charlene Steel for her technical assistance. This work was supported in part by grants from the NIH and NSF.

Received 20 July; accepted 30 September 1983.

1. Leffak, I. M., Grainger, R. & Weintraub, H. *Cell* **12**, 837–845 (1977).
2. Leffak, I. M. *Nucleic Acids Res.* **11**, 2717–2732 (1983).
3. Russev, G. & Hancock, R. *Nucleic Acids Res.* **9**, 4129–4137 (1981).
4. Hancock, R. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2130–2134 (1978).
5. Jackson, V., Marshall, S. & Chalkley, R. *Nucleic Acids Res.* **9**, 4563–81 (1981).
6. Jackson, V. & Chalkley, R. *Cell* **23**, 121–134 (1981).
7. Annunziato, A. T., Schindler, R. K., Riggs, M. K. & Seale, R. L. *J. biol. Chem.* **257**, 8507–8515 (1982).

8. Jackson, V. & Chalkley, R. *J. biol. Chem.* **256,** 5095–5103 (1981).
9. Akiyama, Y. & Kato, S. *Biken J.* **17,** 105–116 (1974).
10. Leffak, I. M. *Nucleic Acids Res.* **11,** 5451–5466 (1983).
11. Prior, C. P., Cantor, C. R., Johnson, E. V. & Allfrey, V. G. *Cell* **20,** 597–608 (1980).
12. Weintraub, H. *Cold Spring Harb. Symp. quant. Biol.* **38,** 247–256 (1973).
13. Weintraub, H. *Cell* **9,** 419–422 (1976).
14. Seale, R. L. *Cell* **9,** 423–429 (1976).
15. Freedlender, E. F., Taichman, L. & Smithies, O. *Biochemistry* **16,** 1802–1808 (1977).
16. Riley, D. & Weintraub, H. *Proc. natn. Acad. Sci. U.S.A.* **76,** 328–332 (1979).
17. Seidman, M. M., Levine, A. J. & Weintraub, H. *Cell* **18,** 439–450 (1979).
18. Roufa, D. *Cell* **13,** 129–138 (1978).
19. Roufa, D. & Marchionni, M. A. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1810–1814 (1982).
20. Fowler, E., Farb, R. & El-Saidy, S. *Nucleic Acids Res.* **10,** 735–748 (1982).
21. Pospelov, V., Russev, G., Vassilev, L. & Tsanev, R. *J. molec. Biol.* **156,** 79–91 (1982).
22. Leffak, I. M. *Analyt. Biochem.* (in the press).
23. Laemmli, U. *Nature* **227,** 680–685 (1970).
24. Laskey, R. A. & Mills, A. D. *Eur. J. Biochem.* **56,** 335–341 (1975).

Timing of the steps in transformation of C3H 10T½ cells by X-irradiation

Ann R. Kennedy, John Cairns & John B. Little

Department of Cancer Biology, Harvard University School of Public Health, Boston, Massachusetts 02115, USA

Transformation of cells in culture by chemical carcinogens or X rays seems to require at least two steps. The initial step is a frequent event; for example, after transient exposure to either methylcholanthrene or X rays, almost every cell of established lines of mouse embryo fibroblasts proved capable of yielding transformed, tumorigenic descendants[1–3]. Although results were interpreted as indicating that 100% of the progeny of methylcholanthrene-treated cells were potentially transformed[1], later experiments showed that only a very small minority of the progeny of cells initiated by X rays[2] or methylcholanthrene[3] actually produced transformed colonies. We thus concluded[2] that there must be a second step in transformation that is a very rare event. We assumed that this event occurred after the cultures became confluent, a time when transformed cells have a selective growth advantage. Since then, however, others have shown that transformation can occur soon after initiation[4] and that clones of transformed cells may already be present by the time initiated cultures become confluent[5]. It has been hypothesized that the second step behaves like a spontaneous mutation in having a constant but small probability of occurring each time an initiated cell divides[3,6]. We show here that the clone size distribution of transformed cells in growing cultures initiated by X rays is, indeed, exactly what would be expected on that hypothesis.

If events such as mutations are scattered at random throughout the lineage of a growing population of cells, the number of mutants finally present will vary greatly from one culture to another; this is the basis for the Luria–Delbrück fluctuation test[7]. With a mutation rate of μ per cell generation, populations undergoing binary fission will on the average have experienced $2\mu N$ mutations by the time they contain N cells, namely, μN events in the last generation, $\mu N/2$ in the previous generation, and so on to give a total of $2\mu N$. The proportion of such populations that have not had an event and therefore contain no mutants will simply be $P_0 = e^{-2\mu N}$. Because clones that contain a single mutant cell can have arisen only among the final generation of cells, the proportion of cultures that contain exactly one mutant will be the proportion that had no mutations before the last generation and exactly one mutation in the last generation, that is, $P_1 = e^{-\mu N}(\mu N\, e^{-\mu N}) = \mu N\, e^{-2\mu N}$. Unfortunately, the expression for the higher terms of the distribution is more complex because any particular number of mutants could have been achieved in several ways; for example, there could be two mutants as the result of two events in the final generation or one event in the previous generation; and as the number of mutants becomes larger, so the ways of achieving that number become more numerous. But the higher terms can be obtained from Lea and Coulson[8] who have tabulated the value of P_r for different values of r and $2\mu N$ (which they call m).

Table 1 Number of transformed cells in cultures exposed to 600 rad, grown to confluence and then reseeded

Treatment group		No. of transformed foci in each dish
Group I		0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
28 cultures, not reseeded		1, 1, 1, 1, 1, 1, 1, 1, 1, 1,
		2, 2, 2, 3, 3, 4, 4, 4
Group II		
27 cultures, each totally		0, 0, 0, 0, 0, 0, 0, 0, 0,
reseeded on to a single dish		1, 1, 1, 1, 1, 1, 1, 1, 2
		2, 3, 4, 4, 5, 5, 7, 12, 19
Group III		
14 cultures, each totally	(*a*)	0, 0, 0, 0, 1, 1, 1, 1, 2, 0, 0, 1, 6, 40
reseeded on to two dishes	(*b*)	0, 0, 0, 0, 0, 1, 2, 4, 5, c, c, c, c, c,
Group IV		0, 0, 0, 0, 0, 0, 0, 0, 1, 1,
28 cultures, each 80%		1, 1, 1, 2, 2, 2, 2, 3, 3,
reseeded on to one dish		4, 4, 5, 5, 5, 13, 15, 22

Stock cultures of C3H 10T½ cells were maintained in 60-mm Petri dishes and were passed by subculturing at a 1:20 dilution every 7 days. The cells used in this experiment were in passage 9. Cells were grown in a humidified 5% CO_2 atmosphere at 37 °C in Eagle's basal medium supplemented with fetal calf serum (10% for the first 2 weeks of the transformation assay period, and 5% for the last 4 weeks) and gentamycin (5 μg ml^{-1}). Dishes were seeded with 12,000 cells and 24 h later were irradiated with 600 rad. As the plating efficiency at this stage in the experiment was 20% and the survival after 600 rad is ~10%, each dish started with an average of 240 surviving irradiated cells. In group I the dishes were not trypsinized. In group II, the cultures were trypsinized as they approached confluence and the cells were then reseeded into their original dishes. In group III, the cultures were trypsinized and reseeded into two separate dishes (*a* and *b*). In group IV, the cells were treated as in group II, except that 20% of the trypsinized suspension was removed to form part of a separate experiment. In the paired sample of group III, c indicates that the culture was lost due to contamination; in groups I, II and IV, dishes lost due to contamination are not included. Types 2 and 3 transformed foci were scored as previously described[2]. When cells are re-plated at the high cell densities used here, the plating efficiency is assumed to be at least 50%. We have previously observed that about 50% of cells transformed by X-irradiation will grow, and form transformed foci when reseeded onto confluent monolayers of untreated C3H10T½ cells[15].

To determine the actual distribution of transformed clone sizes, cultures of C3H 10T½ cells were irradiated with 600 rad of X rays, a dose that kills 90% of the cells, and initiates virtually all those that survive[2]. After growth of the surviving cells to near confluence (about 13 generations), each culture was trypsinized, suspended and totally reseeded on to one or more plates that were maintained for 4 additional weeks, at which time the foci of transformed cells were counted; at the same time, some plates were not reseeded, but were left untouched.

As Table 1 shows, the process of trypsinization and reseeding did not produce any change in the proportion of cultures having one or more transformed foci. Roughly one-third of the cultures yielded no transformed foci. Therefore, $2\mu N$ should be approximately equal to one. Table 2 compares the observed distribution of transformed clone sizes with the values expected on the assumption that the rare step in transformation occurs at random during exponential growth of the progeny of initiated cells and that there is, on the average, one such event per culture. As the differences between observed and expected are not significant, it seems that the second step is indeed occurring at random. The stages in such *in vitro* transformation can therefore be summarized as follows.

Although the initial step is driven by mutagenic carcinogens and creates a fairly stable heritable state, it is unlikely to be a mutation because a single treatment with X rays or methylcholanthrene can initiate almost every cell[1–3,9]. Furthermore, lower doses of initiator seem to place cells in an intermediate

Table 2 Comparison of expected and observed distributions of clone sizes for the data presented in Table 1

	No. of transformed colonies in reseeded groups (r)								
	0	1	2	3–4	5–8	9–16	17–32	33–64	>64
Probability that a culture has r transformants (mutants) when the average number (m) of events (mutations) per culture is 1 (ref. 8, Table 2)	0.3679	0.1839	0.1073	0.1164	0.0961	0.0617	0.0335	0.0168	0.0163
Data for 36 reseeded cultures of groups II and III (i)									
Observed no. of dishes containing r transformed foci	13	9	3	4	5	1	1	0	0
Expected no., from the above probabilities	13.2	6.6	3.9	4.2	3.5	2.2	1.2	0.6	0.6
Data for 69 reseeded dishes of groups II–IV (ii)									
Observed no. of dishes containing r transformed foci	23	14	9	8	8	4	2	0	1
Expected no., from the above probabilities	25.4	12.7	7.4	8.0	6.6	4.3	2.3	1.2	1.1

Comparison (i) excludes those pairs of dishes in group III where one dish was contaminated. The clone size for the uncontaminated pairs was taken to be the sum of the transformed colonies in the two dishes. Comparison (ii) is for all the reseeded groups. The count for any contaminated dish in group III was assumed to be the same as its uncontaminated companion dish. No correction was made in group IV for the fact that only 80% of each culture was reseeded.

condition. In the present experiments, 600 rad produced an initiated state that survived unchanged for at least 13 cell generations, but others have shown that the initiated state produced by exposure to 1.0 μg ml^{-1} methylcholanthrene is unstable, with about a quarter of the cells reverting to normality in each generation[3]. Similarly, in experiments reported elsewhere[9–11], we have found that treating initiated cultures with phorbol esters will greatly increase the number of transformants if the initiation was with 100–400 rad, but has hardly any effect after 600 rad; this too suggests that there are degrees of initiation. At present, the most likely hypothesis is that the initial step consists of some change in the pattern of gene expression that, like the SOS response of bacteria treated with mutagens, determines the frequency of subsequent rare genetic events. Even though initiation is much more stable than the SOS response, this hypothesis is supported by our finding that certain protease inhibitors, which are known to block the SOS response[12], will reverse the initial step in transformation[13].

In contrast, the second step is a rare, random event, occurring in slightly less than one in a million descendants of cells that have taken the first step. In the experiment reported here, the largest clone of transformed cells did not arise until 6 cell divisions after X-irradiation (that is, about 7 generations before the culture was reseeded) and most of the clones arose after 12–13 divisions. Thus, the second step cannot be the direct result of the lesions produced in DNA by the initial dose of radiation. We have observed (unpublished experiments) that the number of transformed foci arising in cultures derived from cells fully initiated with 600 rad of X rays cannot be significantly raised by treating the cultures as they approach confluence with mutagens such as X rays or UV light. If the second step is a mutation (as its frequency and random distribution might suggest), it must represent an unusual class of genetic event that is not readily driven by mutagens.

Although the nature of the steps in the neoplastic transformation of established, immortal cell lines by carcinogens is not understood, what appears to be kinetically a rather similar sequence of events has been observed for radiation-induced recombination in yeast[14]. Additional steps would probably be needed to transform normal cells *in vivo* and the kinetics of these steps have not yet been studied in similar detail.

We thank Mrs Babette Radner for technical assistance in these studies. This research was supported by contract 77EVO4322.A002 from the US Department of Energy and Center Grant ES-00002 from the US National Institute of Environmental Health Sciences.

Received 17 June; accepted 19 October 1983.

1. Mondal, S. & Heidelberger, C. *Proc. natn. Acad. Sci. U.S.A.* **65,** 219–225 (1970).
2. Kennedy, A. R., Fox, M., Murphy, G. & Little, J. B. *Proc. natn. Acad. Sci. U.S.A.* **77,** 7262–7266 (1980).
3. Fernandez, A., Mondal, S. & Heidelberger, C. *Proc. natn. Acad. Sci. U.S.A.* **77,** 7272–7276 (1980).
4. Backer, J. M., Boerzig, M. & Weinstein, I. B. *Nature* **299,** 458–460 (1982).
5. Hall, E. J., Rossi, H. H., Zaider, M., Miller, R. C. & Borek, C. in *Neutron Carcinogenesis* (eds Broerse, J. J. & Gerber, G. B.) 381–405 (Commission of the European Communities, Luxembourg, 1982).
6. Barrett, J. C. & Elmore, E. in *Handbook of Experimental Pharmacology* (eds Andrews, L. S., Lorentzen, R. J. & Flamm, W. G.) (Springer, Berlin, in the press).
7. Luria, S. E. & Delbrück, M. *Genetics* **28,** 491–511 (1943).
8. Lea, D. E. & Coulson, C. A. *J. Genet.* **49,** 264–285 (1949).
9. Kennedy, A. R. & Little, J. B. *Carcinogenesis* **1,** 1039–1047, 1980.
10. Kennedy, A. R., Mondal, S., Heidelberger, C. & Little, J. B. *Cancer Res.* **38,** 439–443 (1978).
11. Yavelow, J., Finlay, T. H., Kennedy, A. R. & Troll, W. *Cancer Res.* **43,** 2545–2459 (1983).
12. Meyn, M. S., Rossman, T. & Troll, W. *Proc. natn. Acad. Sci. U.S.A.* **44,** 1152–1156 (1977).
13. Kennedy, A. R. & Little, J. B. *Nature* **276,** 825–826 (1978).
14. Fabre, F. & Roman, H. *Proc. natn. Acad. Sci. U.S.A.* **74,** 1667–1671 (1977).
15. Kennedy, A. R. & Little, J. B. *Cancer Res.* **41,** 2103–2108 (1981).

ANNOUNCING

NEW FOR 1984

Pharmaceutical Medicine

The contents will include:

★ **Original papers**
- on methods used to evaluate the efficacy and safety of drugs in man
- with emphasis on the validation of these methods
- covering human pharmacology, clinical research, drug epidemiology, and safety surveillance
- and especially the application of new methods to drug testing

★ **Abstracts of methodological studies published elsewhere**
- either used already in the context of drug investigation
- or methods suitable for drug evaluation

★ **Invited reviews on topics related to pharmaceutical medicine**

★ **Editorials and Commentaries**
- offering opinions
- high-lighting problems

★ **Correspondence, Book Reviews, and Calendar**

Editor
Robert N Smith MD FRCP
Director of the Medical Division, Glaxo Group Research Limited, Greenford; formerly Senior Lecturer and Consultant Physician, Clinical Pharmacology and Therapeutics, University of Sheffield

Editorial Advisory Board
Daniel L Azarnoff MD FACP
President, Research and Development, G D Searle & Company, Chicago; formerly Professor of Medicine and Pharmacology, University of Kansas Medical Center
Michael G Carter MB BPharm
Director of Pharmaceutical Division, Roche Products Limited, Welwyn Garden City
Arthur H Hayes, Jr, MD
Dean and Provost, New York Medical College, New York City; formerly FDA Commissioner, Washington DC
William P Maclay MB DTM&H
Director of Medical Affairs, Sandoz Products Limited London
Sir Abraham Goldberg MD FRCP
Regius Professor of the Practice of Medicine, University of Glasgow; Chairman, Committee on Safety of Medicines
J Desmond Fitzgerald MD FRCP
Medical Director, Imperial Chemical Industries plc, Macclesfield; formerly Professor of Medicine, MacMaster University, Hamilton, Ontario
Cyril Maxwell MB ChB
Medical Director and Chief Executive, Clinical Research Services Limited, London; formerly Medical Director, Richardson-Merrell Limited
Paul Turner MD FRCP
Professor of Clinical Pharmacology, St Bartholomew's Hospital Medical College, London
Derek C Quantock PhD MD
Director of Science and Technology, Fisons Limited, Loughborough; formerly Medical Director, Association of the British Pharmaceutical Industry
Peter S Schönhöfer MD
Research Coordination, Institute für Arzneimittel, Bundesgesundheitsamt, Berlin; formerly Professor of Pharmacology, Medizinsche Hochschule, Hannover
Julian H Shelley BSc MRCP
Head of Medical Research, Boehringer Ingelheim International GmbH, Brackneil; Visiting Lecturer in Pharmacology, King's College, London
Geoffrey R Venning BM FRCP
Director of Research and Development, Janssen Pharmaceutical Limited, Marlow; formerly Senior Medical Officer, Medicines Division, DHSS
William M Wardell DM DPhil
Associate Professor of Pharmacology and Assistant Professor of Medicine; Director, Center for the Study of Drug Development, University of Rochester Medical Center

☐ Please send me a guide to contributors
☐ Please send me further information
☐ Please reserve me a sample copy of issue No. 1

NAME _____

ADDRESS _____

To: David N Guthrie,
Scientific and Medical Division,
The Macmillan Press Ltd.,
Houndmills, Basingstoke, Hampshire
RG21 2XS, U.K.

Subscription Information
Frequency: 4 issues per year
Language: English
Rates per volume of 4 issues:
UK & Eire: £45.00
USA/Canada (surface), US$95.00
Rest of the World (surface), £63.00

SCIENTIFIC & MEDICAL | M | MACMILLAN PRESS LTD

Circle No.67 on Reader Service Card.

New Books Spring 1984: Oxford University Press

Oxford Science Publications
Oxford Medical Publications

Astronomy

Gamma Ray Astronomy

Rodney Hillier

An important new text providing a concise and critical account of the observations in gamma ray astronomy, and their interpretation.

0 19 851451 4 £15 460 pp.
Clarendon Press

Civil Engineering

Introduction to the Theory of Thin-walled Structures

N. W. Murray

A monograph for both students and professional civil engineers, introducing the theoretical aspects of the behaviour of thin-walled structures, particularly in relation to their collapse mode.

0 19 856151 2 £55 400 pp.
Clarendon Press

Electrical Engineering

Lectures on the Electrical Properties of Materials, third edition

L. Solymar and D. Walsh

The third edition of this extensively used text book for engineering and materials science students incorporates much updated work in the areas of semiconductors and lasers.

0 19 851163 9 £20
0 19 851162 0 £8.95 Paperback
420 pp. Clarendon Press

Semiconductor Contacts: an approach to ideas and models

Heinz K. Henisch

The only specialized study of semiconductor contacts, this book will bridge the gap between the available high-level abstractions of the modern theory of solids, and the phenomenological treatments employed in device physics.

0 19 852016 6 £50 400 pp.
Clarendon Press

Oxford Studies in Advanced Manufacturing

Metal Cutting Principles

Milton C. Shaw

For students of materials and mechanical sciences, and research and design engineers in industry this book provides a comprehensive account of the important topic of metal cutting as a means of removing unwanted material in the production of mechanical components.

0 19 859002 4 £60 600 pp.
Clarendon Press

Mathematics for Scientists

Free and Moving Boundary Problems

John Crank

The first work to be devoted to a comprehensive coverage of free and moving boundary problems. It includes a full account of the mathematical formulation of these problems, and offers many new methods for their solution.

0 19 853357 8 £40 424 pp.
Clarendon Press

Oxford Logic Guides

Two new titles:

Subrecursion: Functions and Hierarchies

H. E. Rose

An invaluable text for graduate courses, and a reference work for research workers, this book collects together a number of important results in recursion theory developed over the last thirty years.

0 19 853189 3 £20 200 pp.
Clarendon Press

Cantorian Set Theory and Limitation of Size

Michael Hallett

Fulfils the need for a clear understanding of classical set theory which has been necessitated by the philosophical challenges to Cantorian notions of set and of completed infinity.

0 19 853179 6 £25 280 pp.
Clarendon Press

Royal Microscopical Society Microscopy Handbooks

Oxford University Press and the Royal Microscopical Society announce the publication of the Royal Microscopy Handbooks: a major new series for all users of light and electron microscopes which will provide a balanced range of short and inexpensive books that cover both introductory and advanced topics.

Titles to be published Spring 1984

Introduction to the Optical Microscope,
Savile Bradbury
0 19 854401 £2.95 88 pp.

The Operation of the Transmission Electron Microscope,
Dawn Chescoe and Peter Goodhew
0 19 856402 3 £2.95 64 pp.

Specimen Preparation for Transmission Electron Microscopy of Materials,
Peter Goodhew
0 19 856403 1 £4.95 64 pp.

Histochemical Protein Staining Methods,
J. James and J. Tas
0 19 856406 6 £4.95 64 pp.

Lipid Histochemistry,
Olga Bayliss High
0 19 856405 8 £4.95 64 pp.

Animal Behaviour

Insects in Camera

A Photographic Essay on Behaviour
Christopher O'Toole.
Photographs by Ken Preston-Mafam

In the seven sections, each built round a major theme in the lives of insects, this book illustrates the many and sometimes bizarre ways in which they have responded to the great imperative of evolutionary success: 'adapt or perish'.

0 19 217694 3 287 colour plates
£12.95

Social Odours in Mammals
Edited by Richard E. Brown
and David W. Macdonald

The editors here review current knowledge of mammalian olfactory communication, present new information, and provide a synthesis that emphasizes a wider socio-biological framework.

0 19 857546 7 Illustrated £45
Clarendon Press

Forestry

Plantation Forestry in the Tropics

Julian Evans

'Will be particularly welcomed by all those concerned with forestry and land use in the tropics.'
Quarterly Journal of Forestry
0 19 859489 5 Illustrated
New as Paperback £12.00

Soil Survey

Soil Survey for Engineering

A. B. A. Brink, T. C. Partridge,
and A. A. B. Williams

Provides practical information on basic soil survey procedures for engineering projects. 0 19 854583 5 Illustrated New as Paperback £12.50 *Monographs on Soil Survey* Clarendon Press

Psychology

Visual Masking
An Integrative Approach

Bruno G. Breitmeyer

Here for the first time is a comprehensive and integrated account of visual masking and its related phenomena: visual persistence and integration.
0 19 852105 7 Illustrated £22.50
Oxford Psychology Series 4
Clarendon Press

Child's Talk

Learning to Use Language

Jerome Bruner

Explores how the young child acquires the *uses* of his native language, and how by using language first for limited ends, the child comes finally to recognize its more powerful, productive uses.
0 19 857613 7 £11
0 19 857511 4 Paperback £5.95

Medicine

Transplantation Immunology

Clinical and Experimental
Edited by R. Y. Calne

This major book, written by a team of clinicians and immunologists who have pioneered and developed many new transplantation procedures, considers both clinical work and *in vitro* experimental results in each of the five sections.
0 19 261414 2 Illustrated £40

Exotica

A Further Miscellany of Clinical
and Pathological Experiences
William St. Clair Symmers

A collection of unusual clinical and laboratory experiences ranging from the commonplace to the exotic and bizarre.
0 19 261225 5 Illustrated £8.95

Immunology of Reproduction

Edited by Thomas G. Wegmann
and Thomas J. Gill III

Based on the May 1981 International Conference on Reproductive Immunology, this book offers a comprehensive, up-to-date survey of this rapidly expanding field.
0 19 503096 6 Illustrated £30

Aneuploidy

D. J. Bond and A. C. Chandley

Assembles the currently available information about aneuploidy and presents data from a range of species from fungi to man. 0 19 261376 6
Illustrated £25 *Oxford Monographs on Medical Genetics*

Circle No. 10 on Reader Service Card.

What we know about acid rain

Kenneth Mellanby

Acid Rain: A Review of the Phenomenon in the EEC & Europe.
Compiled by Environmental Resources Limited.
Graham & Trotman, Sterling House, 66 Wilton Rd, London SW1/Unipub, POB 433, Murray Hill Station, NY 10157: 1983. Pp.159. £12.50, $23.

In 1982 Environmental Resources Limited (ERL), a private consultancy in London, received a contract from the Commission of the European Communities (EEC) to survey the problem of "acid rain". ERL rightly interpreted their brief to include all acid pollution produced by industry and the burning of fossil fuels. They also considered ozone, another pollutant of industrial origin, which may have its own harmful effects but which may also play a part in the generation of strong acids from other chemicals in the atmosphere.

The result, now published in book format, is a succinct and practical account, summarizing a vast amount of information in 160 pages, which include 15 of references. This bibliography is selective, but contains most useful publications up to 1982. With this volume available, there is no longer any excuse for journalists to continue to produce the uninformed nonsense they have inflicted on the public in recent months.

It is clear that when we are considering the direct phytotoxic effects of acid emissions, it is when they are in the form of gases, and to a lesser extent, of particles, that they are important. It is dry deposition that does the damage. This is something with which we have been familiar for more than a hundred years, but recently opinions may have become confused by allegations that polluted rainwater — "acid rain" — may be even more important. ERL give all the facts to allow the reader to disentangle the effects of dry and wet deposition. They make clear that, as far as direct damage to foliage and buildings is concerned, the acid rain commonly falling in the south of England has little harmful effect. Its acidity is thus something of a red herring, even though it is the property which has caused the greatest public disquiet.

Acute phytotoxicity in Britain has in fact greatly decreased in recent years, though this point is hardly made in the report. The improvement is generally attributed to the Clean Air Act of 1956, but was mainly caused by the substitution of clean, convenient natural gas for dirty coal in homes and industry. It has also been achieved by blowing the flue gases from coal-fired generating stations (still the biggest sources of sulphur dioxide) up tall chimneys so that they are mixed with air and thus diluted below the levels where plants are severely harmed.

This policy, obviously beneficial to Britain, has been severely criticized elsewhere. For instance a recent Swedish leaflet, handed out to tourists visiting that country, alleged that Britain is being selfish, reducing pollution at home by increasing it abroad. Fortunately this is not strictly true. Even with low chimneys only a small (though very damaging) amount of sulphur dioxide is deposited locally; most of it mixes into the atmosphere. So though higher chimneys produce important local reductions of pollution, they do not put much more sulphuric acid into the atmosphere; thus the gaseous concentrations a hundred or more miles from source are much the same whatever the height at which the emission originally took place.

The Swedish countryside's secret: The acid rain is slowly killing it.

Sulphur and nitrogen from the combustion of oil and coal fall with the rain, in the form of acid. This acid damages both the land, and the water. 18,000 lakes in Sweden have already been damaged by acid rain. Thousands of them are practically dead. If nothing is done, many more will soon be badly affected. The soil, the forests, and the subsoil water are also being damaged.

Acidification is our worst single environment problem. And yet many people are still unaware of this. It cannot be seen in the countryside. An acidified lake looks clean and clear, even if it has been poisoned to death.

Acidification is an international environment problem. That is why we say that what is happening in the Swedish countryside matters to you. Air pollutants are carried across countries, and across national frontiers, by the winds. Three quarters of the sulphur falling over Sweden originates from other countries.

The only way of saving our countryside from acidification is to reduce radically discharges in Europe, primarily of sulphur. This can only be achieved by international collaboration. And that is why we are concerned to tell you about acidification — by making the problem known in your own country, you will be helping us to solve it!

Acid rain and international relations — detail from a leaflet, intended for tourists, issued by Swedish conservation and angling bodies.

Until recently, it was thought that the policy of "dilute and disperse" had been completely successful, both locally and on a global scale. However, we now find that some damage may occur to crops and trees from levels of gases below those where acute damage is evident. This seems particularly true when the oxides of sulphur and nitrogen mix with ozone. Nevertheless this is still a local problem, occurring within a radius of perhaps up to two hundred miles from the pollution source. Further off, with greater dilution, no further direct damage to plants or to buildings occurs. Until recently we thought that these very dilute gases were completely harmless, and that the pollution problem had largely been solved.

Unfortunately, it has not proved to be so simple. In Scandinavia, for instance, the levels of sulphur dioxide, a substantial part of which comes from Britain, are indeed low, and cause no direct damage to plant life. In fact very delicate foliose lichens, well known biological indicators of air pollution, flourish. But lakes and rivers are becoming acidic, fish are disappearing, and in some parts of Europe (though not in Scandinavia) trees appear to be damaged. This is all blamed on acid rain, and as the chemicals producing the acidity blow from one country to another, the problem is an international one.

A major cause of damage is believed to be that as the air passes from one area to another, perhaps taking several days to do so, the sulphur dioxide is transformed slowly to sulphate and sulphuric acid. This is washed out of the air, and may then acidify lakes, streams and soils where calcium levels are low and there is poor buffering. The rain itself, even when it contains sulphuric acid, is so dilute that it has no direct phytotoxicity; it is the indirect effect on the soil which is thought to be important.

But there are still many uncertainties. We simply do not understand the quantitative relationships between emissions and their environmental effects. One American report showed a linear relationship, with damage closely correlated with the amounts of pollution emitted. European studies have shown no such correlation. This disparity may be because different phenomena are being investigated. The local effects within the UK of air pollution generated in Britain are roughly proportional to emissions, certainly within a radius of a couple of hundred miles from source. But more distant effects, involving chemical transformations whose rate depends on several factors, cannot be determined so easily.

The value of this publication is that it summarizes the existing information, and shows how little we know and where research is needed. I cannot describe the situation better than by quoting from the summary:

> However, it has not been unequivocably established that these environmental impacts are caused by acid pollutant emissions, nor is the relative importance of other factors properly identified. Also considerable further investigations are required to understand the mechanism involved. Nevertheless circumstantial evidence would suggest that acid emissions and their subsequent chemical transformation and precipitation are at least a partial contributory cause to the observed effects and may be giving rise to as yet unidentified impacts, some of which could be irreversible. □

Kenneth Mellanby is Chairman of the Working Group on Acid Rain of the Watt Committee on Energy. This committee represents 60 British professional engineering and scientific organizations.

Ageing friends at a congenial meeting

Alex Comfort

Aging: An Exploration. By David Barash. *University of Washington Press: 1983. Pp.240. $14.95, £12.75.*

POPULAR books on ageing are rather like conferences on a recondite area of research — they tend to be parades of familiar faces. One greets the quotations and examples like old friends and looks out for those who should be there. Professor Barash's book is an entertaining reworking of Visscher, Ernest and other — largely prescientific — writers on age. Unfortunately, while the literary background is colourful and some of the social comment is acute, the corpus of modern gerontological research has largely passed Barash by: he is still going on about Orgel's error hypothesis and Bidder on growth, and about the Vilcabambans and Abkhasians, when the focus of potential application has shifted to the hypothalamus, to immunology and to other areas largely ignored here.

The book is well-written, amusing and accurate in terms of the gerontology of, say, 1960 (though Barash regrettably attributes Frederick the Great's classic exhortation in battle — "Sons of bitches! D'you want to live forever?" to an American sergeant). But it fails altogether to project ageing research as the going concern which it is: there is little or nothing about research prospects, and a good deal too much about the history of quackery. This is partly because the earlier gerontologists made their point and entered the nature of ageing in the agenda of their colleagues — in neurotransmitter studies, for example — but it makes the book a little dated, and unlikely to inspire younger researchers to take up the subject and secure clinically applicable results. □

Alex Comfort is Consultant in Geriatric Psychiatry to the Veterans' Administration, Los Angeles, and an Adjunct Professor at the University of California, Los Angeles.

Dynamics of dizzy nuclei

Neil Rowley

Fast Nuclear Rotation.
By Zdzisław Szymański.
Oxford University Press: 1983. Pp.250. £35, $39.

IN ATOMIC and solid-state physics much can be learned about the internal dynamics of a system by the application of external electric and magnetic fields. The nucleus, however, is a strongly bound object and, in the laboratory, it is not possible to create fields that are sufficiently strong to perturb its internal structure. One extremely fruitful alternative is to set the nucleus spinning and see how its structure responds to the resulting inertial forces. Our knowledge of nuclear structure has greatly increased over the past ten years or more through the application of this idea, and in *Fast Nuclear Rotation* Szymański has set out to document the relevant theoretical and experimental advances.

For a rotating system one can generalize the concept of a quantum mechanical ground state by talking of the configuration which has the lowest energy for a given value of the angular momentum. Equivalently the set of states defined in this way may be thought of as having the largest possible spins for given excitation energies, and are referred to as the "yrast" states of the system. ("Yrast", as Szymański explains, is the superlative of the Swedish "yr", meaning dizzy.)

For sufficiently rapid rotations the entire nuclear shape will be modified and may undergo violent convulsions in an attempt to minimize its energy while accommodating large amounts of angular momentum. It is finally unable to find any equilibrium configuration and will be torn apart by the enormous centrifugal forces present. The book takes us up the entire yrast line from the low-spin region to this ultimate demise into fission fragments. Along this path many fascinating changes in internal structure take place, and it is the observation of these nuclear "glitches" (e.g. sudden changes in the moment of inertia) and their interpretation in terms of the underlying nucleon motions which is the aim of the field of nuclear high-spin states.

As is described in the book, the most usual way of producing a rapidly rotating nucleus is by the so-called fusion–evaporation mechanism in which two nuclei are caused to collide at energies high enough to overcome their mutual Coulomb repulsion. The fused system produced in this way has a high angular momentum and a large excitation energy. Indeed for any given spin, the energy of the compound nucleus will be significantly higher than the corresponding yrast energy and the system may be thought of as being hot. Just like a hot classical liquid, the droplet of hot nuclear matter cools by "evaporation", i.e. by rapid emission of neutrons, protons and alpha particles which carry away large amounts of energy but little angular momentum. (In addition to producing high-spin states the evaporation process may also be used to produce exotic β-unstable isotopes.) On reaching a lower temperature the nucleus can no longer emit particles and the subsequent energy and spin losses must take place via series of γ-decays into and through the yrast states or down the many other "side-bands", i.e. sequences of higher energy rotational states. It is the experimental observation of the ensuing γ-cascades which is the key to the elucidation of nuclear structure at high spins. The sophistication of experimental devices and techniques has grown enormously in recent years, allowing one to resolve individual γ-decays from states with spins in excess of forty units of angular momentum. Indeed many significant advances have been made since the manuscript of *Fast Nuclear Rotation* was completed, though this is a reflection of the vitality of the subject rather than too serious a criticism of Szymański's treatment in the book.

Many of the phenomena associated with nuclear rotations can be thought of in a semiclassical way. Indeed the very phrase "nuclear rotations" implies that it is possible to think of the nuclei in question as deformed objects rotating with some classical angular velocity, which is in turn related to the frequency of the emitted γ-rays. Although typical rotations may seem rather fast ($\sim 10^{20}$ revolutions per second) they correspond to velocities of the nuclear surface significantly less than those of the individual nucleons through the nuclear interior. It is, therefore, meaningful to think of a particle moving in an average field generated by all the others, and of this field rotating in space. This notion leads to the cranked shell model for high-spin states and Szymański discusses many successes of this model in detail. For example the phenomenon of backbending (a decrease in rotational velocity accompanying an increase in angular momentum) is explained in terms of the effect of the Coriolis force on the individual nucleons. In the nuclear ground state nucleons tend to be coupled in pairs, with zero total angular momentum, while the Coriolis force tends to align their individual angular momenta with the axis of rotation. This increases the total spin of the system even though its collective rotational velocity may drop as a given pair is broken up. The interplay between such collective and single-particle effects is central to this area of study and is well brought out in the book.

In order to understand the relevance of high-spin phenomena it is of course essential to have a certain knowledge of more conventional nuclear-structure physics. Szymański admits that some of the important theoretical ingredients have been treated rather briefly in his Chapter 1. Although this is mitigated to some extent by copious references, the uninitiated will find the book tough going. For those with a reasonable working knowledge of nuclear structure, however, it will be essential reading. □

Neil Rowley is in the Theory and Computational Science Division at the Science and Engineering Research Council's Daresbury Laboratory, Warrington.

Photoreceptors for the beginner

Aubrey Knowles

Photoreceptors: Their Role in Vision.
By Alan Fein and Ete Z. Szuts.
Cambridge University Press: 1983. Pp.212. Hbk £17.50, $34.50; pbk £7.95, $13.95.

OUR knowledge of retinal photoreceptors has increased considerably over the past 20 years, and our present ideas about their function have come from a variety of disciplines ranging from anatomy through chemistry to physics. This book meets a need by providing a comprehensive review at a fairly elementary level. The fact that it appears in the IUPAB Biophysics Series should not put off intending readers: the volume could equally well have been included in a series on zoology or chemistry.

Vision is a sense common to most animals, and it is now clear that the photoreceptors of all species have structural similarities and their visual pigment molecules are closely related. However a gulf exists between workers on the vision of vertebrates and those dealing with invertebrates, due to the different visual characteristics observed and to the use of different techniques. The authors have made a good job of pulling together a wealth of information from these two fields, but this has of necessity involved the critical selection of material.

My principal criticism of the book is of bias in this selection that arises from Drs Fein and Szuts's interest in invertebrate vision. While this may merely reflect my interest in vertebrate photoreceptors, most students take an anthropomorphic view of vision, and can best tackle the subject as an extension of their own experience. The authors recognize this in the introductory chapters, where human visual performance is stressed, but this approach is abandoned as the text develops. In Chapter 8, which covers adaptation, vertebrates are barely mentioned. Cone receptors, colour vision and the visual performance of vertebrates receives poor coverage, although this area is of general interest and is the subject of active current research. The book thus attempts a useful service in bringing together the very different visual characteristics of insects and man, but fails to sustain this approach through to the end.

In another way the balance of the book is also not quite right, the early chapters being cluttered with detail while the later chapters become shorter and more cursory. Much of the early detail is irrelevant and some is contentious — for example, the conjectured sensitizing pigment in fly receptors on p.78 — while recent important work on single cell electrophysiology and the molecular mechanisms of transduction and adaptation are all compressed into the final 30 pages. The references are sensibly arranged at the end of the book and form a useful reading list. Unfortunately this list omits some useful recent reviews and contains a number of outdated and trivial references which will not be helpful to a newcomer to the field.

In summary, the book is a useful addition to the literature of vision, and will be helpful to students and research workers entering the field; I will recommend it to our second-year students in future. It is nicely produced though some of the figures are poor. □

Aubrey Knowles is a Research Fellow in the Department of Biochemistry, University of Bristol.



Adaptive radiation as displayed by Hawaiian sicklebills. The illustration is taken from *Darwin's Finches* by David Lack, recently reissued by Cambridge University Press with notes by Laurene Ratcliffe and Peter Boag. Price is hbk £19.50, $39.50; pbk £7.95, $13.95.

The microfilament system

Uno Lindberg

Mechanisms of Cell Motility: Molecular Aspects of Contractility.
By Peter Sheterline.
Academic: 1983. Pp.180. £14, $23.

RESEARCH on the structural organization of eukaryotic cells in general and cell motility in particular was revolutionized by the development of antibodies against actin by Lazarides and Weber in 1974. Discovery of these antibodies led to a startling new picture of the distribution of actin in different kinds of cells and made it clear that chemo-mechanical transduction by the actomyosin system might be a fundamental process common to all eukaryotic cells. Exciting findings followed rapidly,and within a few years it became evident that eukaryotic cells have a complex and dynamically changing cytoskeleton consisting of finely tuned filament systems (microfilaments, microtubules, and intermediate filaments) through which the life processes can be directed and performed in a precise way. Peter Sheterline's book deals primarily with the microfilament system.

The molecular mechanisms underlying cell motility are still largely unknown. Many proteins which interact with the microfilament system have now been isolated, but the observations concerning their activity *in vitro* give only vague answers to several of the outstanding problems — how microfilaments are formed; how filopodia, microvilli and stress fibres are built by packing of actin filaments in paracrystalline bundles; how membrane lamellae extend themselves from the surface of cells and might cause the observed translocations; and how all of these phenomena are regulated.

When lecturing on these matters I usually begin by describing the diversity of motile activity expressed by eukaryotic cells but emphasize that there might be a common basic mechanism. But to consider this it is necessary to have a relatively detailed picture of the organization of the individual structural elements of different types of cells and the different filament systems. After covering that I explain the difficulties involved in correlating the molecular organization of the cell structure with the behaviour of cells. Only then do I tackle the biochemistry of the components of the filament systems. I feel it is difficult for the beginner to see the molecular details but not lose track of the overall structural organization and behaviour of the cell.

Peter Sheterline has organized his book the other way around. He starts with a thoughtful discussion of the proteins involved in the microfilament system. Then he deals with the ultrastructural organization and with possible mechanisms. One could approach the book by taking a good look at the pictures of cells provided in Chapter 6, and then read Chapters 1–5 to find out more about the proteins involved. Then one might study carefully the concept of "contractile networks", but without taking the different models presented too seriously — they may be far from the reality of the living cell. Unfortunately, the author makes no attempt to tell how the story has developed, and he makes few references to original observations. So while there is something in the book to exercise the specialist, the newcomer should look elsewhere for an introduction to this field. □

Uno Lindberg is a Professor at the Wenner-Gren Institute, University of Stockholm.

Talk of science and philosophy

Donald MacKay

The Philosophy of Popper.
By T.E. Burke.
Manchester University Press: 1983. Pp.220. £6.50, $8.

IN a year that has seen the publication (at last) of Sir Karl Popper's famous three-volume *Postscript* (see *Nature* **300**, 663-664, 1982; **302**, 357-358, 1983), there would be little need for yet another summary of his philosophical position. What Dr Burke has provided (despite his textbookish title) is something quite different. Recognizing that "his own works are by far the best sources of information about the context of [Popper's] thought" (p.vii) he has chosen instead to take some of Popper's key ideas as a starting point for critical enquiries which explore much wider territory. In the process he brings out both the strengths and the weaknesses he sees in Popper's massive contribution to contemporary thinking about the nature of science and scientific reasoning, of human society and human freedom, and indeed of the philosophical enterprise itself.

The book falls into five sections, concerned respectively with the philosophy of philosophy, the philosophy of science, relativism and truth, historicism, and freedom and values. In each, Popper's characteristic emphases are noted, with the help of representative quotations; but on the whole the reader is expected to be familiar with their original context, and to be interested mainly in the way they relate to other strands of contemporary thought.

Popper sees philosophy as a critical or mind-clearing response to crises that arise (typically) in other fields of interest. As such it has genuine problems to tackle; and Popper had no time for the Wittgensteinian idea that its goal should be to dissolve rather than solve them. But what Burke asks is whether, after all, Popper and Wittgenstein had as much to quarrel over as they thought; and he argues that Popper's own philosophizing "can equally well be presented as almost classical examples of Wittgensteinian philosophy". Popper's famous refutation of historicism, for example, "hinges on the claim that, given a sufficiently careful description of the relevant disciplines, and drawing of the demarcation-lines between them, the temptations of historicist programmes disappear" (p.25).

For Popper our main concern, whether as scientists or philosophers, is, or ought to be, the search for truth; no mere instrumentalism could do justice to the drive behind the scientific enterprise. Yet paradoxically he is also famous for the doctrine that though science is seeking truth, it can never have logically sufficient grounds for claiming to have found it. Induction, in particular, he has dismissed as "a myth". Burke, while sympathetic to Popper's reasons for such radical scepticism, argues that "his taste for the dramatic has led him to overstate his case" (p.48). Burke insists that for any serious attack on falsehood or ignorance, we need a basis of established truths, however limited, which can and must be secured in ways that avoid the corrosion of universal scepticism. Without this, he argues, Popper's uncompromising rejection of relativism runs into logical difficulties.

What Popper rejected, of course, was not the principle of relativity as such, but the tendency, especially among social scientists, to turn it into a denial of objectivity. "By relativism", he said, "I mean the theory that the choice between competing theories is arbitrary" (cited on p.82).

Popper's terminology does not make it as clear as he might that a thorough-going relativist can also be a thorough-going objectivist, provided that there are objective transformations linking statements that are valid from different standpoints. Burke, however, goes further and maintains that "there is a case for relativism in the form in which Popper denounces it" (p.86); but the case he makes is not particularly strong.

Scientific theories can indeed be surprisingly resilient in face of contradictory evidence; but this does nothing to justify Feyerabend's claim (cited on p.70) that "we can turn science from a stern and demanding mistress into an attractive and yielding courtesan who tries to anticipate every wish of her lover". It does, however, as Burke points out, tend to soften the sharp epistemic contrast usually drawn by Popperian fallibilists between scientific and metaphysical doctrines.

These examples typify Burke's treatment of his theme — respectful and sympathetic, but refreshingly independent, and eager to report what he can see when standing on the shoulders of his chosen giant. He handles language with a craftsman's skill and precision, and though seldom humorous is never dull. Reading him will be a stimulating experience for anyone who, having absorbed Popper's eloquent message, wonders what remains to be said. □

Donald MacKay is Emeritus Professor of Communication and Neuroscience at the University of Keele.

Planning ahead

Warren R. Jones

Birth Control Technologies: Prospects by the Year 2000.
By Michael J.K. Harper.
Heinemann Medical/University of Texas Press: 1983. Pp.270. £15, $27.50.

THIS timely and comprehensive volume highlights the salient question surrounding global activities in fertility regulation. The social and personal imperatives for effective family planning are as pressing now as they have ever been, but the strategic priorities remain unclear — whether to concentrate finite resources on improving the distribution and acceptability of existing contraceptive methods, or to promote further research into new technologies.

Contraception research and development has now reached a watershed. In recent years, partly as a result of the economic recession, there has been a relative decline in the funding of goal-orientated research into reproduction. This decline began at a time when at least some new contraceptive techniques had reached a critical stage of development. Further progress will now only be made either by dint of serendipitous breakthrough or by more investment of high-risk capital by international agencies and pharmaceutical companies.

The book is built around Dr Harper's critical perception of the current state of the contraceptive art and of prospects for the practical application of new and improved birth control technologies by the year 2000. Each method — current, new and potential — is comprehensively reviewed and supported by an up-to-date and extensive, though critical, survey of relevant literature. The author's predictions are grouped in temporal categories of implementation: highly likely before 1990; possible, but prospects doubtful by 1990; unlikely before 1990 but possible by 2000. Most promise is seen in the area of female contraception with little to offer for the male. The most likely new technology strategies to reach practical application in the next decade are steroid implants, new injectables, better IUCDs, vaginal rings, prostaglandins for abortion and non-steroidal anti-ovulation agents. The fate of radically superior new methods such as contraceptive vaccines, menses-inducing pills or tampons, and pills or injections for the male is uncertain at present.

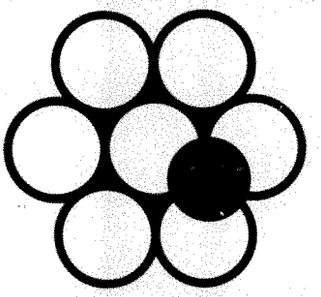
All health professionals who are concerned with the control of human reproduction would do well to read *Birth Control Technologies*. It is scientifically impeccable and, at the same time, eminently readable. In some senses, however, it is depressing. Our contraceptive armoury has progressed little beyond modification of steroidal methods, medicated IUCDs and mechanically simplified contraception. The promise of the future is uncertain, but insofar as advances in science are predictable Dr Harper has given us a blueprint for the next two decades and possibly beyond. □

Warren R. Jones is Professor in the Department of Obstetrics and Gynaecology at Flinders University of South Australia.

The British Journal of Cancer

Published monthly on behalf of the

Cancer Research Campaign



Executive Editor M. Moore,
Paterson Laboratories, Manchester

THE BRITISH JOURNAL OF CANCER is one of the leading cancer journals in the world, publishing articles in all the major scientific disciplines relevant to the subject.

* **papers and short communications submitted by scientists throughout the world**
* **proceedings of the meetings of the British Association for Cancer Research regularly published**
* **other selected national and international meetings also covered**
* **up-to-date book reviews in each issue**

Please subscribe to:
David R. Guthrie
Scientific and Medical Division
The Macmillan Press Ltd., Houndmills,
Basingstoke, Hampshire RG21 2XS, UK.

Subscription rates:
£99 UK and Eire;
$240 (Airmail $285) USA and Canada;
£124 (Airmail £160) Rest of World;

☐ Please enter my subcription to the **British Journal of Cancer** commencing with the _____ (month) issue.

☐ I enclose a cheque for _____ made payable to *Macmillan Journals Ltd.*

☐ I authorise you to charge _____
to my VISA/ACCESS/AMERICAN EXPRESS
(delete as approriate)
Credit Card Account No

Expiry date _____ Signature _____

☐ Please send me a sample copy of the **British Journal of Cancer**

Name _____
Address _____
_____
_____
Postal/zip code _____

SCIENTIFIC & MEDICAL **M** **MACMILLAN PRESS LTD**

Circle No.62 on Reader Service Card.

P k qu lity in chromatography!



The quality of your work deserves the quality of Pharmacia Columns and other compatible equipment from Pharmacia.

- 3-channel Peristaltic Pump
- Dual-Path UV-monitoring system with extreme dynamics
- Single and Dual Channel Potentiometric Recorders
- Gradient Mixer

The convenience of choosing compatible, high quality components, engineered by chromatography experts and based on 20 years of experience saves you time and money.

Ask for our free Catalogue today.

Pharmacia Fine Chemicals AB
Box 175
S-751 04 Uppsala 1
Sweden

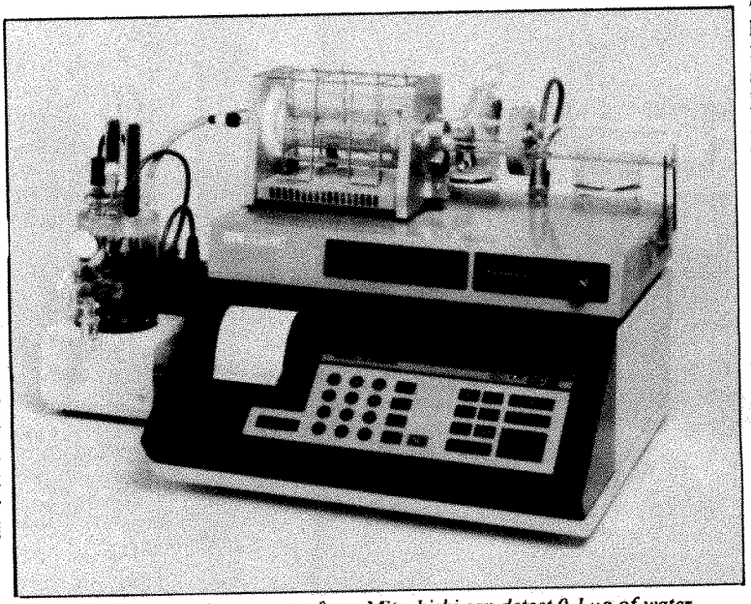
Pharmacia Fine Chemicals

Circle No.21 on Reader Service Card.

The products featured in this week's selection include an air purifier, a refractive index detector and a digital colorimeter

● A new range of all-diffused, fast recovery diodes has been introduced by Westcode Semiconductors. The CXC 924 series is based on 50 mm diameter silicon. It has been developed for use in inverter and chopper circuits. This new diode series is characterized by a repetitive voltage rating of between 1,600 and 2,500 V and an average forward current of 1,265 A at a heat sink temperature of 55°C. Maximum forward current is 2,450 A.
Circle No. 100 on Reader Service Card.

● The Mitsubishi CA-05 moisture meter offers sensitivity to 0.1 μg H_2O. It measures moisture by applying coulometry to the Karl Fischer titration method. The microprocessor controls eight calculation formulae. Samples containing interfering substances and solids insoluble in Karl Fischer reagents are measured by attaching the VA-05 water vaporizer to the CA-05 moisture meter. The VA-05 removes the water from the sample, then transports it to the CA-05 for Karl Fischer titration. The CA/VA combination has a maximum titration speed of 2 mg H_2O min^{-1} and a range of 10 μg to 100 mg H_2O. Precision is ± 5 μg from 10 μg to 1 mg, and 0.5% over 1 mg. A 16-digit alphanumeric display presents calculation results in p.p.m. and percentage H_2O, as well as logging the year, month, day, hour and minute. An integral 20-digit thermal dot matrix printer provides a complete record of calculations.
Circle No. 101 on Reader Service Card.



The CA-05 moisture meter from Mitsubishi can detect 0.1 μg of water.

● A refractive index detector, the SP 6040, is now available from Spectra-Physics. The SP 6040 is a deflection-type differential refractive index detector. Its long focal length increases sensitivity. A temperature control system is built in.
Circle No. 102 on Reader Service Card.

● The 125-test bacteriuria screening kit from Turner Designs is now available from Techmation. The assay is based on the micro-ATP measurement of viable organisms and uses only 50 μl of luciferin-luciferase. The system is claimed to have a false negative rate of around 2% and a false positive rate of 20%. No centrifugation or somatic ATP extraction is required. The model 20 can be used with both the new Turner Designs kit and with most *in-vitro* diagnostic kits.
Circle No. 103 on Reader Service Card.

● The Rodwell Ensign is a free-standing, vertical-loading autoclave with a chamber space of 100 litres. The system does not have to be 'plumbed in', which means that installation only requires an electrical supply. Water that has condensed from the chamber is collected through an integral catchment system. This minimizes water consumption and removes the need for drains and collection vessels.
Circle No. 104 on Reader Service Card.

● Romicon has developed a system for the treatment of water and other fluids before reverse osmosis. The Romicon RO treatment system has wide channel tubes, hollow fibre membranes and operates in a shell and tube geometry at low pressures. It has membrane filters of approximately 0.005 μm.
Circle No. 105 on Reader Service Card.

● An analog multiplexing system is now being produced by Ancom. This DAM (distributed analog multiplexer) system uses a four-wire transmission system and consists of two basic components — a central controller/interface card and a series of remote transmitters. The transmitters accept inputs from transducers such as temperature, humidity, pressure and flow sensors, using local signal conditioning. The Ancom system is controlled through the DAM-C1 card, which can be connected to a thumb-wheel switch and digital display or a microcomputer. The switch is used to select the desired input channel and information is then displayed on the digital readout. A further output connection is available. The four-wire analog transmission system can be up to 1 km in length.
Circle No. 106 on Reader Service Card.

● Micro Instruments has produced a glass microelectrode puller. This new vertical puller is an automatic double-pull type which will take 1 to 4 mm diameter glass tubes. The setting controls allow the heat and the magnets for the initial and the second pull to be adjusted, depending on the thickness of glass tubing being used. Further adjustments are provided to control the heating off time and the pulling time. The glass tubing is held in chucks that are controlled by a 'one-touch' clamping device. The instrument is supplied with a range of chucks and heater coils.
Circle No. 107 on Reader Service Card.

● Astell Hearson has increased its Swiftlock range of autoclaves with the new Swiftlock series 3000. These autoclaves are designed to enable processing of both media and discard material. Three independent adjustable programs are selectable from the front panel and all adjustable controls including a fault reset button are beind a lockable, perspex panel. Supercool models are also being produced.
Circle No. 108 on Reader Service Card.

● Jenway has developed a new digital colorimeter. The Jenway PC01 colorimeter operates in three measurement modes: transmittance, from 0 to 100%, to a resolution of 0.1%; absorbance, which is electronically linearized, from 0 to 1.999 to a resolution of 0.001; and two concentration ranges which provide coarse and fine measurements over the absorbance readings. In this mode the instrument, using most methodologies, provides a direct reading of solution concentration. The PC01 is supplied with eight integral filters to cover the wavelength range 400 to 710 nm. The instrument's bandpass is typically 40 nm.
Circle No. 109 on Reader Service Card.

● A catalogue of specialized equipment for rodent research is now available from Harvard Bioscience. Areas covered in the catalogue include respiration, animal surgery, and housing.
Circle No. 110 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

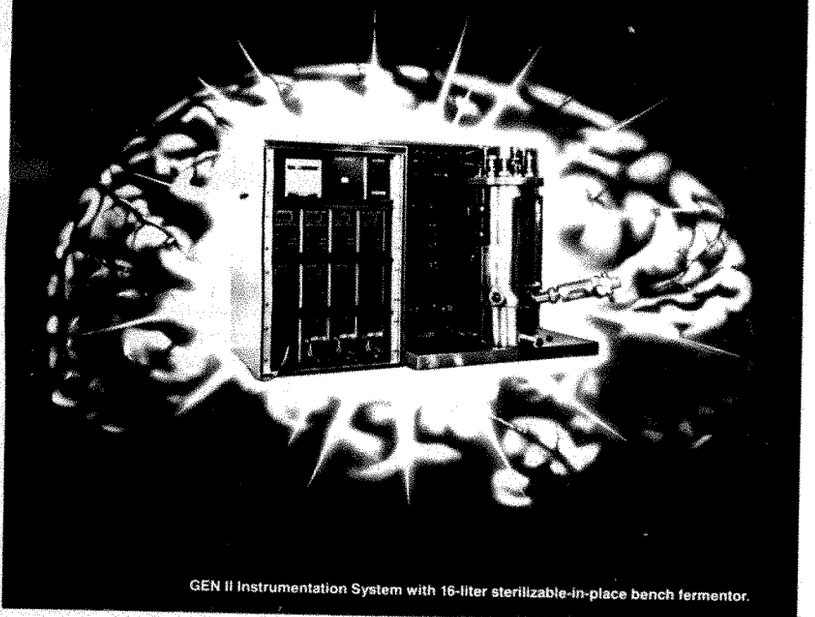
● A new range of Mystaire ward and laboratory air purifiers is now available from Heat Systems Ultrasonics. Interchangeable packs of chemisorbents, activated charcoal and impregnated charcoal are used in conjunction with standard mechanical filter packs to remove specific airborne contaminants.
Circle No. 111 on Reader Service Card.

● Racal Safety has added a power-assisted respiratory system to its Dustmaster series. The Dustmaster 7 includes a mask weighing 50 g with all-round vision. The mask receives a clean air supply through a hose attached to a belt-mounted motor/fan filter.
Circle No. 112 on Reader Service Card.

● Horwell's new Accusplit 920 XP digital LCD quartz wrist watch offers several scientific functions. The countdown timer, pre-settable up to 15 h 59 min, sounds an alarm for 5 s on expiry of the countdown period. Its time-of-day alarm sounds when the pre-set alarm time is reached, but a day exclusion facility stops the alarm going off on days when it is not required. The stopwatch facility times up to 12 h, unless previously stopped, in minutes, seconds and one hundredths of a second up to 20 min and thereafter in hours, minutes and seconds. The watch also tells time of day to the nearest second along with month, date and day of the week. There is an optional hourly bleep.
Circle No. 113 on Reader Service Card.

ADVERTISEMENT

Why buy a "dumb" fermentor when you can have an intelligent fermentation system for the same price



GEN II Instrumentation System with 16-liter sterilizable-in-place bench fermentor.

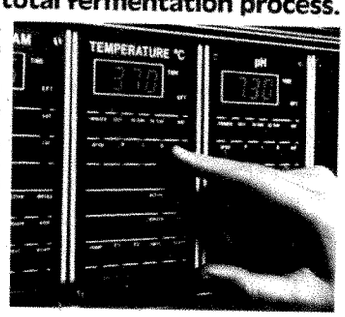
Now! Microprocessor-controlled instruments "talk" to each other to provide interactive control of the total fermentation process.

You don't pay for software and you don't pay for a computer interface — NBS includes them in every instrumentation system.

GEN II is used with bench-top, pilot plant and production fermentors to provide a variety of control strategies including time-based and event-based profiling.

Individual instruments have full PID control capability with proportional gain, as well as integral and derivative time adjustments. The system incorporates fully distributed control and manual back-up of every parameter.

With GEN II, any fermentor has the brainpower it needs for super sophistication and reliability. And, if you can operate your calculator, you can operate GEN II. Send for our new 84 page Fermentation Catalog at P.O. Box 986, Edison, N.J. 08818 or call 800-631-5417.

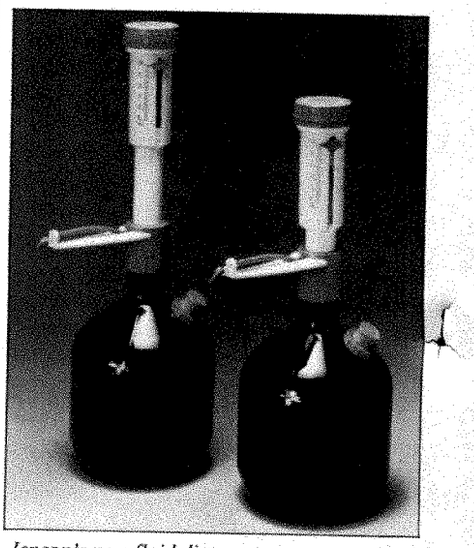


NEW BRUNSWICK SCIENTIFIC CO., INC.
At NBS Advanced Technology is a Way of Life

● Matheson has produced a new electro-pneumatic system that will automatically purge hazardous gas systems. The system is microprocessor-controlled so that valve sequence, number of purge cycles and cycle time can be programmed. The system also responds to pressure, confirmation of valve operation and completion of previous operations. The controller features a shutdown mode in which all valves close when there is a power failure or electrical component malfunction. The purging hardware portion of the system can be up to 200 feet from the controller. The system uses pneumatically-activated Matheson Packless valves which will operate against a cylinder pressure of 2,500 p.s.i. in either direction.
Circle No. 114 on Reader Service Card.

● A fully-automated cassette-to-cassette reactive ion etching system has been introduced by Intertrade Scientific. The LFE Autohex system is equipped with load-lock capability and has been shown to increase throughput and repeatability by 50% compared with manual hex systems. The load-lock is designed to reduce contamination in the process chamber. Operators are not exposed either to the etching gases in the chamber or to residual gases during tray-loading and unloading. A simplified control panel for the system is available from ITS for routine daily operations.
Circle No. 115 on Reader Service Card.

● Jencons Scientific has developed a new duo-style range of fluid dispensers. These models are adapted from Jencons' Zippette and incorporate two types of dispenser. The first is a semi-automatic spring loaded dispenser which refills itself following each delivery. The second is used without the actuator piston spring as a manual model. The duo-style safety Zippette covers the 5 ml, 10 ml, 30 ml and 50 ml range. The MK IV Zippette will dispense acids, aggressive liquids and organic solvents.
Circle No. 116 on Reader Service Card.



Jencon's new fluid dispensers

Biological Sciences

MOSCONA, A.A. and MONROY, A. (eds). Genome Function, Cell Interactions, and Differentiation. Current Topics in Developmental Biology, Vol.18. Pp.227. ISBN 0-12-153118-X. (Academic: 1983.) $32.

MOUNT, M.S. and LACY, G.H. (eds). Phytopathogenic Prokaryotes, Vol.1. Pp.541. ISBN 0-12-509001-3. (Academic: 1982.) $59.50.

MOUNT, M.S. and LACY, G.H. (eds). Phytopathogenic Prokaryotes, Vol.2. Pp.506. ISBN 0-12-509002-1. (Academic: 1982.) $57.

MROSOVSKY, N. Conserving Sea Turtles. Pp.176. Pbk ISBN 0-9507371-1-9. (British Herpetological Society: 1983.) £5, $10.

MULCAHY, D.L. and OTTAVIANO, E. (eds). Pollen: Biology and Implications for Plant Breeding. Proceedings of a Symposium held June 1982, Italy. Pp.446. ISBN 0-444-00738-5. (Elsevier Biomedical/North Holland: 1983.) $70.

MÜLLER, A. and NEWTON, W.E. (eds). Nitrogen Fixation: The Chemical–Biochemical–Genetic Interface. Pp.379. ISBN 0-306-41258-6. (Plenum: 1983.) Np.

NEMEROFF, C.B. and PRANGE, A.J. Jr. (eds). Neurotensin, A Brain and Gastrointestinal Peptide. Annals of the New York Academy of Sciences, Vol.400. Hbk ISBN 0-89766-19-7; pbk ISBN 0-89766-191-5. (New York Academy of Sciences: 1982.) Hbk np; pbk $80.

NOWOTNY, A. (ed.). Pathological Membranes. Biomembranes, Vol.11. Pp.473. ISBN 0-306-41065-6. (Plenum: 1983.) Np.

ORTONNE, J-P, MOSHER, D.B. and FITZPATRICK, T.B. Vitiligo and Other Hypomelanoses of Hair and Skin. Pp.683. ISBN 0-306-40974-7. (Plenum: 1983.) $79.50.

ÖSTERBERG, R. (ed.). Inorganic Biochemistry. Proceedings of the 56th Nobel Symposium held September 1982, Sweden. Pp.160. No ISBN. (The Royal Swedish Academy of Sciences: 1983.) Np.

PALMER, E. A Field Guide to the Trees of Southern Africa, 2nd Edn. Pp.393. ISBN 0-620-05468-9. (Collins: 1983.) £8.95.

PAPAGEORGIOU, G.C. and PACKER, L. (eds). Photosynthetic Prokaryotes: Cell Differentiation and Function. Proceedings of the Special FEBS Meeting held April 1982, Greece. Pp.407. ISBN 0-444-00732-6. (Elsevier Biomedical/North Holland: 1983.) $65.

PARTHIER, B. and BOULTER, D. (eds). Nucleic Acids and Proteins in Plants II: Structure, Biochemistry and Physiology of Nucleic Acids. Encyclopedia of Plant Physiology, New Series, Vol.14. Part B. Pp.774. ISBN 3-540-11140-9/0-387-11140-9. (Springer-Verlag: 1982.) DM 268, $107.20.

PEARSON, M.L. and EPSTEIN, H.F. (eds). Muscle Development: Molecular and Cellular Control. Pp.572. ISBN 0-87969-154-9. (Cold Spring Harbor: 1983.) $57, $68.40 (elsewhere).

PICK, H.L. Jr. and ACREDOLO, L.P. (eds). Spatial Orientation: Theory, Research, and Application. Pp.378. ISBN 0-306-41255-1. (Plenum: 1983.) Np.

PIERCE, C.W. *et al.* (eds). Ir Genes: Past, Present, and Future. Pp.621. ISBN 0-89603-050-4. (Humana Press: 1983.) $64.50 (US), $74.50 (elsewhere).

SMITH, J.E. and LANE, J.D. (ed.). The Neurobiology of Opiate Reward Processes. Pp.475. ISBN 0-444-80430-7. (Elsevier Biomedical/North Holland: 1983.) $144.75, Dfl. 340.

SOTO, R.J., SARTORIO, G. and de FORTEZA, I. (eds). New Concepts in Thyroid Disease. Proceedings of the International Symposium held October 1981, Argentina. Pp.250. ISBN 0-8451-0116-1. (Alan R. Liss: 1983.) £20.

SOUTH, G.R. (ed.). Biogeography and Ecology of the Island of Newfoundland. Monographiae Biologicae, Vol. 48. Pp.736. ISBN 90-6193-101-0. (Dr. W. Junk: 1983.) Dfl. 300, $130.

STEYN, P. Birds of Prey of Southern Africa: Their Identification and Life Histories. Pp.309. ISBN 0-908396-64-3/0-7099-2382-1. (David Philip/Croom Helm: 1983.) £29.50.

STONE, A.R., PLATT, H.M. and KHALIL, L.F. (eds). Concepts in Nematode Systematics. The Systematics Association Special Volume No. 22. Pp.400. ISBN 0-12-672680-9. (Academic: 1983.) £28, $50.

SUBRAMANIAN, C.V. Hyphomycetes: Taxonomy and Biology. Pp.502. ISBN 0-12-675620-1. (Academic: 1982.) £35, $65.

THE CIBA FOUNDATION. Brush Border Membranes. Ciba Foundation Symposium 95. Pp.340. ISBN 0-272-79659-X. (Pitman: 1983.) £25, $35.

VAN TIENHOVE, A. Reproductive Physiology of Vertebrates, 2nd Edn. Pp.491. ISBN 0-8014-128-1. (Cornell University Press: 1983.) £42, $64.35.

VOLPE, E.P. Biology and Human Concerns, 3rd Edn. Pp.675. ISBN 0-697-04734-2. (Wm. C. Brown: 1983.) Np.

WACHTEL, S.S. H–Y Antigen and The Biology of Sex Determination. Pp.302. ISBN 0-8089-1514-2. (Grune & Stratton: 1983.) $44.

WATERHOUSE, J. *et al.* (eds). Cancer Incidence in Five Continents, Vol. 4. Pp.812. ISBN 92-8-321142-1. (IARC: 1982.) SwFr. 100, $50.

WEISS, D.G. and GORIO, A. (eds). Axonplasmic Transport in Physiology and Pathology. Pp.192. ISBN 3-540-11663-X/0-387-11663-X. (Springer-Verlag: 1982.) DM 78, $31.20.

WENG, H.T. Fishes in Varied Reproduction and Sexuality. Pp.60. Pbk ISBN 0-949711-00-4. (Fishing Weng, Australia: 1982.) Np.

YAMAMDA, K.M. (ed.). Cell Interactions and Development: Molecular Mechanisms. Pp.287. ISBN 0-471-07987-1. (Wiley: 1983.) £33, $53.

YOSHIDA, T. and TORISU, M. (eds). Immunobiology of the Eosinophil. Proceedings of the 1st International Symposium held November 1981, Japan. Pp.399. ISBN 0-444-00761-X. (Elsevier Biomedical/North Holland: 1983.) $75, Dfl. 205.

ZRENNER, E. Neurophysiological Aspects of Color Vision in Primates: Comparative Studies on Simian Retinal Ganglion Cells and the Human Visual System. Pp.218. ISBN 3-540-11653-2/0-387-11653-2. (Springer-Verlag: 1983.) DM 89, $35.60.

ZUBAY, G. Biochemistry. Pp.1268. ISBN 0-201-09091-0. (Addison-Wesley: 1983.) £16.95.

History of Science

LLOYD, G.E.R. Science, Folklore and Ideology: Studies in the Life Sciences in Ancient Greece. Pp.260. Hbk ISBN 0-521-25314-4; pbk ISBN 0-521-27307-2. (Cambridge University Press: 1983.) Hbk £25, $49.50; pbk £8.95, $16.95.

RONAN, C.A. The Cambridge Illustrated History of the World's Science. Pp.543. ISBN 0-600-38423-3/0-521-25844-8. (Newnes/Cambridge University Press: 1983.) £12.95.

WESTFALL, R.S. Never at Rest: A Biography of Isaac Newton. Pp.908. Hbk ISBN 0-521-23143-4; pbk ISBN 0-521-27435-4. (Cambridge University Press: 1983.) Hbk np; pbk £12.50.

General

FIRT, V. Statics, Formfinding and Dynamics of Air-Supported Membrane Structures. Pp.449. ISBN 90-247-2672-7. (Martinus Nijhoff: 1983.) Dfl. 190, $83.

FOELL, W.K. and HERVEY, L.A. (eds). National Perspectives on Management of Energy/Environment Systems. International Series on Applied Systems Analysis, Vol. 11. Pp.343. ISBN 0-471-10022-6. (Wiley: 1983.) £32, $59.95.

FOWLER, P.J. The Farming of Prehistoric Britain. Pp.246. Pbk ISBN 0-521-27369-2. (Cambridge University Press: 1983.) £7.50, $14.95.

GOLDSTEIN, G. and RUTHVEN, L. Rehabilitation of the Brain-Damaged Adult. Pp.362. ISBN 0-306-40498-2. (Plenum: 1983.) $27.50.

GOTTFRIED, R.S. The Black Death: Natural and Human Disaster in Medieval Europe. Pp.194. ISBN 0-02-912630-4. (Collier Macmillan: 1983.) $16.95.

HARRIS, C.J. and VALENCA, J.M.E. The Stability of Input-Output Dynamical Systems. Pp.280. ISBN 0-12-327680-2. (Academic: 1983.) £27.20, $45.

HERRMANN, A.G. Radioactive Abfalle: Probleme und Verantwortung. Pp.256. Pbk ISBN 3-540-12028-9/0-387-12028-9. (Springer-Verlag: 1983.) DM 18, $7.20.

HUBBARD, L.R. The Problems of Work: How to Solve Them and Succeed. Pp.179. Hbk ISBN 87-7336-210-7/0-88404-133-6; pbk ISBN 87-7336-121-3/0-88404-132-8. (New Era/Bridge Publications: 1983.) Np.

HUNT, M. The Universe Within: A New Science Explores The Human Mind. Pp.415. Hbk ISBN 0-671-25258-5; pbk ISBN 0-671-25259-3. (Simon & Schuster: 1983.) Hbk np; pbk $9.95.

JENNINGS, J.D. (ed.). Ancient North Americans. Pp.642. ISBN 0-7167-1428-0. (W.H. Freeman: 1983.) £20.95.

JENSEN, J. The Prehistory of Denmark. Pp.331. Hbk ISBN 0-416-34190-X; pbk ISBN 0-416-34200-0. (Methuen: 1982.) Hbk £14.95; pbk £8.95.

KINGHORN, R.R.F. An Introduction to the Physics and Chemistry of Petroleum. Pp.420. ISBN 0-471-90054-0. (Wiley: 1983.) £19.50, $36.50.

KRASILSHCHIKOVA, E.A. A Thin Wing in a Compressible Flow. Pp.247. ISBN 0-306-10972-7. (Plenum: 1983.) $45.

LEHMAN, J.P. (ed.). Hazardous Waste Disposal. NATO Challenges of Modern Society, Vol. 4. Pp.396. ISBN 0-306-41171-7. (Plenum: 1983.) $49.50.

LONGACRE, R.E. The Grammar of Discourse. Pp.423. ISBN 0-306-41273-X. (Plenum: 1983.) $45.

MACLEAN, C. Island on the Edge of the World. The Story of St. Kilda. Pp.160. Pbk ISBN 0-86241-045-2. (Canongate: 1983.) £3.50.

MARSH, G. The Local Plan Inquiry: Its Role in Local Plan Preparation. Pp.167. Pbk ISBN 0-08-030442-7. (Pergamon: 1983.) £11, $20.

McCONNELL, R.B. (ed.). Art, Science and Human Progress. Pp.196. ISBN 0-7195-4018-6. (John Murray: 1983.) £12.50.

MILLER, G. Keepers of the Kingdom. Pp.447. ISBN 0-340-28128-6. (Hodder & Stoughton: 1983.) £8.95.

MITCHELL, R.J. Earth Structures Engineering. Pp.265. Hbk ISBN 0-04-624003-9; pbk ISBN 0-04-624004-7. (George Allen & Unwin: 1983.) Hbk £20; pbk £11.95.

MOSSAKOWSKI, D. and ROTH, G. (eds). Environmental Adaptation and Evolution: A Theoretical and Empirical Approach. Pp.302. Pbk ISBN 3-437-30422-4/0-89574-181-4. (Gustav Fischer Verlag: 1982.) DM 68.

MUJUMDAR, A.S. (ed.). Advances in Drying, Vol. 2. Pp.301. ISBN 0-89116-255-0. (Hemisphere: 1983.) $55.

OLSON, R. Science Deified and Science Defied: The Historical Significance of Science in Western Culture. Pp.329. ISBN 0-520-04621-8. (University of California Press: 1983.) £27.50, $42.

PATTERSON, I. The British Veterinary Profession 1791-1948. Pp.207. ISBN 0-85131-379-5. (J.A. Allen: 1983.) £9.50.

PFEFFER, R. (ed.). Fourth International Conference on Physicochemical Hydrodynamics. pp.535. Hbk ISBN 0-89766-200-8; pbk ISBN 0-89766-201-6. (New York Academy of Sciences: 1983.) Hbk np; pbk $100.

PIGGOTT, S. The Earliest Wheeled Transport: From the Atlantic Coast to the Caspian Sea. Pp.272. ISBN 0-500-01279-2. (Thames & Hudson: 1983.) £20.

PRICE, B. Friends of the Earth Guide to Pollution. Pp.96. Pbk ISBN 0-85117-235-0. (Maurice Temple Smith: 1983.) £1.95.

RILEY, F.L. (ed.). Progress in Nitrogen Ceramics. NATO Advanced Science Institute Series E: Applied Sciences. Pp.814. ISBN 90-247-2828-2. (Martinus Nijhoff: 1983.) Dfl. 230, $100.

ROHMERT, W. and LANDAU, K. A New Technique for Job Analysis. Pp.93. Pbk ISBN 0-85066-251-6. (Taylor & Francis: 1983.) £9.50.

RUEDISILI, L.C. and FIREBAUGH, M.W. (eds). Perspectives on Energy, 3rd Edn: Issues, Ideas, and Environmental Dilemmas. Pp.605. Hbk ISBN 0-19-503289-6; pbk ISBN 0-19-503038-9. (Oxford University Press: 1983.) Hbk np; pbk £9.95.

Key skills and employment growth

Richard Pearson*

For countries left behind by the rapid progress in electronics, prospects for employment will be poor.

THE electronics market is very competitive and is being increasingly dominated by US and Japanese producers, with the Europeans coming a poor third. For example, more than half the world's semiconductor market is supplied by ten companies, five American, four Japanese and one European.

The race is now on for the "fifth-generation computer", and with increasing technical and product integration, "chips" and their derivatives are playing an increasingly critical role in the development of the electronics, manufacturing and service industries. While the semiconductor industry is important in its own right, with world sales of well over $15,000 million a year and a workforce of about 250,000 (Fig.1), the dependent electronics-related markets are of course far bigger, employing several million people.

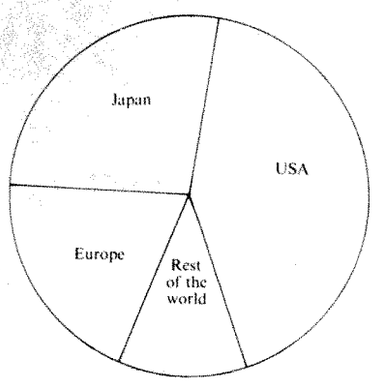


Fig. 1 Manpower in the semiconductor industry. (Institute of Manpower Studies estimates.)

Although the semiconductor industry is particularly sensitive to the economic cycle, it is now experiencing a sharp upturn, and all the forecasters agree that demand will grow significantly in the long term, with output possibly doubling by the end of the decade. Success in these microelectronics markets means large numbers of jobs in the future, yet their success will be critically determined by the availability of a few thousand people with key skills today.

In California, in "Silicon Valley", the birthplace and home of the industry, the companies see two chief threats, growing Japanese competition and shortages of skilled manpower. The recent US Office of Technology Assessment (OTA) report (*Nature* **306**, 307; 1983) may have helped to put the general Japanese threat into perspective but the problems of recruitment are ever present and expected to get worse. In "Silicon Valley", crowded conditions, soaring house prices, rising compensatory salary levels and an inability to attract sufficient skilled staff are leading the major producers to consider moving their factories elsewhere both in the United States, in areas such as Colorado and Austin, Texas, and also in Europe with its rapidly growing semiconductor market.

Semiconductor manufacturers everywhere are struggling with a shortage of key skills and the need to develop a pool of appropriately experienced staff for the future. Thus while an electronics engineer or designer may be highly productive at the relatively young age of 24 or 25, much of his or her necessary experience will have been developed in the two or three years spent in the industry after graduation. Many of the more general skills are interchangeable with the electronics industry, enabling a new or rapidly expanding company which pays top salaries to "poach" experienced staff from a wider pool. However, in the more specialized areas of circuit design, such as very-large-scale integrated circuit (VLSI) design and in process engineering (that is, managing the production line), such skills are only developed on the job, in a semiconductor company. The high costs of equipment and the need to reinvest every three to five years preclude higher education, for example, from contributing effectively to the future supply of experienced people through undergraduate and postgraduate education.

Given the small numbers with such skills, fewer than 2,000 in Europe for example (Fig.2), there are great advantages for semiconductor companies in "clustering" together, facilitating an interchange of skills. This also helps in recruiting staff who can change jobs without relocating. Much of the success of Silicon Valley is put down to the rapid interchange of staff between companies and general ease of career mobility. Such clusters occur "spontaneously", as in California, along Route 128 in Boston, and along the M4 in England. They can also be encouraged by intervention as in Scotland, which is now, through inward investment, a major world electronics centre. Indeed the Scottish Development Agency is capitalizing on this ease of job changing and career development in a small area as part of its recruitment campaign to attract electronics skills to Scotland.

The electronics and related industries are seen as one of the few certain growth sectors for the future. Most governments are therefore encouraging the development of their indigenous industries and are seeking to attract "inward investment" by electronics-based companies. Advances in automation mean that much of the growth in output is being matched, and sometimes overtaken, by increasing productivity levels. The absolute number of assembly and less skilled jobs may well shrink in the future, but the demand for key skills will continue to grow. With up to 10 or more other jobs in the industry, and many more outside it, dependent on each skilled job, the availability of key skills will continue to have a disproportionate effect on attracting and expanding employment in a particular locality.

For many of these skills, semiconductor companies can look to the much wider electronics industry and, provided it can offer attractive salaries and conditions, employers should be able to buy their way out of problems. However, in terms of the few thousand staff needing industry-specific skills, such as VLSI design and process engineering, relevant experience, which can only be acquired in the industry, is critical. Given the long lead times involved in gaining such experience, how will the supply be increased to meet the growing needs of the future? With shortages of skilled staff already apparent, will market forces work resolve these mismatches or will manpower availability and constraints help to determine the industry's winners and losers, and hence the prosperity of particular localities over the next decade? □

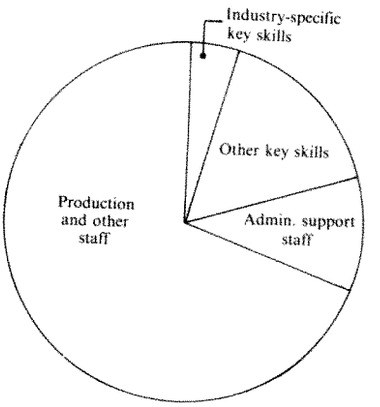


Fig. 2 Balance of skills in European industry.

*Institute of Manpower Studies, University of Sussex, Falmer, Brighton BN1 9RF, UK.

NATURE CLASSIFIED

LONDON OFFICE Keith Walesby 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024)
AMERICAN OFFICES NEW YORK Miss Gene Fein 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900
SAN FRANCISCO JJHS (415) 392 6794 ●**PASADENA** JJHS (213) 796 9200
CHICAGO Didier & Broderick (312) 498 4520 ● **BOSTON** CEL Assoc (617) 848 9306
CANADIAN OFFICE TORONTO Peter Drake 32 Front Street West, Suite 200 Toronto, Ontario M5J1C5 Telephone (416) 364 1623

RATES UK — Rest of world — £11.00 per centimetre. Minimum 3 cm. £2 is charged for the re-direction of replies to advertisements with a box number.

CONDITIONS. The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.

UNIVERSITY OF CALIFORNIA, BERKELEY

DEPARTMENT OF ASTRONOMY AND ASTROPHYSICS

ADJUNCT ASSISTANT PROFESSORSHIP

Pending final budgetary approval, the Department of Astronomy of the University of California at Berkeley expects to make one non-tenure track appointment renewable up to five years in astronomy/astrophysics at the Adjunct Assistant Professor level, which can start as early as July 1, 1984. Applicants should send a curriculum vitae, bibliography and a brief statement of research interests, and should arrange to have three letters of recommendation sent to Jonathan Arons, Chairman, Search Committee, Department of Astronomy, University of California, 601 Campbell Hall, Berkeley, CA 94720. All materials should be received by February 1, 1984.

The University of California is an Equal Opportunity, Affirmative Action Employer. (NW181)A

CHARING CROSS HOSPITAL MEDICAL SCHOOL

(University of London)

SENIOR LECTURER IN BIOCHEMISTRY

Applications are invited for the above post. Candidates should have significant, relevant teaching experience including postgraduate training, and have established themselves as independent scientists as shown both by published work and by ability to attract external funding. They will join the Gene Structure and Function Group being established by A D M Malcolm from whom further particulars may be obtained (address below; telephone 01-748 2040 ext 2835).

Applications (two copies) naming three referees should be sent as soon as possible to The Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP. (905)A

THE UNIVERSITY OF BRITISH COLUMBIA

GRADUATE STUDIES IN CHEMISTRY

Applications are invited from candidates with high academic records who wish to pursue further studies towards the MSc and PhD Degrees in Chemistry. Excellent research facilities are available in all areas of modern Chemical Science, ranging from Biological Chemistry to Chemical Physics. Teaching and research assistantships are available for all students in the programme. Stipends up to $20,800 are available to highly qualified students who hold scholarships; the minimum stipend for 1983/84 is $11,000.

Application forms and further particulars may be obtained from: Dr L Weiler, Professor and Head, Department of Chemistry, The University of British Columbia, 2036 Main Mall, Vancouver, BC, Canada V6T 1Y6. (NW773)A

UNIVERSITY OF LEICESTER

MEDICAL SCHOOL
DEPARTMENT OF SURGERY

NON-CLINICAL LECTURESHIP

Applications are invited from non-medical scientists for a post of Lecturer in the Department of Surgery. Candidates should be post-doctoral and previous experience in tissue culture and immunology would be helpful.

Initial salary will depend upon qualifications and experience on the Lecturers' Scale £7,190 to £14,125.

Further particulars from the Registrar, University of Leicester, Leicester LE1 7RH, to whom applications should be sent on the form provided by 26 January 1984. Informal enquiries may be made to Professor P R F Bell, Department of Surgery, Clinical Sciences Building, Leicester Royal Infirmary, PO Box 65, Leicester (telephone: (0533) 551234 ext 429). (914)A

British Museum (Natural History)

Geochemist

The British Museum (Natural History) is primarily an institution for taxonomic research and has world-wide collections of animals, insects, plants and minerals. There are 5 scientific departments which are supported by a comprehensive library and full technical services including electron microscope and computer facilities.

This opportunity is in the Department of Mineralogy which is well equipped for the analysis of minerals, rocks and meteorites by classical and by instrumental methods. The successful candidate will assist in the running and development of the electron microprobe facility and other instrumental techniques such as neutron activation analysis, particle track analysis and X-ray fluorescence. Duties will also include service analyses by instrumental methods, collaboration in team research and the preparation of rock, mineral and standard samples for analysis.

Candidates should have a good honours degree in chemistry or geology or both; post-graduate experience in mineral chemistry or geochemistry advantageous.



Appointment as Scientific Officer £6930-£9015 (including £1250 Inner London Weighting) with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 27 January 1984) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). (896)A

Please quote ref: SB/77/DK.

CENTRE INTERNATIONAL DE RECHERCHES MEDICALES DE FRANCEVILLE — GABON

RECHERCHE

VETERINAIRE CLINICIEN ET CHIRURGIEN EXPERIMENTE

Adjoint au Responsable de son Centre de Primatologie

FONCTIONS

* Surveillance sanitaire et soins aux primates et autres animaux de Laboratoire.
* Participation aux différents protocoles de recherche en cours, basés principalement sur la Biologie de la Reproduction.
* Participation à la Gestion du Département.

Excellentes conditions de travail.
Contrat début avril 1984 pour 2 ans renouvelables.
Conditions habituelles d'expatriation.

Candidatures à adresser à Monsieur le Directeur Général du CIRMF, 28, Bd de Grenelle, 75015 PARIS, avec Curriculum Vitae, références professionnelles et prétentions. (W620)A

Universiteit van Amsterdam

The Subfaculty of Biology invites applications for a:

visiting professorship in environmental biology (m/f)

with responsibility for teaching and research in environmental biology and for co-ordination of teaching courses leading to professional qualification in Biological Management.

The most important task of the successful candidate will be the integration and co-ordination of current teaching and research in fields related to environmental biology with the goal of creating a unified programme for a new workgroup in Environmental Biology (ex.art. 18 - W.U.B.).

Candidates should hold a Ph.D. degree and posses proven research and teaching ability in environmental biology, in particular in the area of aquatic management. He or she should display good organizational and social qualities and have experience in leadership of a research team. The successful candidate will be expected to make a major contribution to the administrative management of the workgroup, and to establish and maintain contact with other groups working in similar fields.

Appointment to the position will be for a period of 2 years. Part-time appointment is also possible, but full time is prefered.
Salary max. Dfl. 9,283.- per month
(Dutch Civil Servants Code).

Applications, together with a curriculum vitae, list of publications and references, should be sent - within 1 month - quoting number 7389 to the Chairman of the Appointment Committee, Dr. S. Pinkster, Vakgroep Bijzondere Dierkunde, P.O. Box 20125, 1000 HC Amsterdam.

Suggestions for possible candidates may be sent to the Committee.

Woman in particular are invited to apply.

(W624)A

SENIOR ORGANIC GEOCHEMIST

Division Head Required for Interdisciplinary Exploration Team

Woodward-Clyde Oceaneering, an expanding International Geological consulting firm based in Aberdeen, invites applications from individuals with a PhD Degree and a minimum of five years experience in Petroleum Geochemistry. Responsibilities will include developing and managing geochemical hydrocarbon exploration programmes in frontier basins, both terrestrial and marine, utilising current theories in organic geochemistry. Knowledge of a variety of instrumental methods such as GC, GC/MS, Fluorescence, Pyrolysis and Isotope Ratio/MS is desirable.

This position provides the opportunity of working with a multi-disciplinary exploration team consisting of Geologists, Geophysicists and Engineers. Résumés will be treated confidentially. Please state availability. The successful candidate would be expected to assume duties in March/April 1984.

Salary will be negotiated within the range of £15,000 to £22,500, to be commensurate with experience. Interviews will be scheduled in London and Aberdeen during the week of 30 February 1984. Reply to Box No 925 c/o Nature, 4 Little Essex Street, London WC2R 3LF.

(925)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS Centre for Applied Microbiology and Research
MICROBIAL TECHNOLOGY LABORATORY
POST-GRADUATE BIOCHEMIST

To evaluate novel support matrices for the large scale affinity chromatography of proteins. This post is sponsored by the Engineering Sciences Division of AERE Harwell under the auspices of the BIOSEP project, and is available for one year in the first instance. Applicants should have a PhD or an equivalent research qualification. Experience in the purification of proteins on a larger than normal laboratory scale and some understanding of the engineering aspects of large scale chromatography is desirable.

Appointment, subject to qualification and experience will be made on the post-probationary basic grade scale, £6520—£8349. NHS terms and conditions will apply. Applications with full curriculum vitae and the names and addresses of two professional or academic referees should be sent to:
Mrs M Bushby, Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire.

Candidates seeking further information should contact Dr M Scawen on Idmiston (0980) 610391 extension 220.

Closing date for applications will

THE UNIVERSITY OF LEEDS

DEPARTMENT OF EARTH SCIENCES

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post for work on the seismicity and structure of the Cameroon Volcanic Line involving the installation and maintenance of a seismic telemetered network in SW Cameroon and gravity mapping the volcanic province in collaboration with the Institute of Geological and Mining Research (Cameroon). The appointment will be made for a fixed period of up to two years from 1 April 1984. Applicants should have a good honours degree in Geology, Geophysics or Physics and should have or shortly expect to receive a PhD in Geophysics preferably Seismology.

Salary on the IA Range for Research and Analogous Staff (£7,190 — £11,615), according to age, qualifications and experience.

Informal enquiries may be made to Professor Briden (telephone (0532) 431751 ext 6415).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT quoting reference number 49/25/D, to whom applications should be addressed. Closing date for applications 8 February 1984.

Applicants from overseas may apply in the first instance by telex (556473) or telephone (0532) 431751

FACULTY POSITION AVAILABLE

The Department of Earth, Atmospheric, and Planetary Sciences of the Massachusetts Institute of Technology is seeking to appoint a physical oceanographer at the Assistant Professor level. Candidates will be considered from any area of physical oceanography, but the department is particularly interested in developing its programs (both theoretical and observational) in large-scale, deep-sea, physics. Individual chosen would be expected to carry on a vigorous research program and to teach graduate level courses in physical oceanography. Will be a member of the Center for Meteorology and Physical Oceanography.

Please submit a résumé, publication list, statement of interests and names of three references to: W F Brace, Chairman, Dept. of Earth, Atmospheric, and Planetary Sciences, 54-916, MIT, Cambridge, MA 02139.

MIT is an affirmative action/equal opportunity employer. (NW171)A

UNIVERSITY OF PRINCETON

DEPARTMENT OF BIOLOGY

NEUROBIOLOGY

The Department of Biology wishes to appoint one or more neurobiologists. The level is flexible and we are particularly interested in someone working in molecular or cell biological areas of neurobiology. Please write to J.T. Bonner, Chairman, Department of Biology, Princeton University, Princeton, N.J. 08544.

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW178)A

ROYAL POSTGRADUATE MEDICAL SCHOOL

(University of London)

DEPARTMENT OF CLINICAL PHARMACOLOGY

SENIOR RESEARCH OFFICER

A postdoctoral person with experience of protein chemistry is required to join a multi-disciplinary team involved in the purification and characterization of human hepatic cytochromes P-450. The project will involve structural analysis of proteins by biochemical and immunological techniques. The post is supported for a period of 5 years with a salary in the range of £7,190 to £11,615 plus £1,186 London Allowance a year.

Further details of the research programme may be obtained from Professor D S Davies or Dr A R Boobis (Tel: 01-743 2030 ext 454).

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS, quoting reference 20/104/N.

Closing date: 26 January 1984. (910)A

ST JUDE CHILDREN'S RESEARCH HOSPITAL

St Jude Children's Research Hospital is seeking investigators doing innovative research in the areas of contemporary cell or molecular biology to join the faculty in the Divisions of Human Tumor Cell Biology (2 positions) and the Division of Pathology (1 position). Appointments will be at the Assistant or Associate Member level, depending on qualifications and experience, and are intended for individuals interested in investigator-initiated, full-time research. Applicants should have the minimum of two years post-doctoral experience and potential or demonstrated ability to conduct an independent program. Send CV and names of three references to: Charles J. Sherr, M.D., Ph.D. Chairman, Search Committee St Jude Children's Research Hospital Division of Human Tumor Cell Biology 332 N. Lauderdale Memphis, Tennessee 38101

St Jude Children's Research Hospital is an affirmative action employer. (NW173)A

INSTITUTE Of Cancer Research, Cell Transformation Group, Research Officer. Applications are invited for the post of Research Officer in the Section of Chemical Carcinogenesis, working within an established team on the genetic basis of malignant transformation. The work will involve the use of recombinant DNA techniques and eukaryotic gene transfer methods to study the role of dominantly acting transforming genes in the multistage transformation process. Applicants should possess a degree in a biological science plus a minimum of two years relevant laboratory experience, preferably involving some aspect of gene manipulation. At present the laboratories are situated at Chalfont St Giles, Bucks, but will be moving to Fulham Road, London in about six months time. Starting salary will be in the range £6,204 pa — £8,126 pa. Applications in duplicate with the names of two referees to the Personnel Officer, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref 301/B/42. (904)A

POSTDOCTORAL Research Associate. Position open for recent PhD with strong background in molecular biology, biochemistry or cell biology to study DNA damage and repair in human skin, as part of an ongoing collaboration with investigators at the Department of Dermatology, Harvard Medical School, and Skin and Cancer Hospital, Temple University. The successful candidate will be expected to travel occasionally for experiments at these institutions. Initial appointment is for one year beginning on or about March 1, 1984, at a salary of $17,000 per year minimum. Send vita and names of three referees to: Dr Betsy Sutherland, Biology Department, Brookhaven National Laboratory, Associated Universities, Inc., Upton, L I, New York 19973. EOE m/f. (NW182)A

CSIRO

Research Fellows (2 Positions)

$A24,344 — $A35,806

Division of Chemical Physics

Clayton VIC and Lucas Heights NSW — Australia

CSIRO conducts scientific and technological research in Laboratories throughout Australia and employs about 7,500 staff of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Chemical Physics is a Division of the Institute of Physical Sciences.

General: The Division of Chemical Physics is principally located at Clayton, Victoria, with a smaller unit at Lucas Heights, New South Wales. The Division prosecutes a broad programme of research in chemical physics, using fundamental methods. Presently there are twelve sections: atomic, mass and molecular spectroscopy (including specialized optics), electron microscopy, electron diffraction, x-ray diffraction, computing, theoretical chemistry, theoretical physics, crystal defect physics, radiation effects (including radiation chemistry and ion implantation), solid state science, surface science, and instruments and engineering services. Divisional research objectives are both fundamental and applied.

Position 1: No. A2485

Field: Ion Implantation into Solids.

Duties: The appointee will undertake research into ion implantation of semiconductors for microelectronic device fabrication, and ion implantation of metals for enhancement of surface properties, emphasis being placed on the development of an understanding of the detailed processes involved and of their practical applications. The successful candidate will be located at the Division's laboratories at the Lucas Heights Research Laboratories near Sutherland, New South Wales.

Qualifications: A PhD or equivalent qualifications in Physics or a related field together with an ability to undertake independent scientific research. Experience in surface physics and/or ion beam technology is essential.

Position 2: No. A2658

Field: Electron and Ion Physics.

Duties: The Radiation Effects research group at the Division's main David Rivett Research Laboratories, Clayton, Victoria, is involved in part with research and development and the innovative projection of new and sophisticated devices and methods in 'chemical analysis by mass spectrometry'. Current work includes the development of high efficiency ion sources, magnetic momentum analyzers, electrostatic ionic-mass filters and secondary electron multiplier ion detectors. A new surface analysis technique employing secondary ion mass spectrometry is also under investigation.

The successful appointee to this position will be expected to participate in these experimental research activities at the Clayton site, to assist in developing and applying numerical (computer) methods for field determinations and trajectory tracing and, specifically, to take a leading part in the evaluation of devices in the vacuum physics laboratory. The candidate will be expected to be largely responsible for the technology transfer of devices developed successfully in the Division to the appropriate Australian Industry.

Qualifications: A PhD in physics or chemistry or equivalent qualifications in a related field together with an ability to undertake independent scientific research. Some experience or an interest in electron-optical devices would also be desirable.

Tenure: Both positions are offered for a fixed term of 3 years. Australian Government superannuation benefits are available.

Applications: Stating full personal and professional details, the names of at least two referees and quoting the relevant reference number should be directed to: The Chief; CSIRO Division of Chemical Physics; PO Box 160, CLAYTON Vic 3168, AUSTRALIA. By February 3, 1984. (W621)A

POSTDOCTORAL TRAINING PROGRAM

Three postdoctoral positions will be available, commencing July 1, 1984, at Michigan State University in an NIH supported training program in the combined fields of pathology and environmental toxicology. Applicants should have a DVM, MD or DO degree and be willing to undertake training leading to a dual PhD in Pathology/Environmental Toxicology. Deadline for applications is March 1, 1984. Stipends will be based on NIH postdoctoral levels. More complete description of the program is available upon inquiry.

Please address letters of application with three letters of reference to: Dr. R. W. Leader, Associate Dean, Program Director, College of Veterinary Medicine, A-136 East Fee Hall, Michigan State University, East Lansing, Michigan 48824.

MSU IS AN AFFIRMATIVE ACTION/ EQUAL OPPORTUNITY INSTITUTION. (NW118)A

Consultancy

Nutritional Supplements

The Committee for Responsible Nutrition comprises manufacturers of nutritional supplements who believe that there is a national need for responsible communication on health and nutrition. They, therefore, wish to retain a Consultant on a part-time basis who will:

- advise the Committee members on scientific and medically-related matters concerning vitamin and mineral supplementation
- inform consumer groups, government officials, parliamentarians, retailers and others about new developments within the Committee's sphere of interest
- become a source of expertise and focal point for media contact on nutritional issues as they relate to personal healthcare

An average of 12 to 15 hours per week will be required to be devoted to consultancy duties for which a retainer of around £7,000 p.a. plus expenses will be paid. The work may be done from an existing home or office base provided this is within relatively easy access of Surrey.
The successful applicant should have a genuine personal interest in nutritional supplementation and ideally possess a medical qualification or a PhD in a medical or nutrition-related science. He/she will probably be in his/her thirties with a proven record of success in their chosen field. Suitable experience would include research/university teaching or scientific journalism.
Interested persons should write to:

The Secretary, The Committee for Responsible Nutrition, 7a Church Street, Esher, Surrey KT10 8QS.

(929)A

THE UNIVERSITY OF MELBOURNE

SCHOOL OF PHYSICS

RESEARCH FELLOW (GRADE 2) (TEMPORARY)

The Australian Research Grants Scheme has funded a Research Fellowship for work with Professor B H J McKellar on the project "The Potential Approximation in Nuclear Physics".

Applicants should have a PhD in theoretical physics with a good background in quantum field theory. An appointment may be made for up to three years.

Enquiries should be directed to Professor Mckellar, School of Physics (03) 341 5122.

Salary up to $A26,540 per annum within the range $A23,394 to $A30,734 per annum. A contribution towards the cost of travel to and relocation in Melbourne may be made to an appointee from interstate or overseas.

Further information regarding conditions of appointment is available from the Appointments Officer (Academic), University of Melbourne, Parkville, Victoria, Australia 3052, or the Association of Commonwealth Universities, (Appts) 36 Gordon Square, London WC1H 0PF.

Applications close on 29 February

UNIVERSITY OF PITTSBURGH ASSISTANT PROFESSOR OF PHYSICS

A tenure track position as assistant professor in experimental atomic physics will be available starting either in September 1984 or September 1985. Current activities in this area here at the present time include atomic collisions with emphasis on excitation transfer (M A Biondi, R Johnsen), the study of Rydberg atoms and multiphoton effects in atoms (J E Bayfield), and the study of stratospheric processes with laboratory measurements of related cross-sections (E C Zipf). There is theoretical work on collisions (J N Bardsley), high energy radiative proceses (R H Pratt) and multiphoton processes (P Stehle). Preference will be given to candidates whose work would relate to the activities mentioned or to space physics or astrophysics. Credentials should indicate a high promise of establishing a strong research program in atomic physics.

Letters of application, résumés, statements of research interests, and the names of three professional references should be sent to the Chairman, Department of Physics and Astronomy, University of Pittsburgh, Pittsburgh, PA 15260. The university is an equal opportunity/affirmative action employer.

(NW120)A

UNIVERSITY OF WASHINGTON PROTEIN BIOCHEMIST

Assistant Professor, Department of Pathobiology with potential joint appointment in Department of Biochemistry, University of Washington, Seattle. Seeking applicant with strong academic credentials to join biomedical research team involved with the biochemical, genetic, and immunochemical characterization of relevant proteins of microbial and protozoal pathogens. Well-equipped laboratory, start-up funds, and competitive salary available.

Position available by March 1984 or thereafter.

Send curriculum vitae, bibliography, and three references to Dr Thomas M Buchanan, Immunology Research Laboratory, 11th Floor, Pacific Medical Center, 1200 12th Ave South, Seattle, WA 98144, USA.

The University of Washington is an Affirmative Action/Equal Opportunity Employer.

(NW159)A

UNIVERSITY OF WYOMING

BIOCHEMISTRY DEPARTMENT

POST-DOCTORAL AND PRE-DOCTORAL POSITIONS

Positions available to investigate biosynthesis of peptide hormones in adrenal medulla and human small cell carcinoma.

Facilities include HPLC's, gas-phase sequencer, cell culture, and RIA, etc. Growing, dynamic, young department located in beautiful mountain surroundings.

Training in chemistry or biochemistry required. Experience in protein chemistry or cloning required for postdoctoral position.

Send résumé and three reference letters to Dr Randolph V Lewis, Box 3944 University Station, Laramie, WY 82071. (NW164)A

IGNEOUS/METAMORPHIC PETROLOGIST

This is a new faculty position, 12-month appointment, tenure-track. Candidate is expected to develop a strong research program, applying theoretical, experimental, and/or latest analytical methods to petrological problems, particularly those related to the origin and evolution of oceanic crust, islands, and/or volcanic arcs. Research experience with one of the following is desirable: XRF, XRD, stable isotopes (O, N, S), solid source mass spectrometry (Sr, Pb, Nd). Rank is Assistant/Associate Professor. Salary is $27,000 — $43,000, commensurate with experience.

Send résumé and names of three references by 1 March 1984 to: G Ross Heath, Dean, College of Oceanography, Oregon State University, Corvallis, OR 97331.

Oregon State University, an Affirmative Action/Equal Opportunity Employer, complies with section 504 of the Rehabilitation Act of 1973.

(NW160)A

THE UNIVERSITY OF ROCHESTER

SENIOR DEVELOPMENTAL PSYCHOLOGIST

The Department of Psychology of the University of Rochester is seeking a distinguished research-oriented developmental psychologist to direct its program in developmental psychology. Close ties with the Graduate School of Education and Human Development and the Department of Paediatrics bring together a group of nine developmental psychologists. The department, consisting of 23 faculty, has several developmentally related research programs, including clinical, social, cognitive science, developmental neurobiology, and visual science. The specific area of the candidate is not as important as an active program of research.

Interested persons should forward a curriculum vitae, the names of five individuals who would write letters of reference, and reprints of representative publications to Walter Makous, Chair, Developmental Search Committee, Department of Psychology, University of Rochester, Rochester, New York 14627. For questions about the position, please call (716) 275-2460. The deadline for receipt of applications is January 30 1984, although outstanding applications may be considered after this date.

The University of Rochester is an equal opportunity/affirmative action employer. Applications from women and members of minority groups are therefore especially welcome. Salary and fringe benefits depend upon the applicant's experience and are competitive.

(NW152)A

UNIVERSITY OF WALES

Welsh National School of Medicine

DEPARTMENT OF MEDICAL BIOCHEMISTRY

RESEARCH OFFICER

A graduate in biochemistry or a related subject is required for a three-year MRC-funded project to work on the mechanism of toxicity of poly-halogenated aromatic hydrocarbons. The project involves the use of immunochemical, radiochemical and h.p.l.c. techniques to investigate the effect of these agents on haem biosynthesis at the molecular level. The opportunity exists for registration for a higher degree.

Commencing salary £6,310 per annum on the scale for University Research and Analogous Staff Grade 1B.

Further particulars and application forms (quoting Ref. No. C38/4/48) available from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN (0222/755944 Ext. 2296). Closing date for application: 31st January, 1984.

(944)A

POSTDOCTORAL POSITIONS IN CELLULAR AND MOLECULAR BIOLOGY

are available as early as 1 January 1984. Applicants should have a PhD or MD degree with a background in biochemistry, cell biology, or molecular biology. Specific projects include the following: (i) to determine the nature and regulation of subcellular organelle traffic involved int he endocytosis of insulin and other ligands [Dr. Fine and Dr. Pilch; see J. Cell Biol. 96, 133 (1983)]; (ii) to determine the role of growth factor receptor kinases (insulin and IGF-1) in the regulation of cellular metabolism, receptor metabolism, and/or cellular growth [Dr. Pilch, see Biochemistry 22, 717 (1983) and Nature (London) 305, 438 (1983)]; (iii) to clone the insulin receptor and clathrin genes [Dr. Farmer, Dr. Fine, and Dr. Pilch); (iv) to study the regulation of tubulin gene expresion using ß tubulin cDNA clones (Dr. Farmer, see Mol. Cell. Biol. 3, 1333, (1983)]. Applicants should indicate interest in a specific project and send curriculum vitae and the names of three references to one of the following individuals: Dr. Stephen R. Farmer, Dr. Richard E. Fine, Dr. Paul F. Pilche, Department of Biochemistry, Boston University School of Medicine, 80 East Concord Street, Boston, Mass. 02118. An Equal Opportunity Employer, M/F. (NW155)A

The Biotechnology department of Ciba-Geigy Basle has just completed the construction of a new Research Building. In view of our expanding research activities in Biotechnology we now have some

Postdoctoral Positions

open. The duration of the assignments, which are for highly qualified PhDs with research experience in microbial genetics, rDNA technology, cell biology or protein chemistry, will be 1-3 years. Eukaryotic cell transformation, cDNA cloning, heterologous gene expression in prokaryotes, lower eukaryotes and mammalian cells, phage genetics, immunoprecipitation/peptide mapping, posttranslational modification and secretion, are the particular fields in which prospective candidates should qualify. Successful candidates will join an active research team. They will also benefit from an excellent infrastructure and collaboration with the various research institutions in Basle, e.g. the Biozentrum, the Friedrich Miescher-Institute and the Basle Institute of Immunology. They will take part in stimulating research activities on challenging projects.

Applications with curriculum vitae, description of research background and interests and at least two references should be sent to Dr. U. Späti, CIBA-GEIGY Limited, Personnel Department, Postfach, CH-4002 Basle.

CIBA–GEIGY

(935)A

UNIVERSITY OF LIVERPOOL
SERC Daresbury Laboratory
SENIOR RESEARCH ASSISTANT
Synchrotron Radiation Studies of the Structure of Cytochrome C_4

Applications are invited for this post, which is jointly funded by MRC and SERC, with the dual purpose of promoting protein crystallographic research and supporting users of the synchrotron radiation facility. The Senior Research Assistant, working at Daresbury Laboratory, will assist with the development and running of the protein crystallography work stations and will take part in the X-ray crystallographic study of the reduced form of the bacterial protein cytochrome C_4 in association with Dr. Marjorie Harding, Liverpool University, and Dr. Lindsay Sawyer, Napier College, Edinburgh.

Candidates should have a Ph.D. degree in protein crystallography; experience with synchrotron radiation and/or computer programming will be an advantage. The post is tenable from 1 March 1984, for one year in the first instance, with the possibility of renewal for two more years.

Initial salary in the range £7,190 — £8,080 per annum (candidates should not be more than 26 years of age).

Applications, together with the names of three referees should be received not later than 31 January, 1984, by the Registrar, the University, P.O.Box 147, Liverpool. L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/197N. (940)A

THE UNIVERSITY OF MELBOURNE

RESEARCH FELLOW (Grade 1) (Temporary) in the SCHOOL OF PHYSICS

The Australian Research Grants Scheme has funded a Research Associateship for work with Professor B H J McKellar on the project "Weak Interactions".

Applicants should have a PhD in theoretical physics with a background in either particle physics or nuclear structure physics. An appointment may be made for up to two years.

Enquiries should be directed to Professor McKellar, School of Physics (03) 341 5122.

Salary up to $A21,926 per annum within the range $A20,164 to $23,100 per annum. A contribution towards the cost of travel to and relocation in Melbourne may be made to an appointee from interstate or overseas.

Further information regarding conditions of appointment is available from the Appointments Officer (Academic), University of Melbourne, Parkville, Victoria, Australia 3052, or the Association of Commonwealth Universities, (Appts), 36 Gordon Square, London WC1H 0PF.

Applications including names and addresses of at least three referees and quoting position number 640A143 should be addressed to the Staff Officer, University of Melbourne, and close on 31 March 1984. (W618)A

BREWING RESEARCH FOUNDATION

DEPUTY DIRECTOR

Applications are invited for this position, which falls vacant due to the pending retirement of Dr. J. R. Hudson.

Candidates should preferably have some experience of brewing or relevant fermentation processes but otherwise detailed requirements of academic background and experience are open. The person appointed will need to demonstrate a commitment to innovatory research, good understanding and experience of modern methods of research management, and an ability to communicate effectively and persuasively to both scientists and managers at all levels in the industry.

It is anticipated that candidates will already hold a position of responsibility in industry, the university sector or a similar research organisation.

The Brewing Research Foundation is situated in attractive countryside in Surrey yet is within easy reach of London. it was established in 1950 to carry-out co-operative research on behalf of the brewing industry. It is supported primarily by Brewers' Society on behalf of the entire British brewing industry, but has an increasing number of overseas subscribers. The Foundation enjoys an international reputation and is an acknowledged centre of excellence in brewing and fermentation research.

In the first instance, informal enquiries should be made in confidence to: Professor B. Atkinson, Director, Brewing Research Foundation, Nutfield, Surrey. Telephone: 073-782 2413. (898)A

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

SCIENTIST

Division of Marine and Freshwater Science
WELLINGTON
NEW ZEALAND

A vacancy exists in Wellington, New Zealand, at the New Zealand Oceanographic Institute (Division of Marine and Freshwater Science) for a physical oceanographer to study dynamic processes on the continental shelf. Applicants should have a PhD with 1-2 years post-doctoral (or equivalent) experience. A broad knowledge of physical oceanography is required preferably including specific expertise in coastal exchange processes.

Salary up to NZ$30,127 per annum dependent upon qualifications and experience.

DMFS is a division of the Department of Scientific and Industrial Research having a staff of 56 engaged in marine physical geological and biological research housed in a new laboratory in Wellington and 18 at the Freshwater Laboratory at Taupo. Present facilities include a 1000 tonnes research vessel, a comprehensive oceanographic library and a multi-user minicomputer linked to a network of VAX-11/780 computer with access to an IBM 3033 mainframe system.

Further information, application forms, etc., may be obtained from **the Migration Branch, New Zealand High Commission, New Zealand House, Haymarket, London SW1Y 4TQ.** Applicants should quote reference number Imm 2/335/52 (Vac 6169) and forward applications accompanied by a résumé to:

The Migration Branch,
New Zealand High Commission, New Zealand House,
Haymarket, London SW1Y 4TQ.

Closing date for applications February 17th 1984.

(924)A

Remote Sensing and Thematic Information Services

Cartographic Researcher

A vacancy exists in the Cartographic Research Group of the Remote Sensing and Thematic Information Services, Holbrook House, Swindon. The Cartographic Research Group works in co-operation with the Cartographic Production and Image Analysis Groups and also pursues independent lines of research into computer-based methods of handling, processing and display of digital data for thematic mapping. The research is currently centred on data structure design, development of spatial data bases and associated geographic information systems, integration of cartographic and remotely sensed data and colour raster graphics.

Applicants should have a good degree and experience in digital techniques for the display and analysis of geographic data. Programming skills and a knowledge of multivariate statistics would be a definite advantage.

The successful candidate will join at Scientific Officer, or exceptionally, at Higher Scientific Officer level depending on qualifications and experience. Salary scales are, presently, £5,682 to £7,765 for a SO and £7,149 to £9,561 for a HSO depending on age, experience and qualifications.

The NERC is not a Government Department but conditions of service are similar to those of the Civil Service. Applications forms, to be returned by 18 January 1984, are available from Mr. C. I. Brohier, NERC Scientific Services, Holbrook House, Station Road, Swindon, Wilts. SN1 1DE. Tel: 0793 40101 ext 523. Please quote reference: M/P 64.

Further information can be obtained from Dr. M. J. Jackson, Holbrook House, Swindon. (Tel: 0793 40101) ext 495.

NATURAL ENVIRONMENT RESEARCH COUNCIL

(909)A

SWISS TROPICAL INSTITUTE
Field Laboratory Ifakara, Tanzania
BIOLOGIST/ EPIDEMIOLOGISTS

wanted as the head of the Swiss Tropical Institute Field Laboratory at Ifakara, Tanzania.

Candidates should be experienced medical parasitologists, with a thorough background in medical laboratory techniques. They ought to be capable to direct scientific field research, to head a team of co-workers and to take care of all administrative and technical requirements related to the Field Laboratory. They ought to be fluent in English and willing to acquire a good command of Kiswahili.

Scientists, preferably of age 30-40 years, interested in a 2-4 years' contract, should apply to the Director Swiss Tropical Institute, Socinstrasse 57, CH-4051 Basel.

Conditions are negotiable, dependent on the applicant's experience. (W629)A

POSTDOCTORAL POSITION – MOLECULAR CELL BIOLOGY

Available immediately. Work will include characterization of cyto-skeletal high-molecular weight proteins, isolation of m-RNA and c-DNA cloning. Candidates should have strong background in molecular biology preferably in recombinant DNA technology. Send application to: Dr. Gerhard Wiche, Institute of Biochemistry, University of Vienna, Währinger-strasse 17, 1090 Vienna, Austria.

(W641)A

CALIFORNIA INSTITUTE OF TECHNOLOGY
DIVISION OF PHYSICS
ASSISTANT PROFESSORS
ASSOCIATE PROFESSORS
PROFESSORS

Pending budgetary approval, the Division of Physics, Mathematics, and Astronomy of the California Institute of Technology expects during the next two years to make several appointments at professorial ranks. Preferred areas of theoretical, experimental, and observational research include astronomy and astrophysics, condensed matter physics, mathematical physics, nuclear physics, particle physics, and statistical physics.

Applicants should write, as soon as possible but no later than 1 February 1984, to the Chairman of the Division of Physics, Mathematics and Astronomy, 103-33, Caltech, Pasadena, CA 91125, giving curriculum vitae, publication list, and a list of references. Caltech is an Equal Opportunity Affirmative Action Employer. (NW145)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
DEPARTMENT OF CHEMICAL PATHOLOGY
SENIOR RESEARCH OFFICER (POSTDOCTORAL IN MOLECULAR BIOLOGY

Applications are invited for positio of Senior Research Officer (Pos doctoral) to work on a molecula analysis of gene expression in mous brain. The project is funded for years by the Medical Researc Council and is due to start in Marc 1984. Initial salary including Londo Allowance will be in the range £8,37 to £9,716 according to experience.

Informal enquiries may be made Dr S Legon (tel: 01-743 2030 ext 40 and further particulars and appl cation forms are available from th Personnel Office, Royal Pos graduate Medical School, 1 Ducane Road, London W12 0H quoting reference number 4/SL.

Closing date: 26 January 1984.

(911)A

UNIVERSITY OF ALABAM IN BIRMINGHAM
RESEARCH ASSOCIA

Position available immediately f post-doctoral scientist to participa in studies involving molecul cloning and expression of viral gen Should have previous experience wi recombinant DNA technique $16,000 to $27,000 depending experience.

Send curriculum vitae and thr letters of reference to: Dr R Compans, Department of Mic biology, University of Alabama Birmingham, Birmingham, 35294, USA An Affirmative Actio Equal Opportunity Employer.

(NW169)A

UNIVERSITY OF SOUTH CAROLINA/GEOLOGY
DEPARTMENT OF GEOLOGY
invites applications for
TWO ANTICIPATED TENURE-TRACK POSITIO

in two of the following areas: 1) Geophysics; 2) Igneous Petrolo Structure; 3) Coastal Processes; 4) Organic Sediments. It is ant pated that the appointments will b the assistant professor level, applications from more sen persons will also be given strong c siderations.

A PhD degree is required. Start date will be August, 1984, with application deadline of 1 Ma 1984. Applicants should s curriculum vitae, statement research interests, pertinent repri and the name of three references Dr Bjorn Kjerfve, Department Geology, University of So Carolina, Columbia, SC 29 (Phone 803-777 4529).

The University of South Caro is an Equal Opportunity Empl which encourages applicants fr qualified minority groups women. (NW148)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF BIOCHEMISTRY

POSTGRADUATE RESEARCH ASSISTANT

A three year post funded by the SERC Biotechnology Directorate is available from January 1984. The project will be to investigate the leader sequences involved in enzyme excretion in Streptomyces. The DNA encoding some selected extracellular enzymes will be cloned and sequenced. The leader sequences which enable proteins to be exported will be investigated by deletion and site directed mutagenesis.

Starting salary (Research Assistant IB scale) £7,496 (Pt 1) inc London Allowance.

Applications, inc a full cv and the names of two academic referees, are invited from graduates in Microbiology or Biochemistry to Dr John M Ward, Department of Biochemistry, University College London, Gower Street, London WC1E 6BT. (915)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF ZOOLOGY

Postdoctoral position in Neurobiology available immediately to study the physiology, morphology or pharmacology of local interneurones in the insect central nervous system. The project is expected to be funded by N.I.H. for three years. Salary from £7630 pa depending on qualifications and age.

Send C V and names of two referees to Dr M Burrows, Department of Zoology, Downing Street, Cambridge CB2 3EJ. (927)A

PLANT BREEDING INSTITUTE

Maris Lane, Trumpington
Cambridge CB2 2LQ

MOLECULAR/CELL BIOLOGIST

A Molecular/Cell Biologist is required to assist in isolating and characterising plant genes and studying the expression of these genes in prokaryotic and eukaryotic organisms. Duties will consist of maintaining stocks of strains of a variety of organisms, preparing media, isolating plasmids and bacteriophage lambda vectors, constructing recombinant DNA molecules, radioactive labelling and using immunological detection methods.

Appointment will be at Scientific Officer (salary £5,682 to £7,765 pa). Non-contributory pension scheme.

Qualifications are a degree in a relevant biological subject in which biochemistry and microbial genetics are a major feature.

Applications with curriculum vitae and the names and addresses of two referees should be sent to the Establishment Officer by 2 February 1984.

Please quote Reference CYT/166. (899)A

THE UNIVERSITY OF MINNESOTA

THE DEPARTMENT OF PHYSIOLOGY

ASSISTANT PROFESSOR PHYSIOLOGY

The University of Minnesota invites applicants for two tenure-track appointments at the Assistant Professor level beginning about July 1, 1984. Selection will be based primarily on demonstrated research excellence. Applicants must have at least one year of postdoctoral experience and be prepared to carry out independent research. The research may involve any system or tissue but preference will be given to applicants who utilize the methods of cell biology to examine physiological problems.

Areas of particular interest include: membrane transport, intracellular concentration measurements, single channel studies, quantitative biology of excitable membranes, membrane reconstitution of transport proteins, tissue culture.

The applicant will be expected to participate in the graduate and undergraduate teaching programs of the department. Send curriculum vitae, including undergraduate and graduate transcripts, a summary of planned research and the names and telephone numbers of three references to: Search Committee, Department of Physiology, 6-255 Millard Hall, 435 Delaware Street SE, University of Minnesota, Minneapolis, Minnesota 55455.

The University of Minnesota is an equal opportunity educator and employer and specifically invites and encourages applications from women and minorities. (NW149)A

UNIVERSITY OF WASHINGTON

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY AND THE REGIONAL PRIMATE RESEARCH CENTER

IMMUNOLOGIST

Candidates sought for junior faculty position in basic immunology or immunogenetics. The appointee's research activities would include, but not be limited to, identification and exploitation of research opportunities provided by a large, long-established colony of nonhuman primates. A research program of interrelated human and nonhuman primate genetics would be particularly welcome. Previous primate experience not required. The primary selection criteria will be evidence of scientific excellence and promise of independent research of highest academic standard.

Send letter of application, curriculum vitae, and three personal references to: Douglas M Bowden, MD, Chairman, Search Committee, Regional Primate Research Center SJ-50, University of Washington, Seattle, WA98195, Telephone: (206) 543 1430. Affirmative Action/Equal Opportunity Employer. (NW165)A

NORTH ATLANTIC TREATY ORGANIZATION

SACLANT ASW RESEARCH CENTRE

A research organization specializing in underwater acoustics, oceanography, operational research, and system studies is seeking a

SCIENTIFIC EDITOR

to give substantial assistance to the Head of the Scientific and Technical Information Department in aiding research staff and management to prepare written and oral reports of the organization's research work. Duties will include working with authors on the contents of reports and presentations and with technical typists, draughtsmen, photographers and printers on the various aspects of publication. Assistance in the administration of the Department will also be required.

Prospective candidates should possess a scientific or engineering degree or equivalent, preferably followed by research experience and at least 3 years recent experience in editing and/or writing scientific/technical publications. A knowledge of in-house publication techniques would be an advantage. Candidates must be of English mother tongue, with writing skills and ability to edit scientific papers written in English by authors having other mother tongues. A working knowledge of French and Italian would be advantageous.

The successful candidate will initially be offered a fixed appointment of 3 years following which, if mutually agreed, an indefinite duration contract will be offered. Salary on appointment will be in the range of 30 000 000 to 37 000 000 Italian lire per year, depending on family status. All emoluments are tax free.

Applications including curriculum vitae should be forwarded no later than 1 March 1984, addressed to the Civilian Personnel Officer, SACLANT ASW Research Centre, Viale San Bartolomeo 400, 19026 La Spezia, Italy. (W631)A

Technische Universität Berlin

INSTITUT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

3 POSTDOCTORAL POSITIONS BIOCHEMISTRY, MICROBIOLOGY, BIOTECHNOLOGY

To conduct research in the field of bacterial beta-lactam biosynthesis. Positions are available immediately for two years.

The starting salary will depend on age and experience but will be on the Range BAT scale.

Applicants should have PhD in biochemistry, microbiology or biotechnology. Applications, including curriculum vitae and three letters of recommendation, should be sent to: Technische Universität Berlin, Der Präsident, II A 13, Straße des 17. Juni 135, 1000 Berlin 12 (West-Germany). Telephone: (030) 314-4205.

(W616)A

ZWO

The Netherlands organization for the advancement of pure research (Z.W.O.) and the Science and Engineering Research Council (SERC) are collaborating to enhance the experimental facilities for EXAFS and small angle scattering at the Synchrotron Radiation Source (SRS) at the Daresbury Laboratory.

The SRS is the world's first high energy electron storage ring specifically designed to provide synchrotron radiation to a broad range of experiments in the x-ray, vuv and other regions of the electromagnetic spectrum.

Z.W.O. wishes to appoint a

x-ray spectroscopist

to help develop and operate the EXAFS station on the Anglo-Dutch beamline.

The successful applicant will join the in-house team of scientists at Daresbury (of which one member is already a full time employee of Z.W.O.) and will be expected to be actively engaged in the commissioning of the new EXAFS station and its scientific programme. Experience in synchrotron radiation or in a relevant field of x-ray spectroscopy with a practical knowledge of instrumentation and computing would be desirable. Opportunities exist to engage in research with both dutch and british collaboration in fields range from structural studies of catalysts to proteins.

The appointment is initially for a fixed term of 3 years at a starting salary (depending on qualifications and experience) of Dfl. 3.765,— monthly (including foreign service allowance) or, in the case of a british person of £ 837,— monthly without a foreign service allowance.

Applications forms and further particulars are available from professor R. Prins, technical university of Eindhoven, Netherlands (040-473078) or, dr. S. S. Hasnain, Daresbury Laboratory, Warrington WA 4 4 AD, U.K. (0925-65000, extn. 273).

Further information about the post is available from these persons.

Completed applications should be returned by 10 January 1984 to Z.W.O. (reference 68-1), P.O. Box 93138, 2509 AC The Hague, the Netherlands.

(W623)A

Allelix

MICROBIOLOGISTS

Allelix Inc., Canada's first major biotechnology company, is seeking microbiologists to work in fermentation microbiology and plant cell culture. The research will involve fermentation of a variety of microorganisms and plant cells, isolation of mutant strains of bacteria and isolation and identification of microbial and plant cell products. Applicants should have a PhD in microbiology or plant biology and research experience in industry, a university or a research institute.

The founders of Allelix have provided strong financial backing. Research is conducted in a modern, well-equipped laboratory near Toronto, a dynamic, multi-cultural city offering a full scope of activities and interests. Four major universities are easily accessible. Salaries and benefits are at fully competitive levels.

Interested candidates are invited to send complete résumés, including three references and quoting file number 0060 to:

Dr. D. C. Burke
Allelix Inc.
6850 Goreway Drive
Mississauga, Ontario
L4V 1P1 Canada.

(NW168)A

FACULTY POSITIONS IN ASTRONOMY AND ASTROPHYSICS

COLUMBIA UNIVERSITY

The Department of Astronomy and the Astrophysics Laboratory of the Department of Physics at Columbia University plan to make at least four (4) appointments at the assistant professor level, to begin in the summer or fall of 1984. One position in the Astrophysics Lab is for an experimental physicist or an astronomer with an extensive background in instrumentation development to work initially, on funded and planned X-ray and gamma-ray astronomy experiments which will fly on the Space Shuttle and future satellite missions. A second position in Physics is available for anyone with a background and/or interest in high energy astrophysics; research activities currently include analysis of *Einstein* and EXOSAT data, complementary radio and optical observing programs, as well as interpretive and statistical investigations. The Department of Astronomy also has two assistant professor positions open. Candidates with interests in any field of observational and theoretical astronomy are encouraged to apply. Current work in the Department of Astronomy includes observational and millimeter radio astronomy and theoretical investigations in star formation, galactic structure, and the atmosphere and winds of early-type stars. At least one tenure appointment is expected within the next five years, and successful applicants can expect to be considered for this position in due course.

Applicants should include a résumé and letter of interest together with a list of three persons from whom recommendations can be obtained upon request; the letter should state their preference (if any) among the above positions. Columbia University is an equal opportunity employer with a strong affirmative action program, and encourages applications from women and members of minority groups. Mail applications to either Prof. R. Novick, Director, Columbia Astrophysics Laboratory or Prof. L. Lucy, chairman, Search Committee, Department of Astronomy, 538 West 120th Street, New York, New York, 10027.

(NW175)A

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Division of Developmental Biology

Applications are invited from postdoctoral scientists for a non tenured position. The vacancy is for a developmental biologist to work alongside Dr Jonathan Cooke to study the biochemical basis of pattern formation during early amphibian development. The successful candidate will be familiar with theoretical models of development and with modern biochemical techniques.

The appointment will be for five years and the salary will be at an appropriate point on the scales for University non-clinical academic staff, plus £1,186 London allowance and superannuation provision.

Applications, giving a full curriculum vitae, research experience and the names of two professional references, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA, before 27th January 1984 quoting ref: DB/ 1/ S. (907)A

The European Molecular Biology Laboratory, a research institute situated in Heidelberg, West Germany, invites applications from nationals of the member states (Austria, Denmark, France, Federal Republic of Germany, Israel, Italy, The Netherlands, Sweden, Switzerland and The United Kingdom) for the following vacancy

TECHNICAL ASSISTANT

The research of the group is concerned with the determination of three-dimensional structure of protein by means of X-ray diffraction. Applicants will be expected to use the standard laboratory techniques of protein purification. Some knowledge of microbiological or crystallographic techniques would be an advantage. An above average salary will be offered to the successful candidate. Certain allowances are payable in addition, depending on personal circumstances, An initial contract of 3 years' duration which can be renewed, will be offered.

Please write briefly for an application form quoting reference No 83/42 to: EMBL, Personnel Section, Postfach 10.2209, D-6900 Heidelberg. (W615)A

UNIVERSITY OF SALFORD

GEORGE RAMAGE CHAIR IN ORGANIC CHEMISTRY

Applications are invited for the George Ramage Chair in Organic Chemistry. The Department of Chemistry and Applied Chemistry has a very active research programme including that in Organic Chemistry. Funds are also available to appoint a Lecturer in the Organic Section.
Salary within the Professorial range and not less than £17,275 p.a.
Letters of application should be received by the Registrar, University of Salford, Salford M5 4WT from whom further particulars may be obtained by 3rd February 1984. Please quote reference no. CH/287/N. (906)A

UNIVERSITY OF BIRMINGHAM
FACULTY OF SCIENCE AND ENGINEERING
CHAIR OF GEOPHYSICS AND HEADSHIP OF DEPARTMENT OF GEOLOGICAL SCIENCES

Applications are invited for the Chair of Geophysics and Headship of the Department of Geological Sciences, which will become vacant from 1 October 1984 on the retirement of Professor D H Griffiths. The office of the Headship need not necessarily be linked with appointment to the Chair.

Salary in the Professorial range, with superannuation.

Further particulars available from the Registrar, University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (12 copies, 1 from overseas applicants) should be sent by 13 February 1984. (900)A

UNIVERSITY OF CINCINNATI MEDICAL CENTER
PEPTIDE CHEMIST

Selected sequences and analogues of Gastrointestinal and neuronal polypeptides will be synthesized using solid phase methodology. The products will be used for structure function studies and for raising antibodies in collaboration with a research team of biochemists and physiologists. Sequences of apolipoproteins and haemolytic toxins are also being synthesized. The position is for three years beginning as soon as possible after April 1, 1974.

Applicants should send a curriculum vitae and names of referees to Dr R F Murphy, Department of Surgery, University of Cincinnati Medical Center, 231 Bethesda Avenue, Cincinnati, Ohio, 45267. (NW161)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
POST-DOCTORAL RESEARCH ASSOCIATE

Applications are invited for the post of Research Associate to work on a project, funded by The Wellcome Trust, on the properties and regulation of dopamine D_2 receptor-effector mechanisms in the central nervous system. The project includes analysis of intra- and extracellular cyclic nucleotides, inositol phospholipid hydrolysis and receptor labelling techniques.

The successful candidate should possess a PhD (or shortly expect to receive one) and have some experience in biochemical pharmacology/neurochemistry. The appointment will be for three years on the 1A Scale for Research Staff (starting salary £7,630 pa).

Applications (with curriculum vitae and the names of two referees) to and further details from Dr S R Nahorski, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester LE1 7RH, UK (0533) 551234 ext 361.

Closing date for applications 15th February 1984. (892)A

POSTDOCTORAL POSITION IN IMMUNOLOGY

The candidate should be interested in the molecular mechanisms of antigen processing and presentation. Background in membrane and/or protein chemistry desirable.

Interested individuals should send curriculum vitae and the names and telephone numbers of three references to: Dr Howard Grey, Division of Basic Immunology, Department of Medicine, National Jewish Hospital & Research Center, 3800 E Colfax Avenue, Denver, CO 80206. (NW156)A

UNIVERSITY OF OXFORD
DEPARTMENT OF CLINICAL PHARMACOLOGY
Radcliffe Infirmary
Oxford OX2 6HE
Re-advertisement
POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for this position funded by The Wellcome Trust, to study the effects of drugs on 5-hydroxy-tryptamine-mediated phosphatidylinositol breakdown in rat brain tissue and human platelets.

The applicant should have a higher degree in biochemistry or pharmacology and some experience in the area of phospholipid metabolism. The appointment is for 3 years; starting salary will be in the range £7,190 — £8,530.

Applications, together with a curriculum vitae, list of publications and the names of two referees, should be sent to Professor D G Grahame-Smith at the above address. Closing date for applications: 20th January 1984. (917)A

London School of Hygiene and Tropical Medicine
(University of London)

MSc in Human Nutrition

A comprehensive, academic training in the subject of human nutrition is provided through a full-time, one calender year, course. During the first two terms a core course in nutrition, metabolism, physiology, statistics and epidemiology, health-care and economics is taken by all students. The third term consists of options including nutrition in community health and clinical nutrition, nutritional metabolism and socio-economic aspects of nutrition. A research project is completed during July-September.

The curriculum is designed to provide orientation in nutrition for graduates with a range of academic backgrounds (medicine, science or social science) in order to meet the nutritional problems that professionals will encounter in developing and developed countries. The next course commences on October 1st, 1984.

Further details may be obtained from The Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT. (916)A

RESEARCH Associate to carry out research involving cell culture and the isolation of centromeres from metaphase chromosomes, analyzing proteins and DNA from these fractions and preparing monoclonal antibodies. Cloning of DNA is also required. Must have PhD in Cell Biology, Biochemistry or related area with 2 yrs postdoctoral experience. Must also have English speaking and writing skills. $15,000/year. 40 hrs/wk. Apply at the Texas Employment Commission, Houston, Texas, or send résumé to Texas Employment Commission, TEC Building, Austin, TX 78778, JO No 3223086. Ad Paid by an Equal Employment Opportunity Employer. (NW176)A

UNIVERSITY OF FLORIDA
DEPARTMENT OF ASTRONOMY
FACULTY POSITION IN ASTRONOMY

invites applications for a tenure-track position as assistant or associate professor, beginning with the fall term, 1984. The successful candidate must have an outstanding record of research, and if applicable, of outside support.

The salary will be commensurate with qualifications, at a highly competitive level. Although any research speciality will be considered, preference may be given to VLBI or other radio astronomy research, solar system astronomy, or space astronomy.

Send curriculum vitae and names and addresses of at least 3 references to T D Carr, Search Committee Chairman, Department of Astronomy, University of Florida, Gainesville FL 32611, by February 15, 1984. The University of Florida is an equal opportunity/affirmative action employer. (NW144)A

SILVICULTURE — ASSOCIATE Professor or Professor (tenure track, 12-month). Provide instruction (60%) and research (40%) in silviculture. Teach undergraduate and graduate silviculture course. Conduct research related to extensively managed forest. Opportunity for team research on cooperative programs with industry and federal and state agencies. Earned PhD in silviculture or closely related discipline with one degree in forestry required. Demonstrated teaching and research ability required. Send application, résumé, transcripts and three letters of recommendation to: Arnett C Mace, Jr, Director, School of Forest Resources and Conservation, University of Florida, Gainesville, Florida 32611. Closing date is February 2, 1984. (NW146)A

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANT IN MOLECULAR GENETICS

in a research group examining the distribution and interrelationships of Type II restriction-modification systems. Preference will be given to applicants with a sound training in the techniques of either protein chemistry, or bacterial culture, or recombinant DNA work.

The appointment is for three years from 1st March 1984. The starting date may be held open for an excellent candidate. The salary will be in the Range 1A (commencing between £7,190 and £8,530 pa depending on age).

Applications including cv and the names of two academic referees should be made as soon as possible to Dr N L Brown, Department of Biochemistry, University of Bristol, University Walk, Bristol BS8 1TD UK. (893)A

Royal Army Medical College
Millbank, London

Microbiologist

...to manage a small animal unit. Duties involve providing a routine diagnostic service including viral isolation and mycobacterial isolation and identifiction. The successful candidate will also train Army laboratory technicians and participate in the development and assessment of new methods.

Candidates should have a degree/HND/HNC/AIMLS or equivalent in microbiology and at least one year's experience of medical microbiology laboratory work. Concern for the welfare of experimental animals is desirable.

Appointment as Scientific Officer £6930-£9015 (including £1250 Inner London Weighting) with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 27 January 1984) write to Ministry of Defence, CM(S)1b3, Room 310, Savoy Hill House, Savoy Hill, Strand, London WC2R 0BX.

Please quote ref: SA/6/ET.

(897)A **Scientific Civil Service**

UNIVERSITY OF ARIZONA

DEPARTMENT HEAD

The University of Arizona invites applications for the post of Head of the Department of Physics. A distinguished physicist and outstanding administrator is sought to lead this research and teaching department through an anticipated period of intellectual growth over the next decade. Presently the Physics Department consists of around forty faculty members, and it anticipates regular and frequent opportunities to recruit new members during the next several years. A substantial expansion of the physical facilities is currently under way. The University of Arizona is situated in a vital and expanding economic and social environment. The past decade has seen major growth in scientific activities at the University which is presently ranked twenty-first among the nation's universities in the amount of external support received. The Department of Physics is expected to participate in this continuing growth in the years ahead.

Applications, nominations and inquiries should be directed to Professor Henry A. Hill, Chairman of the Search Committee, Department of Physics, University of Arizona, Tucson, Arizona 85721. Informal inquiries are welcome and may be directed to Henry Hill (602) 621-6782. Applications should be accompanied by a complete vita and bibliography, as well as the names of at least three references who can assess the applicant's scientific accomplishments and leadership skills. Letters of recommendation will not be sought until authorized; The University of Arizona is an Equal Opportunity/Affirmative Action Employer. (NW177)A

THE UNIVERSITY OF MUNICH

DEPARTMENT OF NEUROBIOLOGY

POSTDOCTORAL POSITION

Applications are invited for a postdoctoral scientist to work on the localization of transmitters in the extrapyramidal system using mainly immunocytochemical techniques. Experience in EM and/or behavioural methods would be an advantage.

Position funded by the German Research Association (BAT II a, appr. DM 60 000 p.a. before tax) is available immediately for 1 year with possible extension for further two years.

Send complete curriculum vitae and 3 letters of reference to: PD Dr C. Nitsch, Anatomische Anstalt, Pettenkoferstr. 11, D-8000 München 2, West-Germany. (W636)A

ALBERT EINSTEIN COLLEGE

THE DEPARTMENT OF NEUROSCIENCE

seeks several investigators at the

ASSISTANT PROFESSOR

level with interests in cellular and molecular neurobiology. These individuals will be expected to carry out their own research programs, but members of the department have often engaged in collaborative work involving the differing expertise of the participants.

Applicants should contact Michael V L Bennett, Chairman of the Dept of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. An Equal Opportunity Employer. (NW158)A

SOUTHAMPTON PHLS SENIOR MLSO

required to specialise in the assessment of new techniques relevant to diagnostic microbiology. Applications are invited from MLSOs, Scientific Officers or graduates in biochemistry, cell biology, immunology or microbiology to work on the introduction and assessment of molecular techniques relevant to clinical epidemiology and diagnostic microbiology.

The successful applicant would be expected to work on this project for up to three years, during which time appropriate training and expertise will be offered by arrangement with the collaborators in the Southampton PHLS/North American Molecular Epidemiology programme. Experience with radioisotopes, statistics and computing would be an advantage.

Further information can be obtained from Dr A. D. Pearson, PHLS, Level B, South Block, Southampton General Hospital, Tremona Road, Southampton, Hants SO9 4XY, or tel: Southampton (0703) 776177.

(930)A

THE UNIVERSITY OF EDINBURGH

DEPARTMENT OF PHYSICS

RESEARCH FELLOW

Applications are invited for the position of post-doctoral research fellow within the Condensed Matter Group in the Physics Department. The research activities of this group lie largely in the field of phase transitions and critical phenomena, and the successful applicant will be expected to engage in theoretical work in this area, with particular emphasis on Monte Carlo studies using the Edinburgh Distributed Array Processors. Experience in the area of phase transitions and with computing would be an advantage.

The position, which is supported by the S.E.R.C., is available for a period of up to three years, and the appointment will be made on the 1A scale at a point commensurate with age and experience.

Applications, giving full details of the candidate's qualifications and research interests, should be sent to: Dr A.D. Bruce, Physics Department, James Clerk Maxwell Building, Mayfield Road, Edinburgh EH9 3JZ.

Applicants are requested to arrange for letters of support from two referees to be sent directly to the above address. Please quote reference No. 5142. (937)A

UNIVERSITY OF ADELAIDE

invites applications from both m and women for the followi position:

LECTURER in ANIMA SCIENCES (E1073) (Tenurable)

Waite Agricultural Research Institute

in the area of animal nutrition nutritional Physiology.

Applicants should currently actively engaged in studies on so aspect of nutrition or physiology ruminant farm animals, with par ular reference to the grazing anim The appointee will be required to up a research laboratory, and initi research programmes with farm a experimental laboratory animals a there are opportunities for coll oration with other members of Department in the fields of endoc ology and biochemistry. T appointee will also participate teaching in the Animal Scien course at undergraduate level Agricultural Science students will be required to supervise p graduate students.

The position is available from e 1984.

Further information on resea and teaching facilities in Department of Animal Sciences n be obtained from the Chairm Professor B P Setchell.

It is University policy to encour women to apply for considertion appointment to, in particul tenurable academic positions.

Holders of full-time tenured tenurable academic appointm have the opportunity to take le without pay on a half-time basis f specific period of up to ten y where this is necessary for the car children.

Further information about General Conditions of all appo ments may be obtained from Personnel Manager of University.

Salary per annum: Lect $A23394 × 7 — $30734.

Applications, in duplicate, quo reference number and giving personal particulars (includ citizenship, details of acade qualifications and names addresses of three referees, sh reach the Personnel Manager of University of Adelaide, GPO 498, Adelaide, South Australia (Telex: UNIVAD AA89141), later than 17 February 1984.

The University reserves the r not to make an appointment o appoint by invitation. (W634)

POSTDOCTORAL POSITION

available immediately for person with background in membrane biochemistry and cell biology. Expertise in the characterization and tissue localizsation (EM level) of membrane proteins by immunological methods highly desirable. Person will join a group working on membrane protein synthesis and turnover in lact mamary tissue. Salary $16,000 annum.

Send curriculum vitae and t references to Dr I H Mather, Animal Sciences, University Maryland, College Park, Mary 20742. Tel: No. 301-454 3733. Equal Opportunity/Affirma Action Employer. (NW162)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PATHOLOGY
POST DOCTORAL RESEARCH ASSISTANT

pplications are invited from suitably qualified persons for the position of Post Doctoral Research ssistant to join Professor Wildy and r Nash's group working on mmunological responses to herpes mplex virus. The successful candidate will work on the culture and haracterisation of mouse T-cell lones, some of which have already een isolated. Some previous xperience in animal cell culure nd/or immunochemistry is esirable. The project is supported by grant from the Medical Research ouncil for three years. Salary on niversity Scale up to £9875 epending on age. Age not above 30.

Applications, together with curriculum vitae and the names and ddresses of two referees should be ent to The Superintendent, Department of Pathology, Tennis Court oad, Cambridge CB2 1QP. Closing ate 31 January 1984. (926)A

NSTITUTE Of Cancer Research: Senior Technician required to work n the Institute's Human Genetics Section at the Royal Marsden Hospital, Fulham Road, SW3. The applicant would work on a project now in progress to determine the genetic origin of molar pregnancies. The study includes the use of tissue culture, cytogenetics and flow cytometry. A person with experience in tissue culture and/or cytogenetics would be preferred. For further details of the work contact Professor S D Lawler or Miss R A Fisher on 01-352 8171 ext 357. Starting salary will be in the range £7,197 pa — £8,126 pa plus London Allowance of £997 pa. Applications in duplicate with the names of two referees to the Personnel Officer, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref 301/B/41. (903)A

STANFORD UNIVERSITY
DEPARTMENT OF GENETICS
ASSISTANT PROFESSOR

Having scientific interests in areas of human genetics (for example, cytogenetics, human biochemical genetics), cellular genetics (particularly chromosome structure and function) or molecular genetics of higher eukaryotes, especially mammalian organisms. Full-time, tenure-track position that involves teaching and independent research.

Send curriculum vitae, bibliography and names of three referees to: Dr Stanley N Cohen, Chairman, Department of Genetics, Stanford University Medical Center, Stanford, CA 94305. Stanford University is an affirmative action employer and welcomes nominations of women and minority group members and applications from them. (NW151)A

ASSISTANT OR Associate Specialist. It is anticipated funds will be available from a grant from the National Science Foundation or the National Institutes of Health for two years, starting April 1, 1984, with possibility of renewals, to support a full-time individual ($19,848-$22,728/$24,972-$30,084) to study mice at molecular and organismal levels. Emphasis will be on animals from European hybrid zones. Applicant must be experienced at field collection and identification of diverse members of the house mouse complex and at maintenance of a mouse colony. Laboratory proficiency required in mitochondiral DNA purification and restriction analysis, electrophoretic surveys of proteins encoded by many loci, quantitative morphological measurements, karyotypic analysis. Candidate must have at least three years work beyond the PhD, experience in supervisory and instructional capacities, and a publication record testifying to expertise in the areas involved in the project. Knowledge of German, French, Italian desirable. Send résumé, names of three references, copies of relevant publications by 15 January 1984 to: Dr Allan C Wilson, Department of Biochemistry, University of California, Berkeley, CA 94720. UC Berkeley is an Equal Opportunity, Affirmative Action Employer. (NW153)A

THE UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF BIOCHEMISTRY
POSTGRADUATE RESEARCH ASSISTANT

Applications are invited for the above post on an MRC-funded 3 year project to investigate changes in dolichol, phosphodolichol and glycoprotein metabolism in tissues of mammals as they age. Candidates should have a good honours degree in Biochemistry or in a related subject. The possibility of registering for a higher degree would be considered.

Applications including a curriculum vitae and names of two referees should be submitted by January 21st to Professor F W Hemming, Department of Biochemistry, Queen's Medical Centre, Nottingham NG7 2UH. (0602 700111 ext 3619). (901)A

UNIVERSITY OF READING
DEPARTMENT OF AGRICULTURAL BOTANY

Applications are invited for a
LECTURESHIP

with special responsibility for Weed Science. Postgraduate training in ecological or physiological aspects of weed science desirable.

The appointment will be for a fixed period of 5 years from 1 May 1984 or as soon as possible thereafter. Salary scale £7,190 to £14,125 pa plus USS/USDPS benefits.

Further particulars and application forms (2 copies) are available from the Personnel Officer, University of Reading, Whiteknights, Reading, Berks RG6 2AH. Tel: (0734) 875123 ext 220. Please quote Ref AC 841. Closing date 31 January 1984.

(894)A

UNIVERSITY OF GLASGOW

Applications are invited for 2 new Chairs, tenable from 1st October, 1984 or such date as may be arranged.

1. **CHAIR OF MATHEMATICS** (Ref. no. *5285/M*)
The appointment will be in applicable mathematics. Preference may be given to candidates whose main research interests are in some branch of applied analysis, such as (ordinary or partial) differential equations or continum mechanics; but other fields will also be considered.
Closing date for applications: 3rd February, 1984.

2. **CHAIR OF ASTROPHYSICS** (Ref. no. *5186/M*)
Although the successful candidate will be a member of the Department of Astronomy, a major responsibility of the post will be to help guide and develop collaborative research and teaching in the Departments of Natural Philosophy and Astronomy as part of their long term plant for closer association of the two Departments.
Closing date for applications: 13th February, 1984.

Further particulars may be obtained from the Secretary of the University Court, (Room 18), University of Glasgow, Glasgow, G12 8QQ, with whom applications (2 copies), giving the names and addresses of not more than three referees, should be lodged.
In reply please quote appropriate Ref. No. (943)A

UNIVERSITÄT FREIBURG

Am Institut für Biologie III der Albert-Ludwigs-Universität Freiburg i. Br. ist die Stelle eines

PROFESSORS

der Besoldungsgruppe C3 wieder zu besetzen.

Vom Stelleninhaber wird erwartet, daß er sich innerhalb der am Institut vertretenen Gebiete (Genetik, Molekularbiologie einschließlich Gentechnologie, Genexpression bei Eukaryoten, Genetik und Biophysik neuraler Systeme) angemessen an Forschung und Lehre beteiligt. Bei gleicher Qualifikation werden Bewerber bevorzugt, die eine neurobiologische Forschungrichtung mit molekularem und/oder genetischen Ansatz bearbeiten und die in der Lage sind, auch Lehraufgaben im Rahmen des Biophysik-studiengangs zu übernehmen. Eingliederung in einen der in Freiburg bestehenden Sonderforschungsbereiche ist erwünscht.

Bewerbungen mit Publikationsliste, wissenchaftlichem Werdegang und Skizzierung zukünftiger Forschungspläne sind zu richten an den: Dekan der Fakultät für Biologie, der Universität Freiburg i.Br., Schänzlestraße 1, 78 Freiburg i.Br.
Letzter Bewerbungstermin 15. März 1984 (W639)A

UNIVERSITY OF MUNICH
DEPARTMENT OF PHYSIOLOGY
NEUROPHYSIOLOGIST

3 year postdoctoral position available immediately to study neuronal properties in mammalian brain slices under normal and epileptic conditions. In vivo experience with microelectrodes is advantageous. Knowledge of German is not essential. Applications with curriculum vitae and names of 2 referees to: Dr. M. Galvan, Physiologisches Institut, Pettenkoferstr. 12. 8000 Munich 2, W. Germany. Tel: 0049-89-5996250. (W638)A

MEDICAL RESEARCH COUNCIL
Toxicology Unit

TECHNICIAN

Required to work in Laboratory concerned with metabolism of both drugs and natural compounds. The work involves chemical, biochemical and physiological methods.

Qualifications: Degree, HNC or equivalent. Salary according to age, qualifications and experience within the range £5,491 — £7,747 plus London Weighting.

Apply in writing, with curriculum vitae and the names of two referees to: Dr. J. A. Hoskins, Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF.

MRC
Medical Research Council

(936)A

WORLD RESOURCES REPORT EDITOR-IN-CHIEF

The World Resources Institute and the International Institute for Environment and Development seek applications for an editor-in-chief for a new annual publication, the *World Resources Report*, which will be headquartered in Washington, DC. The *Report* will provide timely, objective information on world resource, environmental and population issues for an international audience. It will contain information on resource conditions, trends, and policy developments and will present statistical indicators by country and region.

The editor-in-chief must have strong writing and editing skills, with the ability to synthesize and organize a large body of information, much of it technical, and to communicate that information effectively to a wide and diverse audience, much of it non-technical. Knowledge of the scientific and policy dimensions of international resource, environmental and population issues is essential. The editor-in-chief must also show competence to manage a small staff, operate within a defined budget, effectively use editorial boards and advisors, produce the *Report* on schedule, and promote its international distribution and sale.

Candidates should send complete résumé, publication credits and the names of references to: World Resources Institute, 1735 New York Avenue, NW, Washington, DC 20006. (NW179)A

UNIVERSITY OF LONDON INSTITUTE OF PSYCHIATRY

DEPARTMENT OF NEUROLOGY

RESEARCH PSYCHOLOGIST

Applications are invited for the Post of Research Psychologist for the investigation of the treatment of spasmodic torticollis with electromyographic biofeedback. This study, which is for three years, is funded by the MRC. Clinical training is necessary.

Salary will be on the University Scale 1B for research staff (£6,310 — £8,530 pa plus £1,186 pa London Allowance).

For an application form send stamped addressed envelope to the Assistant Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF quoting ref CDM/N. Closing date for applications — 31 January 1984. (912)A

EPIDEMIOLOGIST

Seek individual with recent PhD, MD or equivalent for appointment as Assistant Professor of Epidemiology to begin Fall Semester 1984. This is tenure-track state-funded position. Recruitment is directed towards epidemiologists with interests that augment and extend research concerns of present faculty. Research interests of current faculty now include arthropod-borne virus diseases, environmental carcinogensis, psychosocial factors and heart disease, occupational diseases, and genetic epidemiology.

Submit curriculum vitae, transcripts, publications, names and addresses of 3 reference persons and a brief statement describing research teaching interests to: professor W C Reeves, School of Public Health, University of California Berkeley, California 94720. Deadline for application is March 15, 1984. The University is an Equal Opportunity Affirmative Action employer. (NW170)A

POSTDOCTORAL POSITION IN IMMUNOLOGY

Two funded postdoctoral positions are available immediately to study the structure and function of the Ia antigens. Specific projects involve establishing the antigenic fine structure of the Ia antigens using monoclonal antibodies, identifying specific interactions of Ia antigens with components of cytoskeleton and plasma membrane, and examining the details of potential interactions between foreign antigen, Ia antigen and T cell receptor during antigen presentation. Salaries to be consistent with NIH guidelines.

Prospective candidates should send curriculum vitae, three references and a summary of research interests to: Dr John H Freed, Division of Basic Immunology, Department of Medicine, National Jewish Hospital & Research Center, 3800 E Colfax Avenue, Denver, CO 80206. (NW157)A

KING ABDULAZIZ UNIVERSITY DEPARTMENT OF BIOLOGY

KING ABDUL AZIZ UNIVERSITY at JEDDAH, SAUDI ARABIA invite PhD holders to apply for vacant posts in Biology Department, Faculty of Science in the following fields:

1 — Molecular Biology.
2 — Population Biology.
3 — Microbial Physiology.
4 — Vertebrate Taxonomy.

Priority will be given to people who are able to teach in Arabic language.

Application should be forwarded to: HEAD, BIOLOGY DEPARTMENT, FACULTY OF SCIENCE, K.A.U. PO BOX 9028 JEDDAH, SAUDI ARABIA. (W630)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF CHILD HEALTH

RESEARCH ASSISTANT

Required for ongoing study, currently funded for further two years, into the process whereby antigen penetrates the pulmonary epithelium and produces immediate hypersensitivity reactions. Ultrastructural autoradiographic and immunohistological techniques are involved.

Salary range 1B (£6,310 — £8,530) and superannuation.

Applications, including a curriculum vitae and the names of two referees, to:
Dr F. Carswell, Reader in Child Health, Department of Child Health, Royal Hospital for Sick Children, St Michael's Hill, Bristol BS2 8BJ. (922)A

MOLECULAR AND Cellular Biologist. A tenure-track position for an individual with postdoctoral experience in molecular and cellular biology. Must have a strong interest in teaching and research at the undergraduate and early postgraduate levels. Responsibilities include teaching molecular and cellular biology, and modern molecular biology technologies to support our programs. The candidate will be expected to have an active, fundable research program. Appointment date: September 1, 1984. Send curriculum vitae, reprints, statement of research goals and three letters of recommendation to: Search Committee, Department of Biological Sciences, Box 152, SUNY Plattsburgh, NY 12901. Deadline: February 15, 1984. Incomplete applications will not be considered. An Equal Opportunity/Affirmative Action Employer. (NW150)A

THE MEDICAL SCHOOL, BRISTOL

DEPARTMENT OF ANATOMY

POSTDOCTORAL BIOCHEMIST

A biochemist with experience in polypeptide biochemistry is required to join a research team investigating the properties of the hormone relaxin. The work involves extraction, purification, and assay of the hormone and there are opportunities to pursue a research project which is appropriate to the expertise of the successful applicant. The salary will be in the range £7190 — £8975 per annum. The post which is funded by the Medical Research Council has some ten months to run. Application will be made for extension or renewal.

Applications together with a curriculum vitae and the names and addresses of two references should be sent to Professor D.G. Porter, Department of Anatomy, The Medical School, Bristol BS8 1TD. (941)A

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF AGRICULTURE

RESEARCH ASSOCIA

Applications are invited for temporary post of Resear Associate in the Department of Ag culture from a date as soon possible until 31st May 1985 to w on a project sponsored by Opencast Executive of the N.C The work will be to evaluate improve the agricultural potential land restored after opencast min and will involve detailed analysis the growth and development cereals and oilseed rape on b restored and undisturbed sit Candidates must have recent ag nomic research experience.

Salary will be on the Range scale (£7,190 — £11,615) accord to age, qualifications and experie with an initial salary not exceed £8,530 per annum.

Further particulars may obtained from the Deputy Regist (F.P.), The University, 6 Kensing Terrace, Newcastle upon Tyne N 7RU, with whom applications copies), together with the names addresses of three referees, shoul lodged not later than 20th Janu 1984. Please quote reference NS. (923)A

RESEARCH ASSOCIATE

to conduct research on the func of poly/ADP-ribose), the me nism of its synthesis in cultured and cell extracts, and its involven in DNA repair. Requirements incl PhD in Biochemistry, two y research experience, and str background in the biochemistry poly(ADP-ribose). Expertise assaying its synthesis, amou distribution and decay. Knowle of DNA repair.

Experience with gel electrophor and high pressure liquid chrom graphy; tissue culture; enz purification and kinetics; radioimmunoassay. Sal \$17,892/yr — 35 hrs/wk.

Send résumé to Mr Willis, Order No 4122, Division of Emp ment Security, Hurley Building, Center, Boston, MA. (NW167)

UNIVERSITY OF PENNSYLVANIA

DEPARTMENT OF SCHOOL OF MEDICINE

POSTDOCTORAL FELLC CELLULAR/STRUCTUR BIOLOGY

Applications are invited for a doctoral fellow, to join our rese on the structure, function, development of microtu containing organelles membrane-microtubule compl The position is supported by an Training Grant. Facilities and ex tise in all aspects of modern struct and cellular biology are avail Send curriculum vitae and names references to J. M. Mur Department of Anatomy, Scho Medicine, University of Pen vania, Philadelphia, PA 19104. (NW180)

DUKE UNIVERSITY
SCHOOL OF MEDICINE
RESEARCH ASSOCIATE

os m available for study of the ha acology and physiology of ip ampal neurotransmission. andidate must have 2-3 years ostdoctoral experience with ntracellular and extracellular ecording from brain slices. position lso requires knowledge of amino cid transmitter pharmacology. alary range: $15,500-$16,300.

Send curriculum vitae and three tters of recommendation to Dr J ictor Nadler, Department of harmacology, Box 3813, Duke niversity Medical Center, Durham, IC 27710. EOE A/A. (NW163)A

Bei der *Fakultät für Physik* der

Albert-Ludwigs-Universität in Freiburg i. Brsg.

ist eine

C 4 — Professur

zu besetzen.

Der Stelleninhaber soll Forschung auf dem Gebiet der Physik der Polymere betreiben. Es besteht die Möglichkeit zur Mitarbeit am SFB 60: "Funktion durch Organisation in makromolekularen Systemen". Es wird volle Beteiligung an der Lehre — auch in der Grundausbildung — der Fakultät für Physik sowie in der Selbstverwaltung erwartet.

Bewerbungen sind bis zum *29.02.1984* zu richten an den

Dekan der Fakultät für Physik
Hermann-Herder-Straße 3, D7800 Freiburg i. Brsg.

(W627)A

SEMINARS and SYMPOSIA

THE SIXTH JOHN INNES SYMPOSIUM: SEPT 17-19 1984

THE CELL SURFACE IN PLANT GROWTH AND DEVELOPMENT



INCORPORATING
THE 11TH BATESON MEMORIAL LECTURE
TO BE GIVEN BY PROF. W. W. FRANKE (HEIDELBERG)
SESSION A — THE CELL SURFACE:
STRUCTURE AND BIOGENESIS
D. H. Northcote (Cambridge); H. Kauss (Kaiserslautern);
Malcolm Brown Jnr. (Texas); K. Roberts & P. Shaw (Norwich);
D. Delmer (California); R. Selvendran (Norwich).
SESSION B — THE CELL SURFACE, THE CYTOSKELETON AND DEVELOPMENT
D. A. Rees (Mill Hill, London); R. Quatrano (Oregon);
B. Gunning (Canberra); P. Green (Stanford);
C. W. Loyd (Norwich).
SESSION C — THE CELL SURFACE IN RECOGNITION
P. Albersheim (Boulder); L. Sequeira (Wisconsin);
J. Callow (Brimingham); J. Robertson (New Zealand);
S. Adair (St. Louis); D. Bauer (Ohio);
A. Clarke (Melbourne); A. W. B. Johnston (Norwich);
H. & J. Heslop-Harrison (Aberystwyth).
FOR FURTHER INFORMATION PLEASE CONTACT:
Jeni A. Fox, Symposium Secretary,
John Innes Institute, Colney Lane, Norwich NR4 7UH.
Tel: (0603) 52571 Telex: 975122 (JIINOR G). (895)M

Field Symposium on the Caledonides of the British Isles
Advanced Studies Institute sponsored by NATO

Director Dr. A. L. Harris (University of Liverpool, UK)

This field symposium will study the localities of classic interest in the Caledonides of Britain.

The field symposium will leave Cardiff, South Wales, on 19th August 1984 and will traverse the Caledonides of Wales, the English Lake District, the Southern Uplands of Scotland and the Scottish Highlands.

Localities visited will include Church Stretton, Aberystwyth, Snowdonia, Anglesey, Lake District, Moffat, Girvan, Fort William and Ullapool. Evening sessions will include discussions and lectures which will review each day's geology and place it in its context within the Caledonide-Appalachian orogen. Lecturers will include Dr. M. G. Bassett, Dr. M. P. Coward, Dr. D. J. Fettes, Professor L. M. Hall, (USA), Professor R. D. Hatcher, (USA), Dr. R. T. Haworth (Canada), Professor G. Kelling, Dr. W. S. McKerrow, Professor B. A. Sturt, (Norway).

The field symposium will end in Glasgow, Scotland, on Sunday 2nd September 1984 to enable participants to join the final symposium of I.G.C.P. Project 27 the Caledonide Orogen.

NATO regulations require those who are interested in participating in the field symposium to write to Dr. A. L. Harris enclosing a brief statement of their geological interests, a list of recent publications and the name of one referee.

Applications must reach Dr. Harris by the 31st March 1984. The number of places available on the field symposium is limited to 40. Priority will be given to applicants from outside the United Kingdom. (932)M

CONFERENCES and COURSES

Federation of European Biochemical Societies Advanced Courses in 1984

Gene Cloning and Sequencing — February 20-29, Helsinki, Finland
Info: Dr J Knowles, Recombinant DNA Group, VTT, Biotechnical Laboratories, Tietotie 2, SF-02150 Helsinki Espoo 15, Finland

Biochemistry of Ageing — March 11-17, Maria Alm, Austria
Info: Professor F Cramer, Max-Planck-Institut für Experimentelle Medizin, Abteilung Chemie, Hermann-Rein-Strasse 3, D-3400 Göttingen, Federal Republic of Germany

Biomolecular Electron Microscopy (BIOMOLEM 1984) — April 5-14, Ulm, Federal Republic of Germany
Info: Professor A K Kleinschmidt, Abteilung Virologie (Mikrobiologie I), Universität Ulm, Oberer Eselsberg, D-7900 Ulm (Donau), Federal Republic of Germany

Structure and Dynamics of Membrane Lipids — April 8-13, Utrecht, The Netherlands
Info: Dr Ben de Kruijff, Institute of Molecular Biology, State University of Utrecht, Transitorium 3, Padualaan 8, NL-3584 CH Utrecht, The Netherlands.

Redox and Energy Transfer Proteins of Coupling Membranes: Structure, Function, and Biogenesis — April 9-19, Bari, Italy
Info: Professor S Papa, Istituto di Chimica Biologica, Facoltà di Medicina e Chirugia, Università di Bari, Policlinico, Piazza G Cesare, I-70124 Bari, Italy

Methods in Yeast Molecular Genetics — Beginning July 1984, two weeks, Düsseldorf, Federal Republic of Germany
Info: Dr C Hollenberg, Universität Dusseldorf, Institut für Mikrobiologie, Universität Strasse 1, Südbereich Geb. 26.23, D-4000 Düsseldorf, Federal Republic of Germany

Molecular Form and Function of Plant Genomes — July 4-15, Renesse, The Netherlands
Info: Dr Lous van Vloten-Doting, Department of Biochemistry, PO Box 9505, NL-2300 RA Leiden, The Netherlands

New Developments and Methods in Membrane Research and Biological Energy Transduction — August 16-29, Spetsai, Greece
Info: Dr Lester Packer, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720, USA

Aspartic Proteinases and their Inhibitors — August 20-24, Prague, Czechoslovakia
Info: Dr Vladimir Kostka, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Flemingovo nam. 2, CS-166 10 Praha 6, Czechoslovakia

Genome Organization and Function — August 30-September 12, Spetsai, Greece
Info: Professor H G Zachau, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, Goethestrasse 33, D-8000 München 2, Federal Republic of Germany

Genetic Transformation — September 2-6, Paris, France
Info: Dr C Anagnostopoulos, Centre de Génétique Moléculaire du CNRS, F-91190 Gif-sur-Yvette, France

Cell Transformation — September 2-12, Sintra-Estoril, Portugal
Info: Dr J Celis, Aarhus University, Kemisk Institut, Langelandsgade 140, DK-8000 Aarhus C, Denmark

The Immune System, Genes, Receptors, and Regulation — September 10-20, Ionian Village, Greece
Info: Dr S Avrameas, Unité d'Immunocytochemie, Institut Pasteur, 28, rue du Docteur Roux, F-75724 Paris Cédex 15, France

Glycoconjugates — September 10-22, Villeneuve d'Ascq, France
Info: Professeur J Montreuil, Université des Science et Techniques de Lille I, Laboratoire de Chimi Biologique, F-59655 Villeneuve d'Ascq, France (W572)C

JAPANESE BIOTECHNOLOGY STUDY TOUR

An in-depth series of site visits, conferences and seminars with some of Japan's leading industrial scientists and executives in biotechnology.

This exclusive study tour is designed to give western scientists and executives an opportunity to become acquainted with the Japanese marketing and research capacity. The mission will be led by Christopher G. Edwards, Editor in Chief of BIO/TECHNOLOGY, and organized by the Technology Transfer Institute. Visits will be conducted throughout Japan from March 31–April 15.

For further information, contact: Technology Transfer Institute—New York, One Penn Plaza, Suite 1411, 250 W. 34th St., New York, N.Y. 10119. 212-947-2648 telex 420057TTIUI. Attention: Mr. Hashizume.

(NW174)C

FEBS International Summer School on "THE IMMUNE SYSTEM: GENES, RECEPTORS AND REGULATION"

at Ionian Village, Peloponn[illegible], Greece, September 10-20, 198[illegible].

Organisers: Drs. Papamichail, Askonas, Avrameas, Rajewsky.

Applications from PhD students and postdoctoral workers (cv, scientific background, research interests, and one recommendation letter) by 20 May 1984 to: Dr. S. Avrameas, Institut Pasteur, 28 rue du Dr. Roux, 75724, Paris Cedex 15, France. The accommodation fee will be US $200.

(921)C

MOLECULAR ENDOCRINOLOGY AND TECHNIQUES FOR HORMONE ACTION
19 to 25 February, 1984

Course will consist of lectures in methodology with comprehensive syllabus covering receptors, gene transcription, mRNA and protein synthesis, recombinant DNA, protein hormones, and cyclic nucleotides.

Co-directors: W. T. Schrader and B. W. O'Malley. Fee: $295/$195 (fellows).

For further information contact: Claudia Payne, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030. Telephone: 713-799 6200. Deadline 6 January 1984.

(NW115)C

WORKSHOP

EMBO WORKSHOP
An EMBO International Workshop on the MOLECULAR AND DEVELOPMENTAL BIOLOGY OF DROSOPHILA

will be held at Kolymbari Crete (Greece) June 24-30, 1984. The workshop will be co-sponsored by the University of Crete and the Molecular Biology and Biotechnology Institute of the Research Center of Crete. Approximately 70 participants will be selected by the organizing committee from applicants. Applications should include a short summary of research interests and should be sent no later than January 20, 1984 to: Dr. S. Artavanis-Tsakonas, Crete Workshop, Yale University, Department of Biology, PO Box 6666, New Haven, Connecticut 06511-8112, USA.

A registration fee of $150 will be remitted upon acceptance. Local expenses in Crete will be covered by the organizers. Participants are expected to finance their travel to Crete. A small number of grants in aid for partial travel support may become available.

The organizing committee: S. Artavanis-Tsakonas (Yale University); M. Ashburner (Cambridge University); A. Carpenter (University of California); M. Gans (CNRS, Gif sur Yvette); A. Garcia-Bellido (Universidad Autonoma, Madrid); W. Gehring (University of Basel); W. Gelbart (Harvard University); D. Hogness (Stanford University); D. Ish-Horowicz (Imperial Cancer Research Fund); F. Kafatos (Crete University); D. Kankel (Yale University); J. Modolell (Universidad Autonoma, Madrid); C. Nusslein (Friedrich Miescher Institute, Tubingen); M. Pardue (Massachusetts Institute of Technology); G. Rubin (University of California). (NW114)V

ASSOCIATESHIPS

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF ORGANIC CHEMISTRY

POST DOCTORAL RESEARCH ASSOCIATESHIPS

Applications are invited from suitably qualified candidates for 2 post doctoral Research Associate posts. The first post is tenable for 3 years and is concerned with the synthesis of biologically active compounds. The second post is tenable for 2 years and is concerned with the study of the mechanism of action of vitamin B12.

Appointments will be made on the Range 1A salary scale £7,190 — £11,615 according to age, qualifications and experience with an initial salary not exceeding £7,630 per annum.

Further information may be obtained from Professor B T Golding, Department of Organic Chemistry, The University, Newcastle upon Tyne NE1 7RU (0632 328511 extension 2959) with whom applications, with the names of 3 referees should be lodged not later than 31st January 1984.

(902)O

STUDENTSHIPS

AFRC FOOD RESEARCH INSTITUTE

COLMAN'S STUDENTSHIP

Applications are invited from graduates with 1st or upper 2nd Class Honours in Microbiology, Biochemistry or related subjects for a 3-year research studentship, funded by Colman's of Norwich, to study the mechanism of inhibitionof yeasts and other acid-resistant microorganisms and by organic acids and other preservatives.

The studentship will be based in Norwich at the Food Research Institute and the work will be submitted for a PhD at the University of East Anglia. Conditions and the remuneration will be similar to those of SERC Case Awards.

Further details and application forms are available from the Secretary, AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA. Closing date: 19 January 1984. (908)F

A STUDENTSHIP leading to a MSc or PhD in biological psychiatry awarded by the Department of Psychiatry, University of Saskatchewan, in a multidisciplinary area of biological psychiatry (neurochemistry, neuropharmacology, enzymology, behaviour) is available. Applications are invited from graduates with a first or upper second class honours degree or equivalent qualifications in a suitable biological subject. Interested persons should write as soon as possible to Dr A V Juorio, Psychiatric Research Division, Cancer and Medical Research Building, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OW0, sending a curriculum vitae and the names and addresses of three referees. Non-Canadian applicants are advised to write before February 28th, 1984 and Canadian nationals or landed immigrants before July 31st, 1984. (NW143)F

THE UNIVERSITY OF SHEFFIELD

(Re-advertisement)

DEPARTMENT OF MEDICAL MICROBIOLOGY

RESEARCH STUDENTSHIP — TUMOUR IMMUNOLOGY

Applications are invited from graduates and final year students for this studentship tenable with immediate effect for 2½ years. The Studentship was previously advertised in June 1983. Applicants should have or anticipate obtaining a good degree in a branch of the biological sciences which preferably included Immunology. The research topic is the effect of tumours on macrophage function. The depression of normal macrophage function by tumour cells will be investigated in terms of induction of the immune response. Applications including c.v. and the names of 2 referees to: Dr. A. Clark, Department of Medical Microbiology, The Medical School, Beech Hill Road, Sheffield S10 2RX (Tel: (0742) 26484, Ext. 2027/2958) from whom further information may be obtained. Quote ref: R24/G.

(933)F

UNIVERSITY OF CAMBRIDGE RESEARCH STUDENTSHIPS

St Edmund's House, a graduate college of Cambridge, offers one or more Research Studentships of £375 to those commencing the PhD. Further details from The Senior Tutor, St. Edmund's House, Cambridge (CB3 0BN). (942)F

FELLOWSHIPS

THE UNIVERSITY OF SUSSEX

RESEARCH FELLOWSHIPS IN THEORETICAL ASTRONOMY

Applications are invited for up to two SERC-supported posts of Research Fellow in Theoretical Astronomy, for a period of two years with possible extension to a third year. The posts are on the Research Fellow IA scale (£7,190 to £11,615 pa). Appointments will be on the lower half of the scale. All posts carry USS benefits. Appointments will normally commence on 1st October 1984.

Candidates should propose an independent research programme related to that of existing members of the Centre. Further information can be obtained from Professor R J Tayler, Astronomy Centre, Physics Building, University of Sussex, Falmer, Brighton BN1 9QH, UK. Applications should arrive by 27th February 1984.

Applicants should ask up to three referees to send confidential reports, to reach the Astronomy Centre by the same date. (913)F

THE UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral research position (MRC-funded) to work on the interaction between tetanus toxin and neural tissue. The project will include studies on (1) the "receptor" for tetanus toxin (2) the biochemical and ultrastructural features of the internalization process and (3) structure function relationships within the toxin molecule itself.

Techniques involved will be in part similar to those previously developed to study the interaction of cholera toxin with target tissues (J Biol Chem 256 (1981) 8724), but will also involve the extensive use of monoclonal antibodies.

Applicants should have a PhD in Biochemistry or related discipline, and preference may be given to those with experience in cell culture and immunochemical techniques, and with an interest in membranes.

Salary: £7,190 to £8,080.

The position is immediately available although the starting date is negotiable, and will be for a maximum of 3 years.

Applications (along with the names of two referees) should be sent to Dr D R Critchley, Department of Biochemistry, University of Leicester, University Road, Leicester LE1 7RN. (939)E

THE AUSTRALIAN National University invites applications for a number of Postdoctoral Fellowships tenable for two years in the Research School of Physical Sciences. These are intended for young scientists under the age of 30 at the time of application who have completed, or expect soon to complete, requirements for a PhD degree or equivalent qualification. Appointments will be taken up during the second half of 1984 and may be made in any field of research of interest to the School which consists of departments of Applied Mathematics, Astronomy (Mount Stromlo and Siding Spring Observatories), Atomic and Molecular Physics, Engineering Physics, Mathematics, Nuclear Physics, Plasma Research Laboratory, Solid State Physics, Systems Engineering and Theoretical Physics. Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow Grade 1 $A20,164 — $A23,100; Postdoctoral Fellow Grade 2 $A23,394 — $A30,734 pa. Current exchange rates: $A1 = $US0.90 = UK62p. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should obtain further particulars from The Registrar, the Australian National University, GPO Box 4, Canberra, ACT 2601, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, before submitting applications which close on 31 January 1984. (W633)F

EMBL

European Molecular Biology Laboratory

PREDOCTORAL FELLOWSHIPS

The European Molecular Biology Laboratory is to award a limited number (6-8) of predoctoral fellowships for graduates wishing to study for a PhD at EMBL Heidelberg or its outstations at DESY, Hamburg or ILL, Grenoble. The areas of research in Heidelberg include membrane biology, cellular differentiation, analysis of the structure of membrane proteins and protein nucleic acid complexes and the structure and regulation of genes in procaryotes and eucaryotes. At the EMBL outstations the research centres on the elucidation of biological structures using either synchrotron radiation (Hamburg) or neutron beams (Grenoble).

To be eligible, candidates must hold, or anticipate receiving in 1984, a first or upper second class honours degree, a masters degree or a diplom in biological sciences, physical sciences or mathematics. Preference will be given to candidates from the member states of EMBL and EMBC namely: Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom.

Short listed candidates will be asked to visit EMBL (travel expenses reimbursed) for an interview. Those awarded predoctoral fellowships will be expected to begin working at EMBL in September 1984. The normal duration of the fellowships will be 3 years subject to annual review.

The closing date for completed applications is February 1st 1984. Successful candidates will be notified by the end of April 1984.

Application forms and a catalogue giving further information about the research at EMBL and the groups participating in the Laboratory's predoctoral programme is available on request from Dr J. Tooze, Predoctoral Programme, EMBL, Postfach 10.2209, 6900 Heidelberg, F.R. Germany. (W511)E

POSTDOCTORAL FELLOWSHIP PhD, or Equivalent — 2 yr position. To participate in active research program on inflammation, arachidonic acid metabolism in blood vessels. Salary $17,000. To begin Jan-June, '84. Send inquiries, curriculum vitae, and 2 references to: Michael A Moskowitz, MD, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114 USA. (NW147)E

VICTORIA UNIVERSITY OF WELLINGTON

New Zealand

UGC POSTDOCTORAL FELLOWSHIPS

In 1984 the University Grants Committe will award Postdoctoral Fellowships — 14 tenable for 1 year, 14 tenable for 3 years, on a national basis. Priority will be given to awards in current growth research areas in science in this country.

The University invites applications for these awards in the following areas: Biochemistry, Botany, Chemistry, Geology, Geophysis, Computer Science, Operations Research/Statistics, Physics, Zoology.

Conditions of Appointment and method of application are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or from Mrs M McCarthy, Personnel Office, Victoria University of Wellington, Private Bag, Wellington, New Zealand, with whom applications close on 10 February 1984. Late applications

MASSEY UNIVERSITY

Palmerston North, New Zealand

DEPARTMENT OF CHEMISTRY, BIOCHEMISTRY AND BIOPHYSICS

POST-DOCTORAL FELLOWSHIP

Applications are invited from chemists or biochemists for a position in the Department of Chemistry, Biochemistry and Biophysics to undertake research for one year commencing as soon as possible in 1984.

The appointee will be part of a team working on the development of new separation methods for large scale protein isolations. A feature of the research will be the use of reversed phase high performance liquid chromatography. The work has considerable commercial potential and is supported by research contracts from the Development Finance Corporation. The appointee could anticipate that additional funding might be available for a continuation of this position.

Emolument will be at the rate of NZ$ 21,660 p.a. inclusive of an allowance of up to NZ$ 3,033 for fares.

Further details of this fellowship and of the University, together with the general conditions of appointment may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or from the Registrar of the University, with whom applications close on 15 February 1984.

FYSSEN FOUNDATION
194, rue de Rivoli, 75001 PARIS

The Fyssen Foundation's general aim is "to encourage all forms of scientific enquiry into cognitive mechanisms, including thought and reasoning, underlying animal and human behaviour, into their biological and cultural bases, and into their phylogenetic and ontogenetic development."

1984 - 1985 FELLOWSHIPS

The Foundation will award a certain number of FELLOWSHIPS.

These fellowships are meant for the training and support of research scientists working in disciplines relevant to the aims of the Foundation such as ethology, paleontology, archaeology, anthropology, psychology, epistemology logic and the neurosciences.

The Foundation wishes to support, more particularly, research in such fields as:

ETHOLOGY AND PSYCHOLOGY: Nature and development of the cognitive processes in man and animals,

NEUROBIOLOGY: Neurobiological bases of cognitive processes and of their embryonic and post-natal development, as well as the elementary mechanisms they involve.

ANTHROPOLOGY-ETHNOLOGY: Study of cognitive foundations:
a) of the representations of the natural and cultural development,
b) of the technical systems developed in the various forms of social organization.

HUMAN PALEONTOLOGY: Origin and evolution of the human brain and human artefacts.

Priority will be given to French scientists wishing to work abroad and to foreign scientists wishing to work in French laboratories. Study grants will normally be granted for one year but may be extended up to three.

Applications should be established according to a form to be obtained from the Foundation which will include:
- the curriculum vitae,
- the *list* of publications of the applicant,
- the names of two senior scientists whom the applicant has asked to send testimonials to the Secretariat of the Foundation by the date indicated below,
- the letter of acceptance of the inviting laboratory.

The completed files should be sent in *15 COPIES* to the Secretariat of the Foundation, 194, rue de Rivoli, 75001 PARIS.

Limit date of receipt of applications: *APRIL 1st, 1984, without fail delivery date at the Foundation.*

1984 INTERNATIONAL PRIZE

A substantial INTERNATIONAL SCIENTIFIC PRIZE shall be given for a major contribution to the progress of knowledge in the fields of research supported by the Foundation. It was awarded in 1980 to Professor Andre LEROI-GOURHAN, in 1981 to Professor William H. THORPE, in 1982 to Professor Vernon B. MOUNTCASTLE and in 1983 to Professor Harold C. CONKLIN.

Disciplines considered for the 1984 Prize: *Cognitive psychology and Epistemology.*

The nominations should include:
- a curriculum vitae of the nominee,
- a list of his publications,
- a summary (four pages maximum) of the research work upon which the nomination is based.

Nominations for the 1984 Prize of the Fyssen Foundation should be sent in *15 COPIES* to the Secretariat of the Foundation, 194, rue de Rivoli, 75001 PARIS.

Limit date of receipt of nominations: *1st SEPTEMBER 1984.*

(W622)E

ANNOUNCEMENT

European Association for Cancer Research (EACR) — Italian Fellowship Programme in Cancer Research — Sponsored by the Italian Association for Cancer Research (Associazione Italiana per la Ricerca sul Cancro, AIRC)

Applications are invited from scientists and clinicians specifically in Europe to undertake cancer research projects in Italy. The Fellowships are designed to provide financial support for researchers to spend from 3 months to 1 year in Italian research institutions and the awards will include travel expenses to and from the host institution. The stipend will be related to age and experience and based upon national pay scales for academic research workers in Italy.

Proposed projects may be concerned with any aspect of research in cancer including chemical and viral carcinogenesis, epidemiology, tumour biology, biochemistry, genetics, immunology, immunotherapy, chemotherapy and radiotherapy. However, preference will be given to investigations on basic topics in cancer research.

Applicants should have worked actively in cancer research for at least two years and they should possess a doctorate degree or the equivalent. Applications should contain a curriculum vitae including a description of the proposed research project, details of travel expenses requested and a letter of approval of the proposed research project and invitation from the host institution in Italy.

The closing date for applications will be 1st April and 1st October of each year and the applicants will be notified of the award about 3 months following each closing date.

Please apply to the Secretariat of EACR at the following address for further details and application forms: Dr. M. R. Price, Secretariat of EACR, Cancer Research Campaign Laboratories, University of Nottingham, Nottingham NG7 2RD, UK. (W635)E

POSTDOCTORAL FELLOWSHIP IN NEUROCHEMISTRY

available for PhD or MD with training in analytical chemistry, especially HPLC. Work to be conducted on clinical research projects in Autistic children. NIMH stipend available beginning July or September 1984.

Interested applicants should contact Roland D Ciaranello MD, Laboratory of Developmental Neurochemistry, Department of Psychiatry, Standford Medical School, Stanford, CA 94305.
(NW166)E

THE QUEEN'S UNIVERSITY OF BELFAST

POST-DOCTORAL RESEARCH FELLOWSHIP IN ORGANIC CHEMISTRY

Applications are invited for a post-doctoral research fellowship to work with Professor R. Grigg on "New Prototropic Processes for the Synthesis of Heterocyclic Compounds". Appointment will be for one year with possible extension for a second year. Salary £7,632 per annum.

Applications, including full curriculum vitae and the names and addresses of two referees, should be addressed to the Personnel Officer, The Queen's University of Belfast, BT7 1NN. Closing date 31st January 1984. (945

EDINBURGH UNIVERSITY
POSTDOCTORAL FELLOWSHIP
Spin Dependent Effects in Low Energy Electron Atom Collisions

Applications are invited for a post of two to three years duration supported by the SERC from experimental physicists with experience in electron atom scattering. The successful applicant will be involved in a sequence of studies of spin dependent effects in the scattering of low energy electrons by atoms, making use of an existing source of polarised electrons.

Salary on the RG1A Scale (£8,080 — £8,975) with placement according to age and experience.

Enquiries and applications with the names of two referees to; Dr D M Campbell, Department of Physics, University of Edinburgh, James Clerk Maxwell Building, The King's Buildings, Mayfield Road, Edinburgh EH9 3JZ.

Please quote Reference No 5129.
(918) E

ISTITUTO NAZIONALE DI FISICA NUCLEARE (I.N.F.N.)

ONE FELLOWSHIP IN NUCLEAR PHYSICS

For one year, starting September 1984 for non Italian citizens. The chosen applicant shall pursue his research at the National Laboratories of Legnaro (Padua).

The main field of activity will be experimental work with the 16 MV Tandem Van de Graaff accelerator of the Legnaro Laboratories.

The salary will be approximately 1.000.000 Italian lire per month for twelve months.

Send applications and requests for further information to: Prof. Cosimo Signorini, Director of the National Laboratories of Legnaro, Via Romea, 4 — 35020 Legnaro (Padua) Italy.

The application deadline is February 15, 1984. (W626)E

SOUTHAMPTON PHLS and UNIVERSITY OF ALBERTA
Department of Medical Microbiology
POST DOCTORAL FELLOWSHIPS

Applications are invited for two Canadian funded positions for working on techniques relevant to the Southampton/North American Molecular Epidemiology Programme. The work will involve the development and evaluation of chromosomal and plasmid probes, endonuclease restriction analysis, plasmid detection and comparison by DNA homology, storage and media requirements for optimisation of molecular techniques.
The posts will relate (1) to the detection and strain identity of Campylobacter jejuni/coli/CLO/pyloridis, and (2) strain identity, antibiotic and antiseptic resistance of proteus species isolated from patients with hospital acquired infection.
Applicants should have an appropriate background in biochemistry, cell biology, immunology or relevant microbiology and be within 3 years of having, or about to obtain a PhD. The post is tenable for up to 3 years in the University of Alberta, Edmonton, Canada. Salary, Canadian $20,000. For further particulars contact Dr A. D. Pearson by telephone in the UK 0703 776177, quoting reference ME3.
No formal application forms. Please send three copies of full curriculum vitae, summary of PhD, names and addresses of three referees to the Director, PHLS, Level B, South Block, Southampton General Hospital, Southampton SO9 4XY, England by 10th February, for consideration March 1984. Appointment will depend on successful competition to an external agency. (931)E

FRIEDRICH MIESCHER-INSTITUT
Basel, Switzerland
RESEARCH FELLOWSHIP

A fellowship is available in a research group studying the role of oncogenes in human leukemogenesis. Appropriate technologies and good clinical contacts are available. We subsidize your travel costs.

Direct your application with CV, names of two referees to Dr Ch Moroni, Friendrich Miescher-Institut, PO Box 2543, CH-4002 Basel, Switzerland. (W628)E

THE JOHNS HOPKINS UNIVERSITY
DEPARTMENT OF EARTH AND PLANETARY SCIENCES
POSTDOCTORAL FELLOWSHIP IN STRUCTURAL GEOLOGY

The Department of Earth and Planetary Sciences seeks a quantitative structural geologist to fill a one-year postdoctoral fellowship (renewable). PhD required. Send resume and three reference letters, by January 31, 1984, to: Assist. Prof. Declan De Paor, Earth & Planetary Sciences, Johns Hopkins University, Baltimore, Maryland 21218. (NW172)E

The Wellcome Trust

Wellcome Trust Senior Research Fellowships in Medical Science in Australia

The Trustees of the Wellcome Trust announce the award in 1984 of 2 five year Senior Research Fellowships to be held in Australia in clinical medical science. Both medically qualified and science graduates, aged probably between 32-38, who wish to make their career in Australia and undertake research in a clinical or para clinical Australian University department are eligible to apply whether or not they are currently working in Australia. Direct personal enquiries by possible candidates are welcome but formal applications must be submitted by the Head of Department in the University where the candidate will work. Candidates will normally be Australian citizens.

The salary offered will be according to age and experience and on appropriate Australian academic scales, with up to $A10,000 p.a. ancillary support.

Nominations must be submitted on the appropriate application form and the closing date for the 1984 awards is 1 March 1984. For information and application forms please contact:

Dr Bridget M Ogilvie, The Wellcome Trust, 1 Park Square West, London NW1 4LJ England, or
Dr B Hudson, The Royal Australasian College of Physicians, 145 Macquarie Street, Sydney NSW 2000, AUSTRALIA.

(919)E

UNIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY
POST-DOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral research position (CRC-FUNDED) to work on an ongoing project directed toward clarifying the relationship between Tyrosine-specific phosphorylation of the cyto-skeletal protein vinculin, and the morphology of cells transformed by Rous sarcoma virus (RSV).

Methods involved will include cell culture (transformation of primary chick cells with RSV), immuno-chemical and immunofluorescence techniques.

Applicants should have a Ph.D. in Biochemistry and experience in some area of Tumour Biology is desirable.

Salary £7,190 to £8,975.

The position is available immed-iately, although the starting date is negotiable, and will be for 2 years.

Applications (along with the names of two referees) should be sent to Dr D. R. Critchley, Department of Biochemistry, University of Leicester, University Road, Leicester LE1 7RN. (938)E

ISTITUTO NAZIONALE DI FISICA NUCLEARE (I.N.F.N.)

TWO POST-DOCTORAL FELLOWSHIPS IN THEORETICAL NUCLEAR AND PARTICLE PHYSICS

For one year, starting October 1984, for non Italian citizens. The chosen applicants can pursue their research at any of the following Laboratories and Sections of INFN:

- National Laboratories of Frascati (Rome)
- National Laboratories of Legnaro (Padua)
- National Southern Laboratory (Catania)
- INFN Section of Turin
- INFN Section of Genoa
- INFN Section of Milan
- INFN Section of Pavia
- INFN Section of Padua
- INFN Section of Trieste
- INFN Section of Bologna
- INFN Section of Florence
- INFN Section of Pisa
- INFN Section of Rome
- INFN Section of Naples
- INFN Section of Bari
- INFN Section of Catania
- INFN Section of Sanità (Rome)

The annual gross salary will be 18.000.000 Italian Lire.

Send applications and requests for further information to: Prof. Nicola Cabibbo, President National Institute of Nuclear Physics (INFN) Piazza dei Caprettari, 70 — 00186 ROME (Italy).

The application deadline is February 28, 1984. (W625)E

AWARDS

Prize competition

announced by the

Anna Monika Foundation

for the investigation of the physical substrate and functional disturbances of endogenous depressions by approval of the Minister of the Interior of Nordrhein-Westfalen, Düsseldorf, 9th of June, 1965.

The Foundation announces the following prizes:

First Prize	**US-Dollar 15.000,-**
Second Prize	**US-Dollar 5.000,-**
Third Prize	**US-Dollar 2.500,-**

preferably for studies of biochemical, histological, neurophysiological, neuropathological, psychopharmacological, psychiatric or psychosomatic nature. The studies should be carried out in close cooperation with a psychiatric clinic, an university or an equivalent scientific institution. As far as possible, the papers should give information about recent advances in knowledge that should be helpful in promoting treatment and would open up new paths of progress. The papers may be written in German, French or English and should be submitted to the Chairman of the Committee, *Professor Dr. P. Kielholz, Basle/Switzerland.* Besides hitherto unpublished studies, papers published in the past two years in a German or international professional journal may also be submitted. *Deadline for submission to the Committee is 30th of September, 1984.* To help the Committee to come to a speedy decision it is requested that a maximum of three publications in four copies as well as a summarizing report (approx. 600 words) of the studies submitted for the competition should be included. *Prizes will be awarded on 30th of June, 1985.* If, in the opinion of the Committee, no papers of sufficient merit are submitted, it reserves the right to make no award. Prizes and their amounts will be awarded according to the merits of the study in question. Subject to the Committee's decision, each prize can be divided between two papers.

The Committee of Judges:

Chairman: Professor Dr. med. P. Kielholz, Psychiatrische Universitätsklinik Basel, Wilhelm-Klein-Straße 27, CH-4056 Basle/Switzerland

Members:

Professor Dr. G. Baumgartner, Neurologische Universitäts- und Poliklinik, Zürich/Switzerland.

Professor Dr. Otto Creutzfeldt, Max-Planck-Institut für Biophysikalische Chemie, Göttingen/Fed. Rep. of Germany.

Professor Dr. H. J. Dengler, Medizinische Universitätsklinik, Bonn-Venusberg/Fed. Rep. of Germany

Professor Dr. Alfred M. Freedman, Department of Psychiatry, New York Medical College Valhalla, New York/USA.

Professor Dr. K. Heinrich, Psychiatrische Universitätsklinik, Düsseldorf/Fed. Rep. of Germany.

Professor Dr. Albert Herz, Max-Planck-Institut für Psychiatrie, München/Fed. Rep. of Germany.

Professor Dr. H. Hippius, Psychiatrische Klinik und Poliklinik der Universität München/Fed. Rep. of Germany.

Professor Dr. Horst Jatzkewitz, Max-Planck-Institut für Psychiatrie, München/Fed. Rep. of Germany.

Professor Dr. Dr. Palm, Zentrum für Pharmakologie der Johann-Wolfgang-Goethe-Universität, Frankfurt/Main.

Professor Pierre Pichot, Chaire de Psychologie Médicale, Centre Psychiatrique Sainte-Anne, Paris/France.

Professor Dr. Dr. Wilhelm Stoffel, Institut für Physiologische Chemie der Universität Köln/Fed. Rep. of Germany.

Managing Director: Professor Dr. Benno Hess, Töllnerstraße 9/11, D-4600 Dortmund 1/Fed. Rep. of Germany.

(W632)N

GRANTS and SCHOLARSHIPS

GRANTS and SCHOLARSHIPS

The HUBRECHT LABORATORY an international institute for fund mental research in *developmen biology* (early amphibian ar mammalian embryogenesis organismal, cellular and molecul levels).

Short-term maintenance gran (1-6 m.) are available for your developmental biologis (preferably under 30) to carry o research fitting in with t Laboratory's programme. F further info. and appl. form wri to Deputy Dir., Uppsalalaan 3584 CT Utrecht, Netherland Deadline for applications 1 Ju and 1 January. (W640)H

THE NATIONAL KIDNEY RESEARC FUND

invites applications for re research grants for consideration the Meeting on April 17th 1984.

Application forms for these a travel grants available fr N.K.R.F. 184B Station Roa Harrow HA1 2RH (Tel: 01-8 4460).

Completed forms required by 5 March, 1983.

(920)H

WANTED AND FOR SAL

Scientific and Technical Reviews.

Please inquire for runs or single volumes.

SANTO VANASIA,
58 Via M. Macchi,
20124 MILANO, Italy.

(W547)

APPOINTMENTS WANTE

advertise your qualities and qua cations through the most influen science weekly in the world a special reduced cost of *50 cen word.* (Personal Box Numb $4.00).

Advertisements must be pre-paid sent to: Nature Classified (A Wtd), 15 East 26 Street, New Y NY 10010. (890)

Please mention nature when replying to these advertisements

Typesetting: J.W. Arrowsmith Ltd, Bristol and Outline Graphics Ltd. London EC4Y 0HP. Printing: Kingsdale Press Ltd, Reading, Berks, in association with Godfrey Lang Ltd. London WC1R 4EB and The William Byrd Press Inc., Richmond, Virginia, USA Published by MACMILLAN JOURNALS LTD, 4 Little Essex Street, London WC2R 3LF. — 5 January 1984.

nature
is available in Microform.



Please send additional information for_____
(name of publication)

Name_____

Institution_____

Street_____

City_____

State_____ Zip_____

300 North Zeeb Road, Dept. P.R., Ann Arbor, Mi. 48106

University Microfilms International

Circle No.43 on Reader Service Card.



nature is keeping ahead

Are you being left behind?

New research results, the very latest international science news, key papers by the world's leading scientists and speed of publication ensure *Nature* remains one of the foremost weekly scientific journals in the world.

But if you're tied in to a circulation list at work, all this vital weekly information will undoubtedly reach you late, often incomplete and sometimes not at all.

With your own personal copy, you solve the problem. Every week you'll have all the latest research results and science news in your hands within days of publication:

News reports from throughout the world of science and technology.

A **News and Views** section, where new ideas are analysed and discussed by leading researchers.

Articles and Letters in which key investigators disclose their work for the first time.

Book Reviews – Eminent figures offer expert judgments on a wide variety of recent publications.

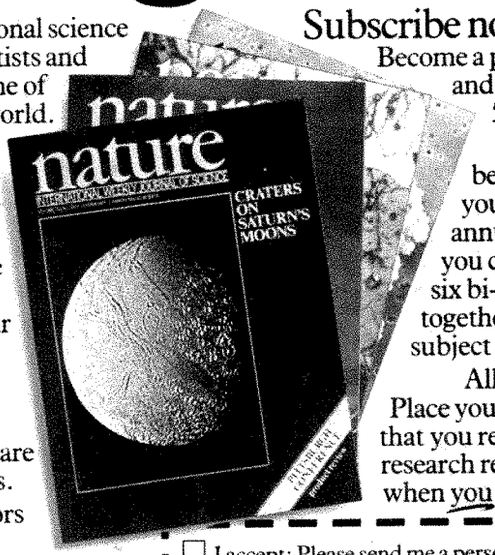
Special Issues providing an in-depth treatment of topics of importance.

Product Reviews – Detailed descriptions of laboratory equipment newly available.

Classified Advertisements for a wide variety of positions all over the world.

You will no longer be dependent upon other people passing down the 'list' copy, and because you will automatically receive every issue, you won't risk missing a single copy through absence from work. Moreover, you keep all this key information for permanent reference.

Through your door every week



Subscribe now – at Half Price

Become a personal subscriber now and you will receive a discount of 50% off the full rate.

All 51 weekly issues will be delivered to the address of your choice. You can start your annual subscription with any issue you choose. And you will receive six bi-monthly author indexes, together with the annual index of subject and author.

All this for just 94p a week. Place your order now and make certain that you receive the scientific news and research results crucial to your work when you want them.

☐ I accept: Please send me a personal subscription to *Nature* for one year (51 issues) at the special half-price rate of £49.00.

☐ I enclose my personal cheque for £49.00 made payable to *Nature*. (Note: Institutional cheques cannot be accepted at this special rate).

☐ Please charge to my credit card account.
Access/American Express/Visa/Diners Club.

Account No._____

Card Expiry Date_____

Signature_____

Name_____

Address_____

_____

_____

_____

Date_____ 7B18

Please return this form to:
Circulation Manager
Nature
Brunel Road, Houndmi
Basingstoke
Hants RG21 2XS

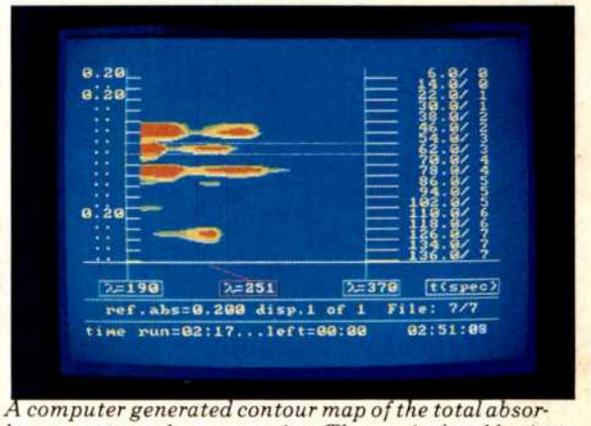
This offer applies to UK and North America only. Please allow approximately 4 weeks for delivery of the first issue in your subscription year.

nature

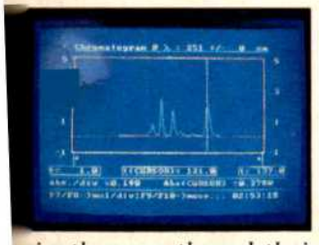
T MISS PEAKS

E ARRAY DETECTOR

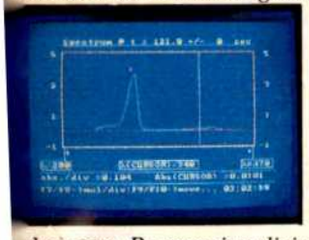
ısh-button access to chromatograms and spectra, combined with an innovative cing technique, automatically surveys and explores the whole chromatographic ıdscape. Equips you with colour-graphic ed back for an analytical follow-through no her system offers. Power to track, expose d pinpoint all your peaks. Only LKB's Rapid ectral Detector lets you rapidly pursue, sily discover and clearly reveal every single ak—and then deliver it blown-up in perfect detail. So however small, or wherever it's hidden, you'll just never miss a peak—you just can't! The Rapid Spectral Detector is a computer based diode array detection system that offers dramatic breakthroughs in HPLC. With real time display and raw data storage Rapid Spectral provides the true visual command you need for complete analytical insight. Lets you probe new perspectives to give you more information and better results. So if you want to find those missing peaks, get in touch with LKB. We'll send you a broschure that describes the very best in diode array technology. You'll find it infinitely revealing, just like our Rapid Spectral Detector.



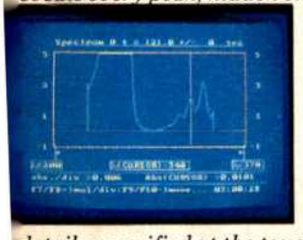
A computer generated contour map of the total absorbance pattern after separation. The vertical and horizontal axes are time and wavelength respectively. Colour coding is used to represent different levels of absorbance for each isogram.



ive the cursor through the isoıms to access chromatograms...



d spectra. Progressive slicing rapeveals every peak, hidden or not.



detail, magnified at the touch of ton.



LKB BROMMA

Circle No.42 on Reader Service Card.

Head office: LKB-Produkter AB, Box 305, S-161 26 Bromma, Sweden. Tel. 08-98 00 40, telex 10492
IS sales office: LKB instruments, Inc. 9319 Gaither Road, Gaithersburg, Maryland 20877. Tel. 301-963-3200, telex 909870 (dom.), 64634 (intern.)
UK sales office: LKB Instruments Ltd., 232 Addington Road, S. Croydon, Surrey CR2 8YD, England. Tel. 01-657 8822, telex 264414
Other sales offices in: Athens (for Middle East), Copenhagen, Ghent, Zoetermeer, Hong Kong, Luzern, Munich, Paris, Rome, Turku, Vienna

Packagene™

Lambda DNA Packaging System

The Packagene DNA Packaging System delivers the long-awaited combination of convenience and performance.

Promega Biotec

2800 S. Fish Hatchery Road
Madison, WI 53711 U.S.A.
Toll Free 800-356-9526
In Wisconsin 608-274-4330
TWX 910-286-2738

Packagene™ packaging system is also available from any of the following:

In Holland, Ireland & U.K.:
P & S Biochemicals Ltd.
38 Queensland Street
Liverpool L7 3JG, **England**
Telephone: 051-709-4701
Telex: 627694 HYDRO G

In Finland:
Yliopiston Apteekki
Kemikaaliosasto
Kalliolanrinne 6
00510 Helsinki, **Finland**
Telephone: 90-717100
Telex: 121708 UNIVE SF

In Belgium & France:
Coger
79 Rue des Morillons
75015 Paris, **France**
Telephone: 1-533-67-17
Telex: COGER 204046F

In Israel:
Eisenberg Brothers, Ltd.
Asia House
P.O. Box 33638
Tel-Aviv, **Israel**
Telephone: 3-219111
Telex: 33-511 EISGR IL

In the Far East:
Seikagaku Kogyo Co. Ltd.
Tokyo Yakugyo Bldg.
9-8, Nihonbashi-Honcho 2-chrome,
Chuo-ku, Tokyo, 103 **Japan**
Telephone: 3-270-0966
Telex: SKK J27140

In Denmark, Norway & Sweden:
SDS
Scandinavian Diagnostic Services
Box 40
S-311 01 Falkenberg, **Sweden**
Telephone: 0346-83050
Telex: 38230 DIAGNO S

In France, Germany, Italy Spain & Switzerland:
Genofit, S. A.
5 Rue des Falaises
1205 Geneva, **Switzerland**
Telephone: 022-29-84-30
Telex: 423226 STER CH

Literature references available upon request.

Circle No.05 on Reader Service Card

Packagene™ Extract System

- Frees up the time you now spend making packaging extracts. You can spend your time producing and analyzing data.
- Provides high efficiencies and consistent, reliable performance. The **Packagene** System routinely achieves packaging efficiencies of greater than 10^8 pfu/μg.
- Is more convenient to use than any other commercially available lambda packaging extract system. It's a single tube, to which only substrate DNA need be added.

The **Packagene** extract system is an efficient, single-extract *in vitro* lambda DNA packaging system. It is derived from a complementary mixture of induced *E. coli* strains (1, 2). These strains are lysogenic for lambda mutants, which are blocked at different stages of virus maturation. The extract contains all the components and buffers you need to perform the packaging reaction, and requires only the addition of substrate DNA.

Performance

Efficiency—A typical **Packagene** Extract System gives the following efficiencies in a standard packaging reaction using 0.5 μg of the indicated vector DNA.

DNA	Efficiency (plaque forming units/μg)
ligated lambda cI857 Sam7	2 x 10^8
ligated lambda 1059	1 x 10^8
ligated EMBL 3 or 4	1 x 10^8

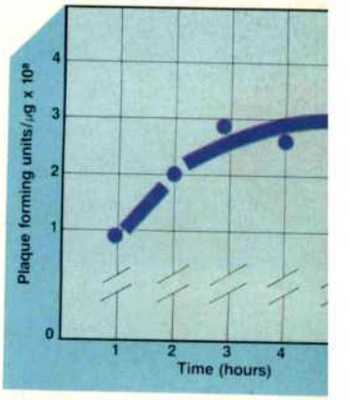


Figure 1. Time course analysis of a la packaging experiment. At time zero, C of lambda cI857 Sam7 DNA was add duplicate **Packagene** Extract System the times indicated one reaction at a was assayed for plaque forming units data indicate the **Packagene** System is complete after 3 hours.

Capacity: As much as 5 μg of DNA can be packaged in a sing **Packagene** extract system vial.

Low Background: The level of endogenous background plaqu the extract comprises less than .0001% of the expected phage

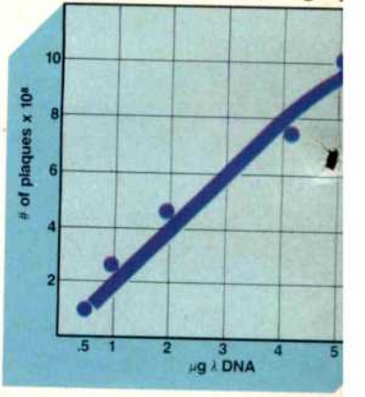


Figure 2. Analysis of the **Packagene** E System capacity. Different amounts of lambda cI857 Sam7 DNA were added t **Packagene** Extract System vials as indi After 2 hours the extracts were assayed plaque forming units. The increase in th number of plaques obtained is directly p portional to the amount of DNA adde 3-5 μg of DNA in each extract vial.

New! Eliminate the need to prepare lambda arms with the state-of-th EMBL 3 and EMBL 4 lambda vectors now available from Promega. Co us or one of our distributors for further information.

Packagene

Lambda DNA Packaging System

Convenient, highly efficient
extract system



Peptide antiser for immunocytochemistry



Anti-CCK 39 on porcine antrum (paraffin embedded section, Bouin's fixative). Detected with anti-rabbit Ig, horseradish peroxidase-linked, $F(ab')_2$ fragment.

Amersham now offers a wide range of antisera designed for the localization of peptides by immunocytochemistry. These new products are fully compatible with our anti-rabbit antibodies with enzyme or fluorescent labels.

Please contact Amersham or your local representative for more details.

Amersham Corporation
2636 South Clearbrook Drive, Arlington Heights, IL 60005
(312) 364-7100 or (800) 323-9750 (Toll Free)

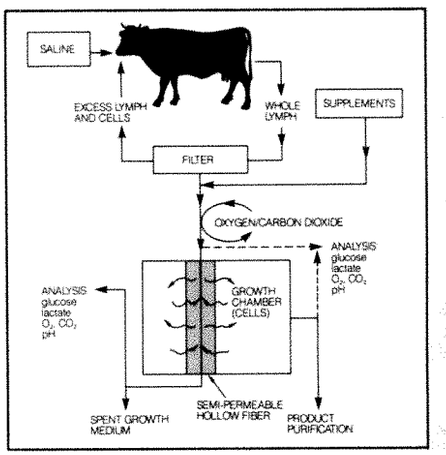
In Canada 505 Iroquois Shore Road, Oakville ONT L6H 2R3 (416) 842-2720/(800) 268-5061

Amersham

Circle No.56 on Reader Service Card.

NATURE'S BIOREACTOR

The New Bio-Response MCT* (Mass Culturing Technique) System Delivers Monoclonal Antibodies And Other Mammalian Cell Products When You Need Them—From Grams To Kilograms.



Insufficient quantities of desired peptides are a key cause of expensive research and production delays. The MCT system is a *new* and *unique* mammalian cell culturing process designed to overcome the obstacle of insufficient quantities of peptides by delivering the quantity you need... *when you need it!* Additionally, every step in MCT production has been developed to contain costs now and in the future while maintaining exceptional purity and quality in mammalian cell proteins.

Quantity

Unlike conventional cell-culture methods, the MCT system uses "fresh," free-flowing lymph directly from a cow. Following treatment, the lymph is continuously diffused into and out of a growth chamber. This nearly *in vivo* growth environment stimulates continuous and optimal protein secretion. The result? Large quantities of desired proteins enabling you to move to the clinic or market... *faster.*

Cost

Unlike conventional cell-culture methods that require extensive labor forces and expensive growth mediums, Bio-Response's MCT is a closed, steady-state system. In essence, the MCT system starts with a low-cost lifeline—a feeding cow—and ends with a pure, quality product. MCT *...an optimal system at low cost.*

Purity

Unlike conventional cell-culture methods, the MCT system is designed to insure purity without tedious processing. By directing the lymph across semi-permeable membranes, MCT provides both separation of media protein from desired cell products, and a continuous nutrient flow. The ability to add or withdraw from the chamber to maintain optimal cell density permits continuous harvest of a highly pure product.

Quality

Unlike conventional methods of production that attempt to create biologically active mammalian cell products, the MCT system provides a pure-mammalian product—an exact copy of the original protein and, in the case of non-antibody polypeptides, uncompromised by inappropriate glycosylation and disulfide bond formation. There is a growing consensus favoring products produced in a mammalian cell-culture system, such as the Bio-Response MCT system.

Circle No.14 on Reader Service Card.

For further information, call Bio-Response, Inc. at (415) 786-9744.

BIO-RESPONSE, INC.

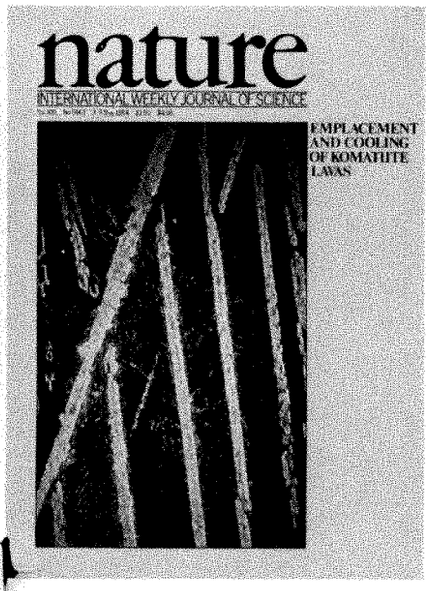
*MCT (Mass Culturing Technique) is a trademark of Bio-Response, Inc.

© 1984 Bio-Response, Inc. All Rights Reserved

nature

NATURE VOL. 309 3 MAY 1984

COVER



A thin section of a spinifex-textured komatiite from Marshall Pool, Australia. For a discussion of the flow of komatiite lavas in Archaean times and an explanation of spinifex texture see page 19.

OPINION

Attrition, not conquest, of disease 1
Double-talk on animals 2

NEWS

Star wars 3
Genetic engineering
Cancer research 4
NIH to bolt the stable door
Agent Orange 5
Chinese nuclear industry
Australian technology 6
Radioactive atolls
European pharmaceuticals
China in space 7
Unesco
Animal welfare 8
Israeli–Egyptian relations
Amersham International 9
Taxing problems

CORRESPONDENCE

Testing laboratories/Abstracts/Multiple authors/etc. 10

NEWS AND VIEWS

Benefits of measuring time
John Maddox 11
Retroviruses linked with AIDS
Robin Weiss 12
Fixation of CO_2 in dark midwater zones of the ocean
Paul Tett 14
Turbulence in petrology — the behaviour of komatiites
E G Nisbet 14
A budding interest in the yeast cytoskeleton
Jeremy Hyams 15
Fluctuations in microwave background radiation
M S Longair 16
Black holes, accretion discs and Seyfert galaxies
C Martin Gaskell 17
Oxygen carriers of a third kind
K E van Holde 18

BOOK REVIEWS

Catastrophe Theory
by V I Arnold and
Mathematical Models of Morphogenesis
by R Thom
Brian Goodwin 93
Sun Traps
by J Elkington
Ian Smart 94
An Introduction to the Physical Chemistry of Biological Organization
by A R Peacocke
George Oster
The Natural History Prose Writing of John Clare
M Grainger, ed.
Richard Mabey 95
Theoretical Glaciology
by K Hutter
Charles F Raymond 96
Parallel Processing in the Visual System
by J Stone
D J Tolhurst

ARTICLES

Emplacement and cooling of komatiite lavas
H E Huppert, R S J Sparks, J S Turner & N T Arndt 19

3.2Å structure of the copper-containing, oxygen-carrying protein *Panulirus interruptus* haemocyanin
W P J Gaykema, W G J Hol, J M Vereijken, N M Soeter, H J Bak & J J Beintema 23

Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule
M D Pierschbacher & E Ruoslahti 30

LETTERS TO NATURE

The Sunyaev-Zeldovich effect towards three clusters of galaxies
M Birkinshaw, S F Gull & H Hardebeck 34

Alignment of distant radio sources
R H Sanders 35

The case for antiparticles in the extragalactic cosmic radiation
F W Stecker, & A W Wolfendale 37

Neutrino tomography: Tevatron mapping versus the neutrino sky
T L Wilson 38

Molecular confrontation in surfactant micelles
K A Dill, D E Koppel, R S Cantor, J D Dill, D Bendedouch & Sow-Hsin Chen 42

Synchrotron X-ray diffraction from a 800μm^3 zeolite microcrystal
P Eisenberger, J M Newsam, M E Leonowicz & D E W Vaughan 45

Propagated temperature changes during onset and recovery of the 1982–83 El Niño
F P Chavez, R T Barber & H Soldi 47

A wetter climate in eastern Sudan 2,000 years ago?
R Mawson & M A J Williams 49

Nutrient depletion indicates high primary productivity in the Weddell Sea
J C Jennings Jr, L I Gordon & D M Nelson 51

Bacterial chemolithotropy in the ocean is associated with sinking particles
D M Karl, G A Knauer, J H Martin & B B Ward 54

Human large granular lymphocytes are potent producers of interleukin-1
G Scala, P Allavena, J Y Djeu, T Kasahara, J R Ortaldo, R B Herberman & J J Oppenheim 56

Mouse Ia antigens are receptors for lactate dehydrogenase virus
T Inada & C A Mims 59

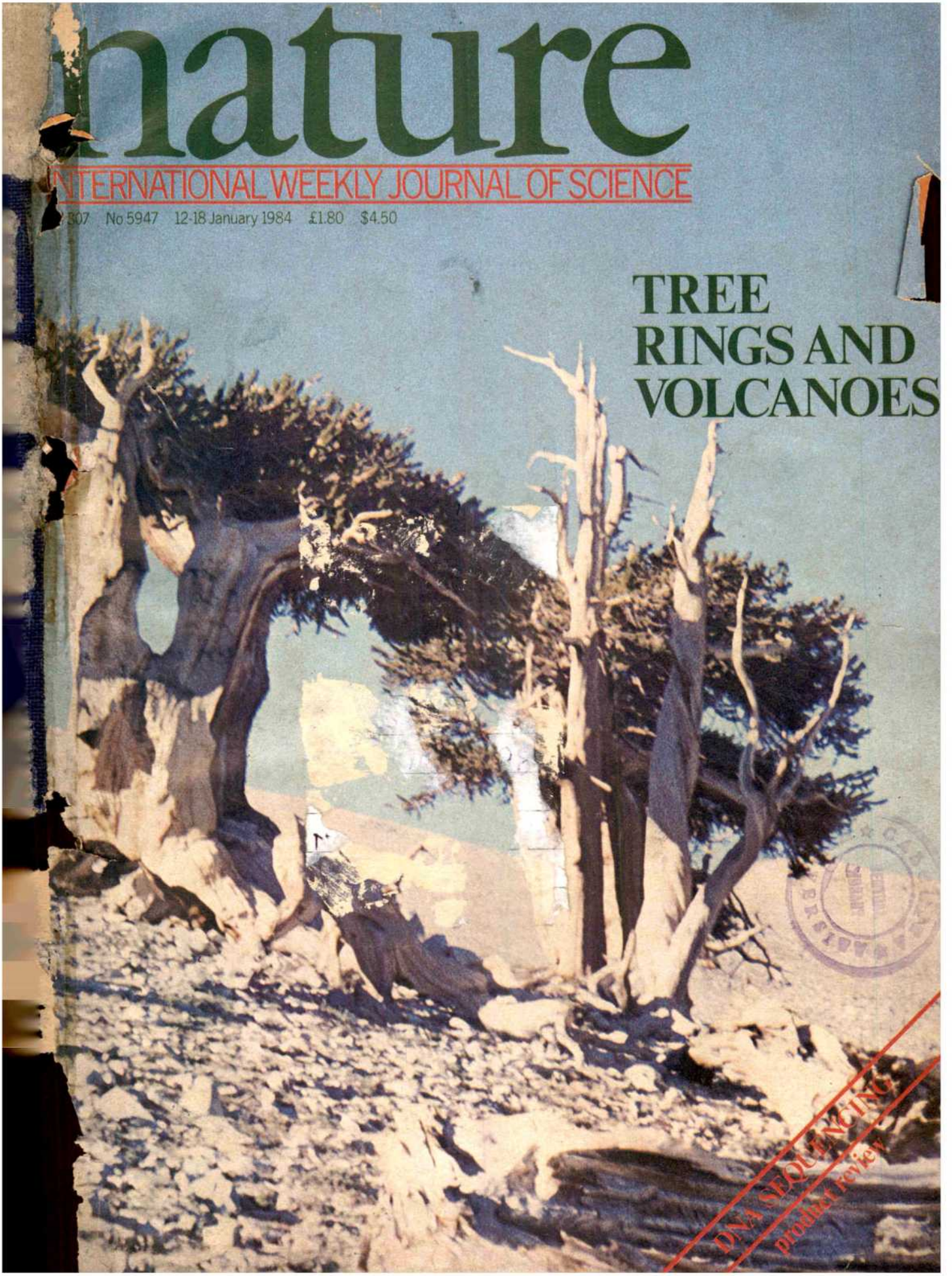
Contents continued overleaf

Nature® (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Annual Index (mailed in February). Annual subscription for USA and Canada US $230 (for subscription prices elsewhere, see next page). USA and Canadian orders only to: *Nature*, Subscription Dept, PO Box 1018, Manasquan, New Jersey 08736, USA. All other orders to *Nature*, Brunel Road, Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY 10010 and additional mailing offices. Authorization to photocopy material for internal or personal use, or the internal or personal use of specific clients, is granted by *Nature* to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of $1.00 per copy plus $0.10 per page is paid directly to CCC, 21 Congress Street, Salem, Massachusetts 01970, USA. The identification code for *Nature* is 0028-0836/84 $1.00 + $0.10. US Postmaster send form 3579 to: *Nature*, 15 East 26 Street, New York, NY 10010. Published in Japan by Macmillan Shuppan K.K., 5th Floor Eikow Building, 1-10-9 Hongo, Bunkyo-ku, Tokyo, Japan.

© 1984 Macmillan Journals Ltd.

nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
307 No 5947 12-18 January 1984 £1.80 $4.50

TREE RINGS AND VOLCANOES

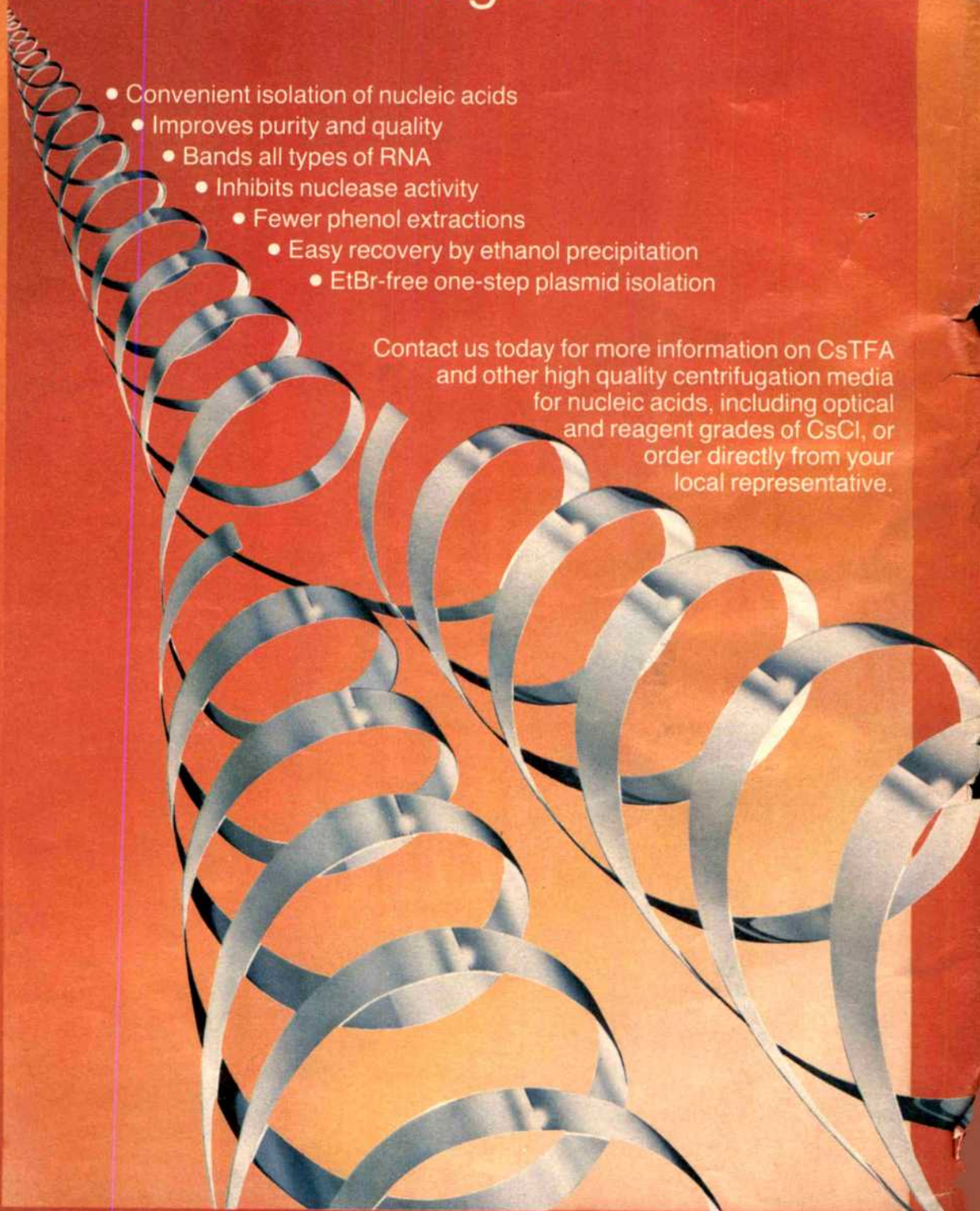


DNA SEQUENCING
product review

Isolate DNA and RNA with CsTFA™ a new centrifugation medium

- Convenient isolation of nucleic acids
- Improves purity and quality
- Bands all types of RNA
- Inhibits nuclease activity
- Fewer phenol extractions
- Easy recovery by ethanol precipitation
- EtBr-free one-step plasmid isolation

Contact us today for more information on CsTFA and other high quality centrifugation media for nucleic acids, including optical and reagent grades of CsCl, or order directly from your local representative.



Pharmacia Fine Chemicals AB
Box 175
S-751 04 Uppsala 1
Sweden

Pharmacia
Fine Chemicals

Circle No. 13 on Reader Service Card

HEPES

THE MOST WIDELY-USED "GOOD" BUFFER IN PHYSIOLOGICAL & RELATED RESEARCH.

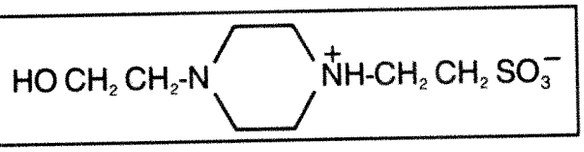
HEPES, with a pKa value of 7.31 at 37°C has become the most widely used of the "Good buffers" in physiological and related research. Its emergence is no surprise considering the shortcomings of the buffers commonly in use prior to the introduction of these zwitterionic buffers by N.E. Good and colleagues in 1966. The various systems in use up til that time have a variety of disadvantages which limit their usefulness and make them inconvenient in cell or tissue culture techniques. For example, the carbonate system has a pKa value more than a full pH unit away from that required for efficient cell growth. In addition, an atmosphere of carbon dioxide is required to maintain the pH of the media by minimizing CO_2 loss from the solution. Phosphate buffers tend to precipitate many polyvalent cations and have poor buffering capacity above a pH of 7.5. Tris buffers have a high coefficient of pH variation with temperature and are frequently inhibitory. For a thorough review of these and other commonly used buffer systems see Good et al.

HEPES on the other hand, with its pKa of 7.31 at 37°C, has its maximum buffering capacity very near the pH of mammalian systems. It exhibits no metal binding and has a low pKa temperature coefficient of −0.014/°C. HEPES is readily soluble up to 2.25 M at 0°C and HEPES buffer systems can be maintained open to the atmosphere.

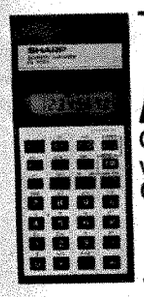
The success of HEPES and other zwitterionic buffers in cell culture systems has led to their widespread use in many fields. Several examples include their use in: 1) improved methods for propagating influenza A2 (Hong Kong) virus and in routine methods for virus assay and identification, 2) improving methods for extending survival times during organ culture, 3) increasing post-freeze motility of cryogenically stored semen, 4) reducing loss of blood factor activity from freeze drying during preparation, 5) improving media for bacteria culture, particularly using MOPS and HEPES and 6) improving the quality of preservation and stabilization for cell components from plant and mammalian tissue prepared for electron microscopy.

U.S. Biochemical Corporation has been a major supplier of HEPES and other "Good buffers" since our inception. Consistency of our product is assured by rigorous quality control of each batch before it is approved for use, including a low UV absorbance specification as well as tests for residual halide and trace heavy metal contaminants.

HEPES
M.W. 238.31
pK_a at 20°C. — 7.55



16926 HEPES
(N-2-Hydroxyethylpiperazine-N′-2-Ethanesulfonic Acid)
1 kg 115.00



A SPECIAL PRICE, A SPECIAL OFFER:

Our 1 Kg. quantity of HEPES (USB Product No. 16926) is specially-priced at just $115.00. And when you order 1 Kg. HEPES, you'll receive a Sharp EL-508A 32-Function Scientific Calculator FREE!

USB

We're part of the solution.

United States Biochemical Corporation

P.O. Box 22400 • Cleveland, Ohio 44122 • 800-321-9322
Ohio and Canada call collect 216-663-0330 • Telex: 980718

Write for your free USB Catalog containing listings of over 5,000 biochemicals.

A 'TdT' THAT REALLY WORKS NEEDN'T BE AN 'ET' ONE

The introduction of a
TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE
that is monoclonal affinity
purified from calf thymus
to be greater than 99% pure

Specific activity in excess of 120,000 units/mg

Door to door delivery
within 48 hours anywhere in Europe

Anglian Biotechnology Limited
Hawkins Road, Colchester, Essex, England CO2 8JX
Telephone (England) (0206) 44469
Telex 987181 BIOTEC G

Circle No.21 on Reader Service Card.



50 years of the gold standard

In 1934 a lasting symbol of quality and purity was founded – AnalaR.

AnalaR quickly became accepted as the internationally recognised standard for analytical reagents. Today in its Golden Jubilee year of 1984 there are over 400 AnalaR reagents, of guaranteed purity and reliability, available through BDH, the largest and most research orientated manufacturer of laboratory chemicals and biochemicals in Britain.

BDH

ng the Standard

micals Ltd, Broom Road, Poole, BH12 4NN. Tel: 0202 745520.
186 or 418123 TETRA G. Cables: TETRADOME POOLE.

BDH AnalaR

BDH Chemicals Ltd Poole England

Nitric acid sp. gr. 1.42

Advertisement

BRL® PRODUCT REVIEW

Bethesda Research Laboratories, Life Technologies Inc. P.O. Box 6009 • Gaithersburg, MD 20877
Bethesda Research Laboratories GmbH Offenbacher Strasse 113 • 6078 Neu Isenburg • West Germany
Bethesda Research Laboratories (UK) Ltd. P.O. Box 145 • Science Park • Cambridge • United Kingdom CB4 3
Bethesda Research Laboratories SARL F-95220 Herblay France

Two Superior Color Indicators

Bluo-Gal

For cloning into the lac gene, choose Bluo-Gal, a histochemical substrate for β-D-galactosidase. As with the widely-used substrate X-Gal, lac⁺ bacterial colonies are blue in its presence. However, Bluo-Gal contains a unique chromophor that rapidly produces a darker blue color than X-Gal. BRL's exclusive Bluo-Gal is the ideal indicator for M13 cloning and whenever β-galactosidase activity is altered. Already an integral part of the BRL M13 Cloning System, Bluo-Gal now is available separately for the first time.

Circle No. 40 on Reader Service Card.

AMPSCREEN™

Use BRL's exclusive AMPSCREEN, and detect ampicillin-sensitive recombinant DNA-containing colonies in only 10 minutes without replica plating. AMPSCREEN is a sterile disk, sized to fit into a standard 85 mm petri dish, and treated with a chromogenic substrate. After the colonies growing on a petri dish are blotted onto AMPSCREEN, recombinant DNA-containing colonies remain blue, while non-recombinant ones change from blue to yellow. The complete color change takes place in less than 10 minutes.

Circle No. 41 on Reader Service Card.

DNase I, RNase free

BRL DNase I is free of contaminating ribonucleases, making it ideal for isolating RNA. Isolated from bovine pancreas and purified to homogeneity by affinity chromatography, DNase I degrades DNA to oligonucleotides without degrading RNA or proteins. With each lot of DNase I, BRL provides exclusive lot-specific functional data specifying the amount of DNA digested by one μg of DNase I in one minute at 37°C.

Circle No. 42 on Reader Service Card.

Dpn I

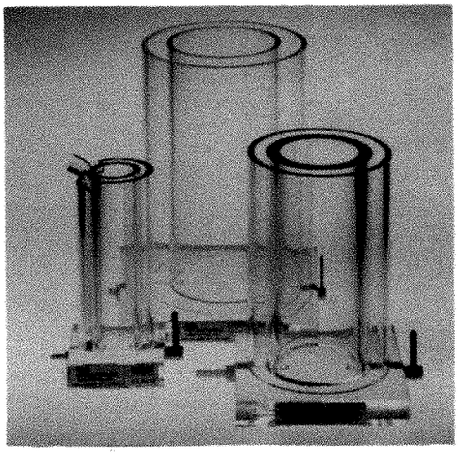
5′-G^{Me}A$^{\downarrow}$T C-3′

3′-C T$_{\uparrow}$A^{Me}G-5′

The newest addition to BRL's line of high-quality restriction enzymes is *Dpn* I. *Dpn* I cleaves its recognition sequence *only* when the adenine is methylated, and is especially useful in isolating unmethylated DNA from a mixture of both methylated and unmethylated DNA. Isolated from a variant strain of *Diplococcus pneumoniae*, BRL *Dpn* I is purified free of contaminating nucleases and is qualified as a cloning-grade enzyme.

Circle No. 43 on Reader Service Card.

Save 15% on BRL Gradient Formers January 1 Through March 31, 1984



BRL Gradient Formers provide an inexpensive, easy-to-use means of generating accurate, highly reproducible gradients of aqueous fluids. The compact, concentric reservoir design makes it easy to match fluid heights and requires no cumbersome supports for precise leveling. Each gradient former is individually calibrated and adjusted to assure accurate linear gradients (± 1% of absolute linearity). In addition, concave, convex and exponential gradients can be readily generated. BRL Gradient Formers are available in three sizes, 150 ml, 750 ml, and 2 liter total capacities.

Circle No. 44 on Reader Service Card.

In-House Mycoplasma Detection and Identification

With MycoTect and MycoSpec, availab *exclusively* from BRL, you can detec and identify species of mycoplasma quickly, easily, and inexpensively right in your laboratory.

MycoTect is cytotoxic to mycoplasm infected cells. After incubation of cel samples with MycoTect and exposur to crystal violet, cell monolayers i uninfected wells stain more intensive ly and can be easily distinguished fron the sparse cell layers found i mycoplasma-infected wells. The entir process takes only five days—les than one fifth the time needed fo detection by conventional methods The cost of a MycoTect assay is only fraction of the cost of current tests and the only equipment needed is 24-well plate.

MycoSpec is a rapid, simple and inex pensive assay for the five majo mycoplasma species that account fo more than 95% of all laborator mycoplasma infections. Mycoplasm samples are incubated separately wit monoclonal antibodies to each of th five species, then with a biotinylate secondary antibody and finally with streptavidin-horseradish peroxi complex. The sample that stains diaminobenzidine is added co the monoclonal to the infectin species. With MycoSpec, identificatio is complete within five hours.

Circle No. 45 on Reader Service Card.

T4 Polynucleotide Kinase

Prepared by an exclusive new metho BRL Kinase is entirely free of co taminating endonucleases and exon cleases and is functionally free RNase. Each lot of BRL Kinase is fun tionally assayed for its ability to en label DNA fragments in both exchan and forward reactions, and data f these tests are provided with e order. BRL provides Kinase in solub form and, for the researcher's conv nience, guarantees the concentratio of each lot of enzyme at 10 units/μl.

Circle No. 46 on Reader Service Card.

nature

NATURE VOL 307 12 JANUARY 1984

COVER



Bristlecone pine (*Pinus longaeva* D. K. Bailey) at the upper treeline in the White Mountains, California. Variations in the properties of annual growth rings in such trees may be correlated with major volcanic eruptions (see p. 121) and can indicate changes in solar activity (p. 141) and climate (p. 143).

OPINION

Unesco's crisis is for all of us 95

NEWS

Monoclonal patent dispute 97
European forests
French research support 98
Concerted selectivity
Unesco
Solar energy 99
A place in the Sun
Indian science congress 100
Educational computing
Greenhouse effect 101
Acid rain
UK research councils 102
Biotechnology
***Nature* index of biotechnology stocks**
Scientific Correspondence defined

CORRESPONDENCE

Chile under Pinochet/US creationists/ Paying for CERN/etc. 103

COMMENTARY

Antarctic mining regime at risk
John Maddox 105

NEWS AND VIEWS

From Santorini to Armageddon
John Maddox 107
Prospects for transforming monocot crop plants
Richard Flavell and Raymond Mathias 108
The origins of men with two X chromosomes
Paul Burgoyne 109
Scattering by fractal objects
Eric Jakeman 110
Two kinds of halo gas identified
Joel Bregman 111
Small companions for early dinosaurs
Michael Benton
Stonehenge comes alive
Julian Richards 112
***Notch* mapping function obscure**
Michael Akam 113
Chemical inhomogeneity of mantle above 670 km transition
Don L. Anderson 114

SCIENTIFIC CORRESPONDENCE

Seed viability/Cultured plants/Balanced salts/Familial cancers/etc. 115

BOOK REVIEWS

The Nature of Mathematical Knowledge *by P Kitcher*
Stephen Toulmin 189
Mammal Skin *by V E Sokolov*
Sam Shuster 190
Surface Physics, 2nd Edn *by M Prutton*
J E Inglesfield
Lizard Ecology: Studies of a Model Organism *R B Huey, E R Pianka and T W Schoener eds*
Mark Williamson 191
The Biology of Religion *by V Reynolds and R E S Tanner*
John Habgood
Muscles and their Neural Control *by G Hoyle*
P N R Usherwood 192
Quark Matter Formation and Heavy Ion Collisions *M Jacob and H Satz eds*
Louis Lyons

ARTICLES

Aspherical heterogeneity of the mantle from phase velocities of mantle waves
I Nakanishi & D L Anderson 117

Frost rings in trees as records of major volcanic eruptions
V C LaMarche Jr & K K Hirschboeck 121

DNA sequence of the maize transposable element *Dissociation*
H P Döring, E Tillmann & P Starlinger 127

Mode of proviral activation of a putative mammary oncogene (*int*-1) on mouse chromosome 15
R Nusse, A van Ooyen, D Cox, Y K T Fung & H Varmus 131

Correlation between segmental flexibility and effector function of antibodies
V T Oi, T M Vuong, R Hardy, J Reidler, J Dangl, L A Herzenberg & L Stryer 136

LETTERS TO NATURE

Large anisotropy in the Universe does not prevent inflation
M Demianski 140

Correlation of bristlecone pine ring widths with atmospheric ^{14}C variations: a climate–Sun relation
C P Sonett & H E Suess 141

Reconstruction of precipitation history in North American corn belt using tree rings
T J Blasing & D Duvick 143

Elemental constituents of atmospheric particulates and particle density
A Sugimae 145

Photochemical production of carbonyl sulphide in marine surface waters
R J Ferek & M O Andreae 148

First observations of the interaction of ocean swell with sea ice using satellite radar altimeter data
C G Rapley 150

Earthquake seasonality before the 1906 San Francisco earthquake
P H McClellan 153

Localized uplift in the Scottish Dalradian
T J Dempster 156

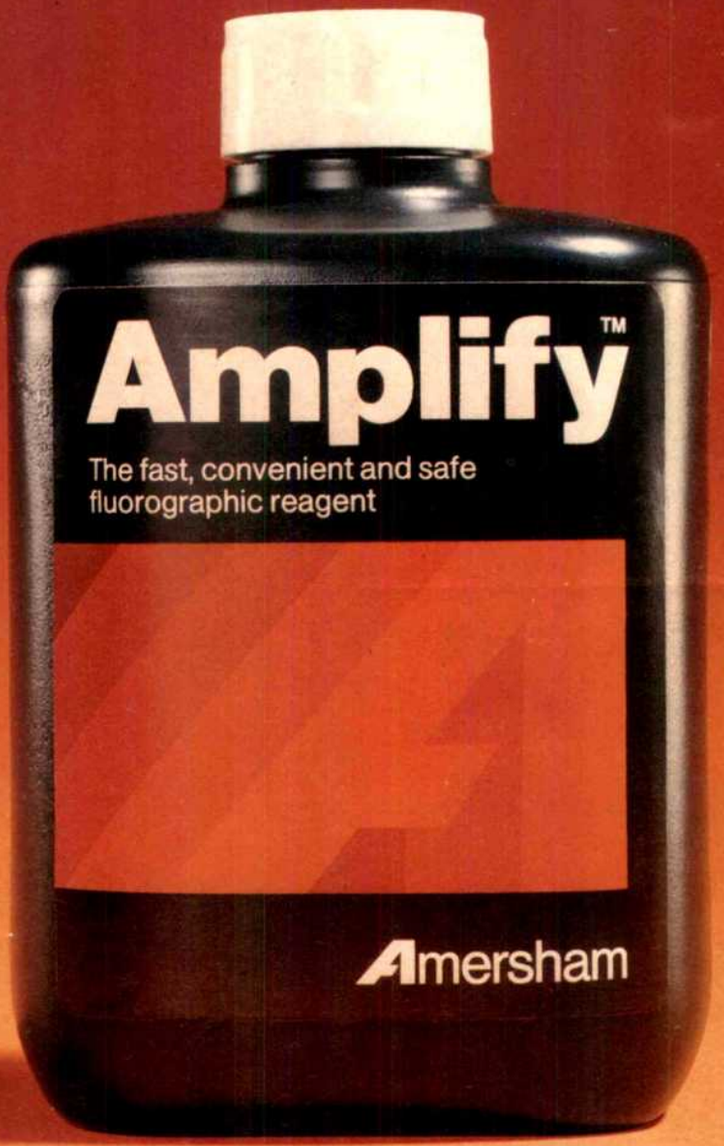
Period multupling-evidence for nonlinear behaviour of the canine heart
A L Ritzenberg, D R Adam & R J Cohen 159

Contents continued overleaf

Nature (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Annual Index (mailed in February). Annual subscription for USA and Canada US $230 (for subscription prices elsewhere, see next page). USA and Canadian orders only to: *Nature*, Subscription Dept PO Box 1018, Manasquan, New Jersey 08736, USA. All other orders to *Nature*, Brunel Road Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY10010 and additional mailing offices. US Postmaster send form for 3579 to: *Nature*, 15 East 26 Street, New York, NY 10010. Published in Japan by Macmillan Shuppan K.K., 5th FloorEikow Building, 1-10-9 Hongo, Bunkyo-ku, Tokyo, Japan.

© 1984 Macmillan Journals Ltd.

A quantum jump in fluorography



Please contact Amersham or your local representative for further details.

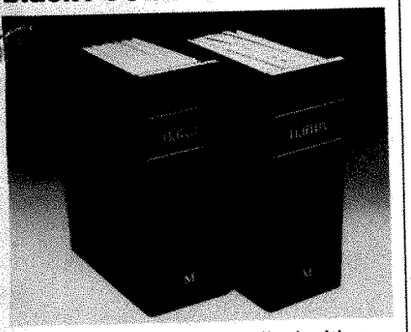
Amersham International plc
Amersham England HP7 9LL
telephone Little Chalfont (024 04) 4444

Amersham

Amersham Australia PTY Limited Sydney **Amersham Belgium SA/NV** Brussels **Amersham Buchler GmbH & Co. KG** Braunschweig W Germany
Amersham Corporation Arlington Heights USA **Amersham Denmark ApS** Birkerød **Amersham France SA** Paris **Amersham Japan** Tokyo **Amersham Nederland BV** Utrecht

Circle No.50 on Reader Service Card.

Keep your copies of *Nature* protected in these tough black PVC binders



- Each binder is supplied with a set of wires for the easiest possible loading
- *Nature* logo blocked in gold on spine
- Transparent plastic pocket provided for your own reference
- Each binder takes two volumes of *Nature*; three binders will serve for a year
- Prices and address for orders are set out below

Annual Subscription Prices

UK & Irish Republic		£98
USA & Canada		US$230
Australia & NZ	Airspeed	£150
Continental Europe	Airspeed	£120
India	Airspeed	£115
Japan	Airspeed	Y85000
Rest of World*	Surface	£120
Rest of World*	Airmail	£180

**(not USA, Canada, Europe & Japan)*

Orders (with remittance) to:

USA & Canada
Nature
Subscription Dept
PO Box 1018
Manasquan
NJ 08736
USA

UK & Rest of World
Nature
Circulation Dept
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to:
Japan Publications Trading Company Ltd
2-1 Sarugaku-cho 1-chome
Chiyoda-ku, Tokyo, Japan
Tel: 03-292-3755

Personal subscription rates
These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
15 East 26 Street
New York,
NY 10010, USA
Tel: (212) 689-5900

UK & Europe
Felicity Parker, Nature
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

Credit card orders only (in USA):
Call toll-free: (800) 824-7888 (Operator 246)
In California: (800) 852-7777 (Operator 246)

Back issues
UK, £2.50; USA & Canada, US$6.00 (surface), US$9.00 (air); Rest of World, £3.00 (surface), £4.00 (air)

Binders
Single Binders: UK, £4.50; Rest of World, $10.00
Set(s) of 3 Binders; UK, £12.00; Rest of World, $25.00

Annual indexes (1971–1982)
UK, £5.00 each; Rest of World, $10.00

Nature First Issue Facsimile
UK, 75p; Rest of World (surface), $1.50; (air) $2.00

Nature in microform
For Information:
University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

Brain potentials during reading reflect word expectancy and semantic association
M Kutas & S A Hillyard 161

Cell determination and regulation during development of neuroblasts and neurones in grasshopper embryo
P H Taghert, C Q Doe & C S Goodman 163

Prostaglandin I_3 is formed *in vivo* in man after dietary eicosapentaenoic acid
S Fischer & P C Weber 165

Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts
S T Ildstad & D H Sachs 168

Genetic evidence of X–Y interchange in a human XX male
A de la Chapelle, P A Tippett, G Wetterstrand & D Page 170

Human XX males with Y single-copy DNA fragments
G Guellaen, M Casanova, C Bishop, D Geldwerth, G Andre, M Fellous & J Weissenbach 172

The establishment and maintenance of pregnancy using *in vitro* fertilization and embryo donation in a patient with primary ovarian failure
P Lutjen, A Trounson, J Leeton, J Findlay, C Wood & P Renou 174

Similar level of polyteny in bands and interbands of *Drosophila* giant chromosomes
A Spierer & P Spierer 176

Human hepatitis B vaccine from recombinant yeast
W J McAleer, E B Buynak, R Z Maigetter, D E Wampler, W J Miller & M R Hilleman 178

Antisera to a synthetic peptide of the *sis* viral oncogene product recognize human platelet-derived growth factor
H L Niman 180

Primary structure homology between the product of yeast cell division control gene *CDC28* and vertebrate oncogenes
A T Lörincz & S I Reed 183

Similarity of the *Cin1* repetitive family of *Zea mays* to eukaryotic transposable elements
N S Shepherd, Z Schwarz-Sommer, J Blumberg vel Spalve, M Gupta, U Wienand & H Saedler 185

A large increase in enzyme–substrate affinity by protein engineering
A J Wilkinson, A R Fersht, D M Blow, P Carter & G Winter 187

MISCELLANY

Product review: Automatic DNA sequencing
A Wada 193

NATURE CLASSIFIED

Professional appointments — Research posts — Studentships — Fellowships — Conferences — Courses — Seminars — Symposia: Back pages

Next week in *Nature*:

- Light from dark side of Venus
- Supernova remnants
- New kind of radioactivity
- Soviet observations of solar oscillations
- Primaeval aqueous dissolution and chemical reactions in meteorites
- More iridium at Cretaceous-Tertiary boundary
- Interleukin-3 cloned
- Kilobase mapping of complement genes

GUIDE TO AUTHORS

Authors should be aware of the diversity of *Nature*'s readership and should strive to be as widely understood as possible.

Review articles should be accessible to the whole readership. Most are commissioned, but unsolicited reviews are welcome (in which case prior consultation with the office is desirable).

Scientific articles are research reports whose conclusions are of general interest or which represent substantial advances of understanding. The text should not exceed 3,000 words and six displayed items (figures plus tables). The article should include an abstract of about 50 words.

Letters to *Nature* are ordinarily 1,000 words long with no more than four displayed items. The first paragraph (not exceeding 150 words) should say what the letter is about, why the study it reports was undertaken and what the conclusions are.

Matters arising are brief comments (up to 500 words) on articles and letters recently published in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted.

Manuscripts may be submitted either to London or Washington (decisions being made only in London). Manuscripts should be typed (double spacing) on one side of the paper only. Three copies are required, each accompanied by copies of lettered artwork. No title should exceed 80 characters in length. Reference lists, figure legends, etc. should be on separate sheets, all of which should be numbered. Abbreviations, symbols, units, etc. should be identified on one copy of the manuscript at their first appearance.

References should appear sequentially indicated by superscripts in the text and should be abbreviated according to the *World List of Scientific Periodicals*, fourth edition (Butterworth 1963–65). The first and last page numbers of each reference should be cited. References to books should clearly indicate the publisher and the date and place of publication. Unpublished articles should not be formally referred to unless accepted or submitted for publication, but may be mentioned in the text.

Each piece of artwork should be clearly marked with the author's name and the figure number. Original artwork should be unlettered. Suggestions for cover illustrations are welcome. Original artwork (and one copy of the manuscript) will be returned when manuscripts cannot be published.

Requests for permission to reproduce material from Nature *should be accompanied by a self-addressed (and, in the case of the United Kingdom and United States, stamped) envelope.*

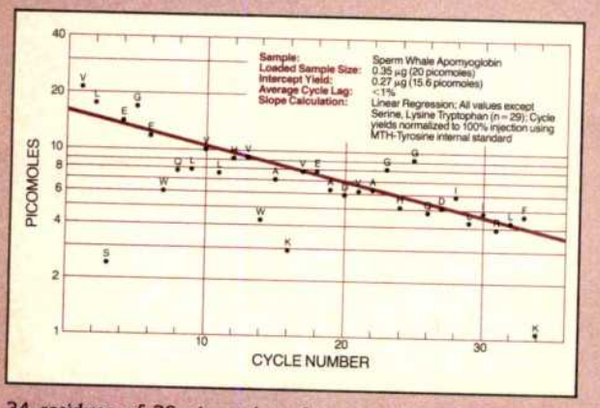
BIOSYSTEMS UPDATE

Gas-Phase Protein Sequencing

A Progress Report

Less than two years ago, Applied Biosystems introduced new gas-phase protein sequencing technology which, though in its infancy, set new standards of analytical performance and sensitivity. We are now pleased to announce the first in a series of improvements in chemistry, programming and hardware which begin to further realize the still untapped potential of the Model 470A Gas-Phase Protein Sequencer.

Changes in the coupling, cleavage and solvent extraction times, and use of an optional miniature sample cartridge, substantially increase repetitive yields and decrease lag on picomole level samples. Sequencer artifacts which can interfere with high sensitivity PTH-AA chromatography are also reduced by a factor of three to five.

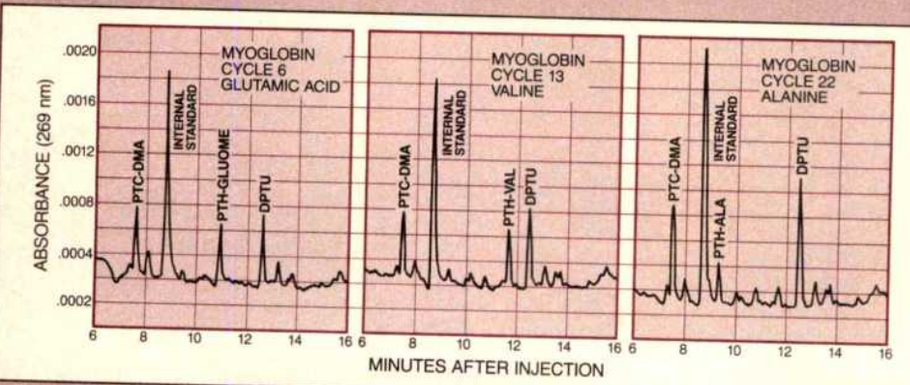


34 residues of 20 picomoles of sperm whale apomyoglobin. Repetitive yield is 96% calculated from the least squares linear regression plot of individual amino acids quantitatively recovered at each sequenced cycle. Lag averages less than 1%/cycle.

The improvements are particularly dramatic with peptides. A user evaluating our new chemistry has reported sequencing the octapeptide angiotensin II to completion with only 25 picomoles of sample.

A new miniature PTH conversion flask increases typical PTH-AA recovery to greater than 95%. The improvement is most significant on serine, threonine and tryptophan since drying times are reduced threefold.

Best of all, these improvements, and others still under development, are designed to be compatible with every instrument we've built so our current users will continue to be at the leading edge of protein sequencing technology. The 470A Gas-Phase Protein Sequencer is capable of setting even higher performance standards and Applied Biosystems, in collaboration with many of our users, is continuing to investigate further improvements to this remarkable instrument. If you'd like more information, please circle number 8.



Actual HPLC chromatograms, without background subtraction or data enhancement, of residues 6, 13 and 22 from sequencing analysis of 20 picomoles of sperm whale apomyoglobin. The data illustrate the low background and high sensitivity of the Model 470A Gas-Phase Protein Sequencer.*

*Chromatography: IBM Cyano Column. Internal Standard: MTH tyrosine. PTH yields normalized to 100% injection: Cycle 6 (glutamic acid)—11.7 pmol, cycle 13 (valine)—9.1 pmol, cycle 22 (alanine) 6.2 pmol. PTC-DMA, phenylthiocarbamyldimethylamine; DPTU, diphenylthiourea.

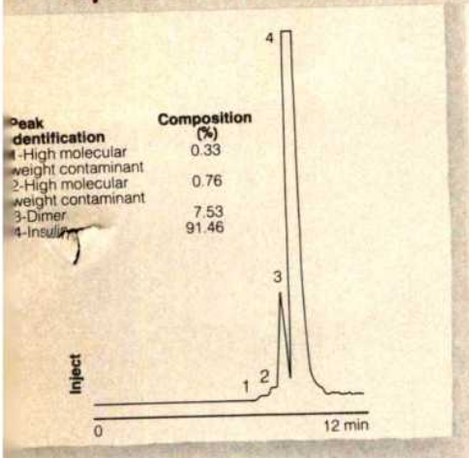
Applied Biosystems

APPLIED BIOSYSTEMS, INC., 850 Lincoln Centre Drive, Foster City, CA 94404 • (415) 570-6667 • (800) 874-9868
IN EUROPE: APPLIED BIOSYSTEMS GMBH, Bergstrasse 104, D6102 Pfungstadt, West Germany • 06157-6036

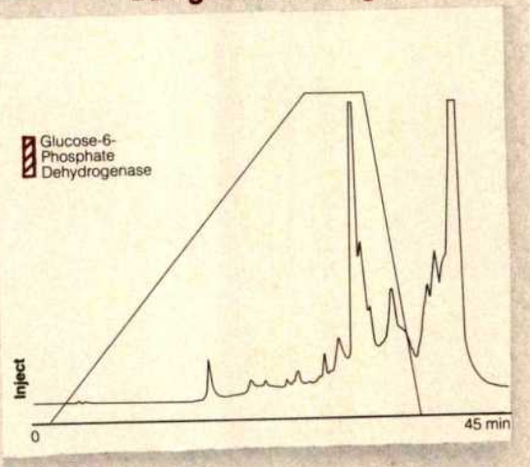
Now isolate and analyze proteins faster, with higher resolution and recovery.

Waters HPLC systems with new ion exchange, gel filtration and hydrophobic packings for biological separations.

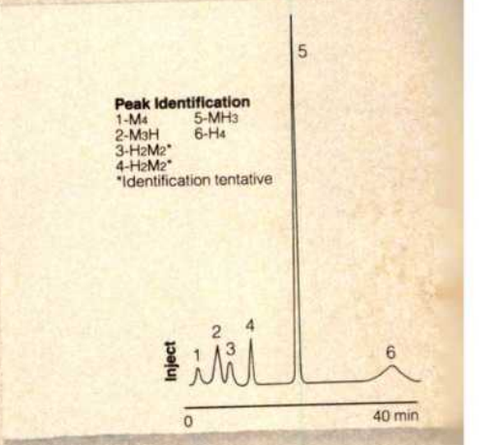
High speed gel filtration separation of insulin.



Total recovery of enzymatic activity from a crude bacterial extract using ion exchange.



High resolution ion exchange separation of isoenzymes.



Separation time: 12 minutes.

Waters new high speed gel ltration methods allow rapid ractionation of protein samples. his same gel filtration method lets ou profile sample composition, olate all components and verify ne purity of collected fractions.

ample: 10 µl Insulin, 5 mg/ml
Column: PROTEIN-PAK 125
luent: 0.1% Trifluoroacetic Acid, pH 2.1
low Rate: 1.0 ml/min
etection: 214 nm, 0.5 AUFS

100% recovery of biologically active protein.

The large pore size of Waters polymeric ion exchange packings (1,000 Å) gives you recoveries of enzymatic activity approaching 100% for even high molecular weight enzymes.

Sample: 100 µl Bacterial Extract, 3 mg/ml
Activity: Glucose-6-Phosphate Dehydrogenase
Column: PROTEIN-PAK DEAE 5PW
Gradient Conditions
Eluent A: 0.02M Tris HCl, pH 8.5
Eluent B: 0.02M Tris HCl, pH 8.5 + 0.5M NaCl
0-100% B, curve 6, 25 min
Flow Rate: 0.5 ml/min
Detection: 280 nm, 0.1 AUFS
Specific Activity Increase: 4.9
Activity Recovery: 100%

Complete resolution of LDH isoenzymes.

The resolving power of Waters high performance ion exchange packings provides resolution of the closely related isoenzymes c lactate dehydrogenase.

Sample: 100 µl LDH Isoenzymes, 1 mg/ml
Column: PROTEIN-PAK DEAE 5PW
Gradient Conditions
Eluent A: 0.02M Tris Acetate, pH 8.0
Eluent B: 0.02M Tris Acetate, pH 8.0
+ 1.0M NaOAc
0-100% B, curve 6, 45 min
Flow Rate: 0.5 ml/min
Detection: 280 nm, 0.05 AUFS

Add the performance of Waters HPLC to your separations. And achieve greater success in protein isolation and analysis.

For a demonstration or additional information, contact your local Waters representative or call 617-478-2000 (X 2665).

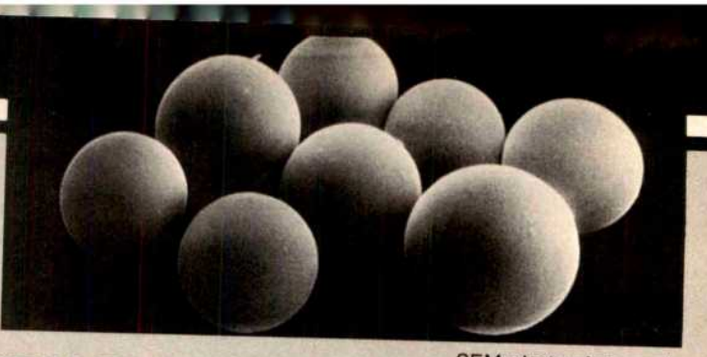
Waters

Waters The Liquid Chromatography People®

For a demonstration circle No. 27 on Reader Service Card
For further information circle No. 28 on Reader Service Card

VYDAC™

C MPLEMENTARY RE ERSE PHASE M 'ERIALS NO RES ARCH LAB C N AFFORD TO BE WITHOUT.



SEM photomicrograph of VYDAC HS silica.

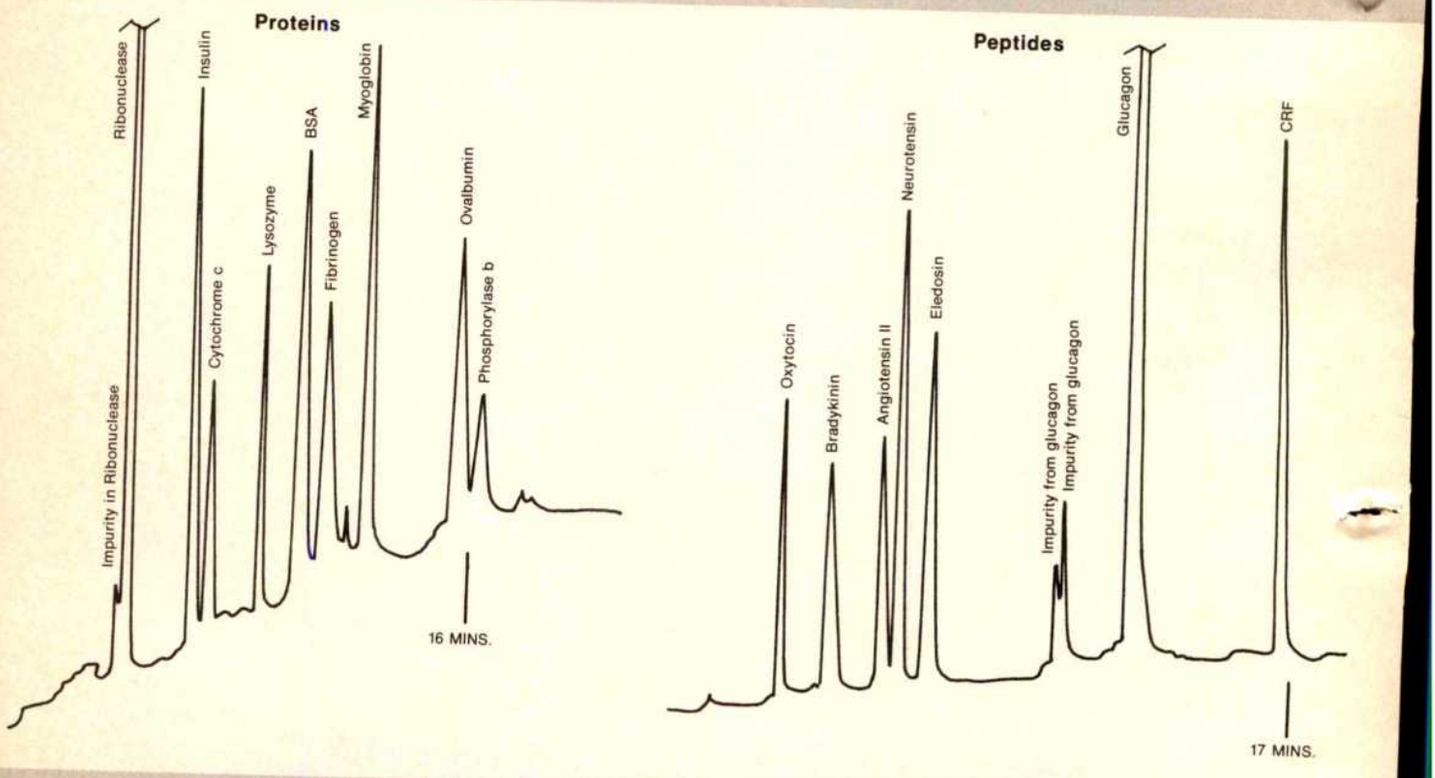
VYDAC TP 300 Angstroms
Spherical Silica based reverse phase

Applications
Polyaromatic hydrocarbons
Vitamins D_2 and D_3
Large Peptides
Proteins
Anti-oxidants in plastics
Vitamin A Isomers

and/or

VYDAC HS 80 Angstroms
Spherical Silica based reverse phase

Applications
Nucleosides, purines, pyrimidines
Catecholamines
Small Peptides
Vitamin Bs and C
Organic Acids



Only the Separations Group offers C_{18} reverse phase materials bonded to distinctly different spherical silica substrates yielding uniquely different selectivities for the HPLC chromatographer.

For your free copy of our "Comprehensive Guide to Reverse Phase Materials for HPLC," contact the Separations Group. Do not hesitate to call collect.

THE SEP|A|RA|TIONS GROUP

MANUFACTURERS OF VYDAC
Box 867 • 16695 Spruce St. • Hesperia, CA 92345 USA
Telephone (619) 244-6107

SPHERICAL SILICAS and **separation materials** for **high performance liquid chromatography** since 1971

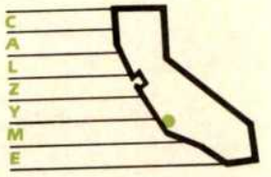
Circle No. 06 on Reader Service Card

CALZYME
LABORATORIES, INC.

Manufacturers of enzymes and related biochemicals, human proteins, and enzymatically prepared substrates of the highest possible purity.

With confidential agreements, offers custom work scaling up your procedures from laboratory scale to industrial scale.

3443 Miguelito Court
San Luis Obispo, CA 93401
(805) 541-5754 Telex: 4994640

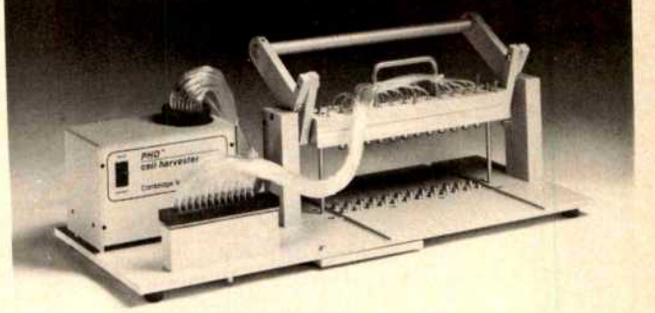


LOCATED ON THE CENTRAL COAST
BETWEEN SAN FRANCISCO AND LOS ANGELES

Write or phone for free catalogue

Circle No.29 on Reader Service Card.

Cell Harvesting
fast and error free



- Impossible to mix-up samples
- Punches samples out and deposits them directly into vials
- Built to last a lifetime
- Supernatant collection and many other accessories available
- Affordable price

Find out how the PHD™ Cell Harvester can make your work easier.

Write or call for further details.

Cambridge Technology, Inc.

2464 Massachusetts Avenue
Cambridge, MA 02140 U.S.A. TEL. (617) 876-0891

Circle No.2 on Reader Service Card.

Concentrate 200 Samples without BUMPING and achieve 100% sample recovery



Most laboratories report to us that they never realized how much sample they were losing until they used "Speed Vac"... The ease of sample preparation is also a big advantage... Place your samples in individual tubes or vials and turn "Speed Vac" "ON"... No cross contamination, no losses, no creeping, no foaming and the samples will be concentrated at the bottom of the tube...

How else can you concentrate "1 or 200" samples with such ease and efficiency.

- **HPLC** sample preparation where a dried, concentrated sample is important.
- **GLC** sample preparation... as above.
- **PHOSPHOLIPID** separation by HPLC — total evaporation of Acetonitrile — Water Solvent.
- **INTERFERON** purification by dialysis and evaporation.
- **TRI-CYCLIC** anti-depressant drug blood levels measured after a series of extractions and evaporations to dryness.
- **HORMONE** and Enzyme assays.



U.S. Patent 4226669

NOW 2 COMPLETE SYSTEMS FOR 100 & 200 SAMPLES

Write for Illustrated Catalog

Savant Instruments, Inc.

221 Park Ave., Hicksville, N.Y. 11801 • 516-935-8774

Circle No.07 on Reader Service Card.



The Superpower in Power Supplies for Electrophoresis

All the power you need for today's electrophoresis applications can now be generated by a single power supply—the Bio-Rad Model 3000/300.

This one-of-a-kind power supply provides the following high outputs:

- Constant voltage to 3000 volts
- Constant current to 300 mA
- Constant power to 400 watts

Here are a few of the key electrophoresis techniques you can perform with the high outputs from the Model 3000/300.

High voltage: high performance electrofocusing (HPEF) in ultra-thin polyacrylamide gels.

High power: DNA sequencing

High current: protein electroblotting

Being brand new, this power supply has many convenience features. These include three independent digital readouts, so that voltage, current, and power are displayed simultaneously. And a crossover feature that ensures maximum resolution and minimum run time and prevents gel overheating.

Of course you're concerned about safety and so are we. Model 3000/300 conforms to all relevant safety standards to protect both the operator and the instrument.

On a price/performance basis, the Model 3000/300's price tag is the best in the industry. You can't beat it from any standpoint.

For more information about the single most useful power supply ever, contact:

BIO-RAD

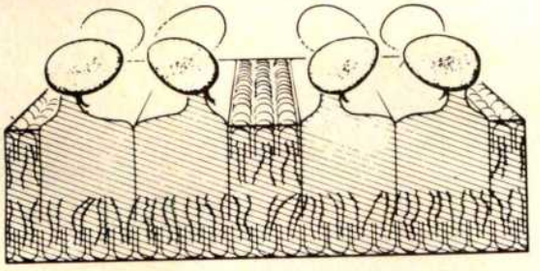
Bio-Rad Laboratories
2200 Wright Avenue
Richmond, CA 94804
Phone (415) 234-4130

Circle No.09 on Reader Service Card.

Also in Rockville Centre, New York, Australia, Austria, Canada, England, Germany, Italy, Japan, Netherlands, and Switzerland.

Attention all biophysicists, molecular biologists and biochemists!

Biomembrane Structure and Function



Edited by
DENNIS CHAPMAN
Professor of Biophysical Chemistry
Royal Free Hospital
School of Medicine, London

Volume 4 in the TOPICS IN MOLECULAR AND STRUCTURAL BIOLOGY Series
Series editors:
Professor Watson Fuller, Dr Stephen Neidle and Professor Tony Atkinson

The application of physical techniques – such as X-ray diffraction and Raman spectroscopy – has revolutionised much of our thinking on the structure and function of membranes. In this important new study, contributors of international standing review a topical selection of approaches to the elucidation of **biomembrane structure and function.** The emphasis, throughout, is on the in-depth analysis of recent experimental work.

Published January 1984 Price £50.00 432pp
ISBN 0 333 31869 2

Contents

Calorimetric studies of model and natural biomembranes – *Diana Bach*, The Weizman Institute of Science, Israel; X-ray diffraction and electron microscope studies of the molecular structure of biological membranes – *Lee Makowski and Jade Li*, Columbia University, USA; Raman spectroscopy of lipids and biomembranes – *Surendra P. Verma and Donald F.H. Wallach*, Tufts-New England Medical Centre, USA; Infrared spectroscopic studies of model and natural biomembranes – *Ralph L. Amey*, Occidental College, USA and *Dennis Chapman*; Rotational and lateral diffusion of membrane proteins as determined by laser techniques – *Winfried Hoffman*, NUKEM GmbH, E.Germany, and *Colin J. Restall*, Royal Free Hospital, London; Theoretical models of monolayers, bilayers and biological membranes – *David A. Pink*, St Francis Xavier University, Canada; The Ca^{2+} dependent ATPase of sarcoplasmic reticulum – *G. Inesi and M. Kurzmack*, University of Maryland, USA.

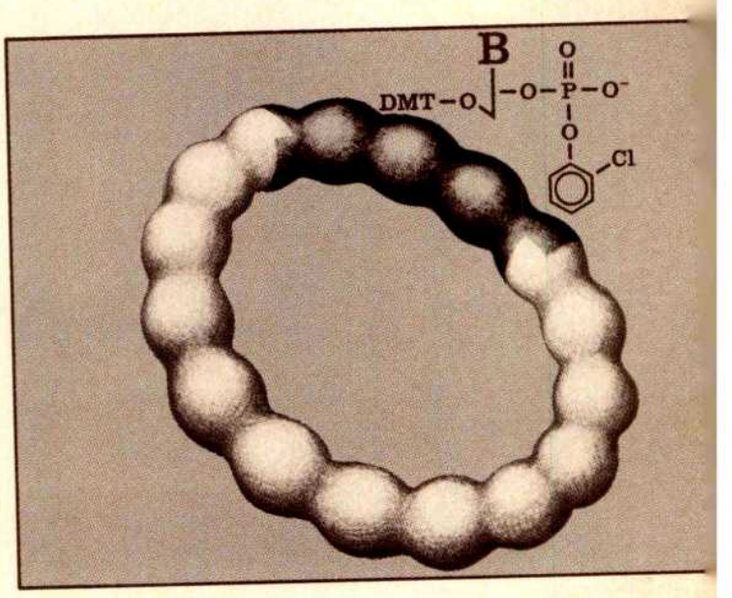
Please send orders and requests for information to:
Carolyn Leuw, Product Manager,
Scientific and Medical Division,
The Macmillan Press Ltd.,
Houndmills, Basingstoke, Hants RG21 2XS, England

SCIENTIFIC & MEDICAL | M | MACMILLAN PRESS LTD

Circle No.70 on Reader Service Card.

Genetic engineering

Building blocks and Reagents for a leading technology



Protected nucleosides and nucleotides for the synthesis of oligodeoxynucleotides according to the phosphotriester method and the phosphite triester method

- 5'-OH protected nucleosides
- 3'-OH protected nucleosides
- 3'-phosphate and 5'-OH protected nucleotides
- 3'-phosphate duplex protected nucleotides
- Fully protected phosphoamidites (morpholido)
- carrier bound protected nucleosides as starter for solid phase synthesis

Chromatographically pure and subject to application tests

Special reagents for coupling condensation and selectively splitting off protective groups

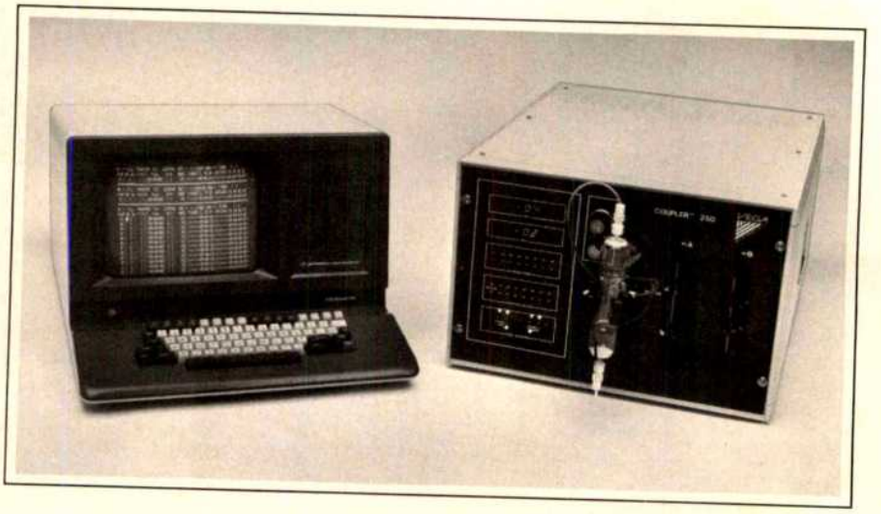
Please ask for our detailed information

Circle No.23 on Reader Service Card.

Reagents
MERCK

E. Merck, Frankfurter Strasse 250, D-6100 Darmstadt 1
Federal Republic of Germany

What do Scripps, Revlon, Fujiya, and Syntex have in common?



Vega synthesizers.

Fully automated peptide synthesizers.

The most reliable and economical peptide synthesizers available.

That's saying a lot for our systems. But they do a lot. For Scripps, Revlon, Fujiya, Syntex and scores of others.

Take the Coupler™ 250. Microprocessor controlled with floppy disk storage. Dependable. Easy to program. Capable of operating up to four chemistry modules with just one microprocessor. Around the clock, if you want. Without missing a coupling.

Or the Coupler™ 296. Rugged. Large capacity. Able to produce quality peptides on a massive scale. All with the same reliable sophistication as the Coupler 250...only bigger.

There's more, too. Chemically-inert plumbing. User programmability. Pre-formatted synthesis software. A 1 year warranty on parts, 6 months on labor. And remarkably low prices.

So if you're looking for an automated peptide synthesizer, follow Scripps, Revlon, Fujiya, and Syntex.

Choose Vega.

You'll be in good company.

Vega Biotechnologies, Inc.
P.O. Box 11648, Tucson, AZ 85734
(602) 746-1401 (800) 528-4882 Outside AZ
TELEX: 165572 (VEGA BIO TUC)

The link between science and tomorrow.

VEGA™ BIOTECHNOLOGIES

© 1983 by Vega Biotechnologies, Inc. All rights reserved. Coupler™ is a trademark of Vega Biotechnologies, Inc.

Circle No.37 on Reader Service Card.

The transfer membranes with a memory.

GeneScreen*Plus*™

GeneScreen*Plus*™ is the new transfer membrane that gives you not only highest sensitivity and excellent band resolution, but trouble-free handling as well.

The slight natural curl of the membrane makes it easier to select from the package and easier to place on a moist surface. After drying it returns to its original shape to remind you which side is carrying the bound material. Wet or dry, GeneScreen*Plus*™ is highly resistant to cracking, distorting, tearing, or shrinking.

Binding of all sizes of DNA is so strong you don't need to waste time baking the membranes. No need for refrigeration either; just keep the box handy on the shelf.

Colony/PlaqueScreen™ Discs

Colony/PlaqueScreen™ Discs have all of the performance and convenience features of GeneScreen*Plus*™ and are precut to culture dish sizes. The gentle curl of the discs makes them easier to place on the agar plate without smudging, and moisture causes them to lie flat with minimal air entrapment. Removal is easier too, because they are strong and tear resistant.



Our Applications Laboratory is available to answer your questions about hybridization methods using either of these products. Meanwhile we'll be glad to send you detailed information on their performance characteristics. Simply call New England Nuclear or circle the Reader Service Number.

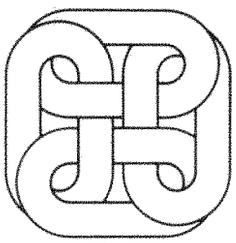
U.S. method of use patent pending

New England Nuclear
549 Albany Street, Boston, MA 02118
Call toll free: 800-225-1572, Telex: 94-0996
Mass. and Internat'l: 617-482-9595
Europe: NEN Chemicals GmbH, D-6072, Dreieich, W. Germany
Postfach 401240, Tel. (06103) 803-0, Telex 4-17993 NEN D
NEN Canada: 2453 46th Avenue, Lachine, Que H8T 3C9
Tel. 514-636-4971, Telex 05-821808 © 1983 NEN

Circle No.60 on Reader Service Card.

NEN New England Nuclear®

DUPONT



Associated Biolabs Limited

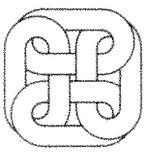
A NEW FORCE IS WITH US

Associated Biolabs is a new UK company dedicated to simplifying established techniques and developing new materials and tests in the fields of:

Tissue Culture
Chromatography
Radioimmunoassay
Water purification
Mass screening
Veterinary diagnostics

Find out how Associated Biolabs can help you – send today for information on the complete range of products.

Companies interested in the distribution of Associated Biolabs' products in foreign markets are invited to contact us.



Associated Biolabs Limited
The Novo Centre 9-11 London Lane London E8 3PR
Telephone 01 986 4717 Telex 896746

Circle No.19 on Reader Service Card.

EDITORIAL OFFICES

London Office
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024

Washington Office
1134 National Press Building, DC 20045
Telephone: (202) 737-2355

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff: Alun Anderson, Tim Beardsley, Philip Campbell, Isobel Collins, Peter Gambles, Melanie Kee, Tim Lincoln, Naomi Molson, Sara Nash, Geoffrey North, Peta Pickering, Miranda Robertson, Robert Walgate, Charles Wenz, Nigel Williams, Guil Winchester

Washington Editor: Peter David
Washington Correspondent: Stephen Budiansky

Art and Design: Joan Godwin, Joel Chernin, Theresa George, Paul Norris, Marie Xeridat

Administration: Mary Sheehan, Jacqueline Cole, Ruth Cutts, Joanna Czechowska, Ruth Goldenberg, Sharon Isaacs and Penny Walker (Washington)

Publisher: Elizabeth Hughes

Marketing Director: Ray Barker

Promotion Manager: Felicity Parker

International Advertising Manager: Andy Sutherland
Features Advertising Manager: Marion Delaney

Group Classified Advertising Manager: John Barnes — Telephone: (01) 240 1101

Nature New York Office
15 East 26 Street, New York, NY 10010
Telephone: (212) 689-5900 Telex: 668497

General Manager: Gary Rekstad

American Advertising Manager: Henry Dale

Classified Advertising Manager: Miss Gene Fein

Circulation Manager: Mirta Soto

Canadian Display and Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1—(416) 690-2423

Display Advertising Representatives

New York: Nature Office

Chicago: Didier & Broderick, Inc.—(312) 498-4520

San Francisco: Jobson/Jordan/Harrison/Schulz, Inc.—(415) 392-6794

Los Angeles & Pasadena: Jobson/Jordan/Harrison/Schulz, Inc.—(213) 796-9200

Philadelphia: Daniel Adams Associates, Inc.—(215) 353-6191

Boston: CEL Associates—(617) 848-9306

Fort Lauderdale: Brinker & Brinker—(305) 771-0064

New Jersey: Mary Grunmeier—(201) 572 9018

Dalton, Pennsylvania: Donald E. Northup Associates—(717) 563 2572

West Germany and Austria: Franz Schrecklinger TMW Top Media Werbegesellschaft GmbH Frankfurt—(611) 726046

Holland and Belgium: Adele Struyck G. Arnold Teesing BV Amsterdam—(020) 2636 15

Switzerland: Werner Stähli Agentur Iff AG Schaffhausen—(053) 45821

Scandinavia: Andrew Karnig Andrew Karnig & Associates AB Stockholm—(08) 51 68 70

Japan: Mashy Yoshikawa Orient Echo Inc Tokyo—541-4923

Australia: Gus Bartel & Associates PO Box 1755, Southport, Queensland 4215 (075) 466 577



Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office

©1984 Macmillan Journals Ltd
Vol.307 No. 5947 12 January 1984

nature

Unesco's crisis is for all of us

The US withdrawal from Unesco is intelligible and may be wise. The remaining members should abandon gentility in the hope of winning overdue reform. Unesco alone is not the worry.

DISMAY about the proposed withdrawal of the United States from the United Nations Educational, Scientific and Cultural Organization (Unesco) is natural. Especially when the US Administration is more than usually insensitive to the needs of collaboration overseas, the prospect that the United States may withdraw from a surviving network of international collaboration must be a matter for regret. But those in Washington who have been wringing their hands about the proposed withdrawal (see p.98), officers and officials of the National Science Foundation and the National Academy of Sciences, should not go too far, or they will find themselves in the same case as those, in the 1930s, who applauded Mussolini because he had arranged that Italian trains should run on time. The fact is that the US withdrawal from Unesco has been decided for reasons that have nothing to do with the organization's scientific programme and that, if valid, not merely justify the withdrawal but make it necessary.

For the past three years, the central complaint against Unesco has been its espousal of what it calls the "new world information and communications order", NWICO for short. This notion, which has been rumbling about in Le Corbusier's building in Paris since 1972, is a vivid illustration of the mischief that can be done when people set out to translate good but ill-defined intentions into rules that the whole world is then expected to follow. The argument has gone like this. Free speech is a common good, right? *Of course.* So everybody should be free to communicate, right? *Well . . . yes.* But it's not like that, because the world's successful news agencies and television networks are mostly owned by capitalists and put out distorted information about countries against which they are prejudiced but which lack the means to answer back; so should there not be some way in which governments not in control of international news agencies or television networks can influence what is said about them by others, and in the meantime should not journalists operating internationally be licensed by those about whom they would write? *Well . . . no.*

Doublespeak

Unesco's remedy would be worse than the disease it is meant to cure. To regard as synonymous the principle of free speech for people and the doctrine of information control by governments is, in *1984* language, doublespeak. To nurse the ambition, as Unesco has done for the past few years, to embody this mischievous obfuscation in some kind of international convention would run flatly against its own objectives of helping to make the world a more civil place. Unesco's management has been obtusely unwilling or unable to see the difficulty, citing the acknowledged imperfections of news agencies, newspapers and television networks as proof that something should be done. It has steadfastly refused to understand that many of its member governments, but especially that of the United States, are constitutionally bound to the view that the imperfections of the international press, in any case remediable by means other than NWICO, are the price that must be paid for free speech. To general surprise, the management seems to have soft-pedalled on the issue at Unesco's General Conference in Paris last November, even accepting a British gloss on the description of NWICO as an "evolving and continuous process", which is apparently a diplomatic device for making sure that the new order cannot now be brought in by *fiat*. Yet the issue has not gone away, and those remaining within Unesco will be battling with it for years to come.

If the State Department's calculation were that pulling out of Unesco is the best way of making sure that this tedious matter does not rear its head again, or that the threat of doing so may be the most effective way of changing Unesco's management, the loss to the scientific community would be a small price to pay — as would have been unpunctuality on the Italian railways in the 1930s for some other kind of political leadership than that provided by Mussolini.

The weakness in the position the United States has taken is threefold. Resignation would have been more convincing at the end of 1982, when the row about NWICO was at its height, than now, when the issue seems to have gone off the boil. In present circumstances, it is hard to tell to what extent the decision reflects the general impatience with United Nations institutions that keeps bubbling up within the administration. And there is bound to be a suspicion that resignation from Unesco, or the threat thereof, is an early move in November's presidential election campaign. If it wishes to avoid the shabby conclusions to which these suspicions point, the State Department should quickly give substance to the hints it has been dropping in Washington and put up the money that will be needed before the end of the year if the important parts of Unesco's programme are not to founder. The need that the International Council of Scienfific Unions (ICSU) should not be endangered typifies what must be done.

Disenchantment

There is also an urgent need that the State Department, and the US Administration as a whole, should come to a clearer and more guileful understanding of the reasons why its relations with UN organizations have become so irksome. One is that the United States has allowed its discovery in the 1940s that the United Nations did not obviate the need for international organizations, its alarm at the total cost (to which it is the chief contributor) and its dislike of being bested by the political manoeuvrings of its critics, to engender a disenchantment and a detachment that only exaggerates legitimate discontents. The United States would be better advised to define what is wrong and to seek to put it right.

There are general complaints against the UN system as a whole, and particular complaints against specific UN agencies, all of which unhappily apply to luckless Unesco. First, the agencies are needlessly bureaucratic, with decisions on minor matters rubber-stamped (or sometimes capriciously overturned) by officials in a complicated hierarchy whose exaltation and competence are often imperfectly correlated. Second, and not simply as a consequence, many of the agencies are outrageously expensive ways of accomplishing inexpensive tasks. Third, the agencies are often disastrously politicized, which means that issues entirely irrelevant to the objectives of an agency (such as whether Israel should continue as a member of the World Health Organization, a bone of contention three years ago) dominate proceedings. In this connection, it is only proper to acknowledge that Unesco's concern with the international traffic in information is not irrelevant to its terms of reference, although a majority of Unesco's member governments are guilty of imprudence for having used their voting power to advance the issue, just as it was shabby of them to use the same voting power to lift the minimum number of a member's nationals whom Unesco must employ from four to five.

0028-0836/84/020095-01$01.00

© 1984 Macmillan Journals Ltd

All of these complaints are legitimate, but can be sensibly assessed only against the knowledge that the UN system and its agencies are the only systematic means by which nations with very different interests are regularly required to meet each other. In recent years, the process has done very little to sustain the hope of the United Nations' founders that the system would somehow abolish conflicts large and small, but it has become a powerful channel for the flow of multilateral technical and material assistance from developed to developing countries, itself an indispensable complement to what countries are able to do bilaterally. Latterly, the UN system has become indispensable (if infuriating) as the only systematic meeting ground between the two kinds of governments. The value of all this is not negligible even if the present cost is too high.

Moreover, the complaints against the United Nations and its agencies are not irremediable, as the performance of the more efficient agencies shows clearly enough. The World Meteorological Organization is top of most people's lists of good performers, the International Atomic Energy Agency plays an indispensable part in the administration of the Non-Proliferation Treaty, the International Telecommunications Union is indispensable (if too much a prisoner of national telecommunications monopolies and thus in need of reform), the Tropical Diseases Programme of the World Health Organization has surprised the sceptics by having emerged as a sensibly directed programme of research and development and not the slush fund that seemed in prospect ten years ago. Arranging that Unesco should function that well is bound to be difficult, given that this agency's terms of reference are more fuzzy than any other's, but there is much that can and should be done to simplify Unesco's housekeeping.

The administrative problems should be more easily soluble than most Unesco delegates will allow. The bureaucracy and high cost, consequences though they are of the way in which the United Nations and its agencies are instruments of the collective will of member governments, should with wit and perseverance be remediable. Unesco as it has become is two things — a talking shop and an executive agency. It is inevitable and even proper, within the agency's terms of reference, that all member governments should feel free to have their say on grand matters of policy, but intolerable and unnecessary that the same should apply to the selection of people who should benefit from training programmes in special fields, or to the management of collaborative scientific programmes of the several kinds on Unesco's books. For an agency whose ambitions forever outstrip resources and even common sense, but whose staff is bloated by the small army of professional people hired to second-guess hostile governmental delegations, the sensible and frugal course would be to delegate much of this work. The International Council of Scientific Unions, already a powerful source of help to Unesco (but which antedates it) could for example take over much of what is intended. Then at least there would be some hope that the overhead cost, which often amounts to a third or more of what Unesco has to spend on its programmes, would be reduced.

Puzzlement

Many other Unesco projects should, on the other hand, be promptly brought to an end. Even in the relatively tangible field of science and technology, it is hard to see why Unesco is planning to spend $85,000 this year on helping member states to draw up "national programmes for research in biotechnology and its applications", and hard to imagine what special expertise it pretends to have in this competitive field. It is similarly mystifying that it should wish to spend $89,000 to help national governments to work out strategies for the development of information technology (or "informatics" in Unesco language). If there were even the most meagre evidence that member governments were anything but over-aware of the opportunities in these fields, the modest sums involved might be forgiven, but as things are they will literally be wasted. Unesco, unfortunately, has never been able to exercise self-restraint but instead feels bound to jump on every new bandwagon that comes along, usually with too small an amount of money to be decisive, often with too woolly an idea of what might be done to be helpful and sometimes with the ill-concealed intention of stealing other people's thunder so as to be able to magnify its own appearance on the scene.

But how can an incorrigibly expansionist organization such as Unesco be made to behave more sensibly? Those member governments dismayed by such developments must not underestimate their power. While the massed votes of the developing countries in the group of 77 (misnamed because there are many more of them) can almost always force the general conference to accept unpalatable proposals, particular wrongheadedness can be rooted out whenever delegations dig in their heels, abandon the customary gentilities of UN meetings which require that the chairman of every committee should be applauded for his eminence and congratulated for his wisdom, throw out the dangerous notion that a nonsense will become sensible if enough people vote for it, and simply declare that they will not be parties to proposals that they know to be objectionable. That, of course, would demand more of delegations than they have in the past been able to provide. The present crisis springs from members' past neglect.

Politicization

The politicization of Unesco is a more difficult problem, although not very different in its essentials. The first need is that all member governments should recognize that an organization with such wide terms of reference cannot be apolitical. If Unesco's programme on the free flow of information had not turned out to offend North America and Western Europe, it would no doubt have caused similar offence to the governments that practise censorship. Yet who will say that the free flow of information is not relevant to the interests of a body with science, education and culture in its title? The now-complainant governments have weakened their own positions in the past by failing to anticipate that such conflicts are unavoidable, to argue with conviction that there are two sides to most of the coins turned up — and then to insist on what is plain for all to see, that neither Unesco nor any other such representative body is equipped to be a negotiating forum for the removal of irreconcilable differences.

The second need is somehow to neutralize the irresponsible use of the votes of the group of 77, both in Unesco and the other UN agencies. Here again, the complainant governments are not nearly as powerless as they think. If, as this year, Unesco is determined to hold a major conference on the future development of information technology of which those with the most to contribute disapprove, they have a simple remedy — simply not to participate. If the massed votes should commit Unesco to a resolution calling for the restoration of all cultural property to its place of origin, or to a demand that intellectual property should be freely available to some class among the membership, they can do more than merely decline to cooperate — they can, more openly than has been their habit, argue for the notion that equity is not unidirectional. The complainant governments have in the past been too unreflective in their response to the demand that all countries should at all times be qualitatively equal, when the best that can be hoped for is dynamic diversity. Plain speaking is again the answer.

The future for Unesco is in all the circumstances not nearly as gloomy as the threatened withdrawal of 27 per cent of its budget would suggest. The ideal is that the hard times ahead should persuade the organization and its member governments that three-quarters of the budget is ample to support the present programme. Only last week, the director-general was asking his staff to reduce spending by 10 per cent this year — nearly half the battle. The complainant governments not planning to withdraw know what they have to do to encourage him in this direction — be less polite and better-informed. The United States should plan seriously to practise what Mr George Schulz promised in the State Department's letter of resignation — that the United States will collaborate with Unesco in (and help pay for?) those programmes it approves of. The important need is that disenchantment with Unesco should not infect US policy to the rest of the UN system. □

0028-0836/84/020096-01$01.00

© 1984 Macmillan Journals Ltd

Monoclonal patent disputed

Disclosure threat to Japanese rights

Washington

THE Wistar Institute's efforts to patent monoclonal antibodies have run into trouble in Japan and the United Kingdom. Twenty-seven separate challenges have been filed in Japan to a Wistar patent for monoclonals that specifically attack tumour cells. The patent is based on research by Hilary Koprowski and Carlo Croce of the Wistar Institute and was assigned to Wistar by the researchers. And the British Patent Court recently affirmed the rejection by the Patent Office there of a second patent that covers monoclonals specific for viral antigens. Both have been successfully patented in the United States.

The challenge to the tumour antibody patent in Japan may prove the more costly of these setbacks to Wistar. A test kit that makes use of the antibodies for diagnosing solid tumours is already on the market in Japan, and Wistar has already sold three licences to the US patent. Clinical trials of anticancer agents that use the antibodies to home in on tumour cells are under way in the United States. Although Wistar's associate director, Warren Cheston, says that "realistically" he expects the patent to bring in less than $100,000 a year to the institute, he acknowledges that "if you believe the people on the commercial side, there's potentially huge sales out there". Cheston points out that the non-monoclonal diagnostic for colo-rectal cancer now in use is a multi-million dollar market.

Those challenging the patent are basing their case largely on an article that appeared in *Nature* the day before the US patent application was filed. The US patent is not threatened by the Japanese challenge, but the US filing established the priority date of the invention. And unlike the United States, which allows an inventor to apply for a patent up to a year after disclosing his invention, Japan (like Western Europe) demands "absolute novelty" — no disclosure before the first filing. The patent was filed for in the United States on 28 April 1978; in the 27 April issue of *Nature*, a News and Views article appeared with a brief description of Koprowski's work.

According to Helmuth Wegner, a Washington patent attorney who is representing one of the challengers, that disclosure, even if it is no more than "a fairly broad suggestion", is enough to invalidate the Japanese patent. The *Nature* article provides no experimental details; it notes in a single sentence that Koprowski had produced hybridomas that secrete antibodies with tumour-specific activity. The legal issue is whether that disclosure is in theory sufficient to allow someone else to duplicate Koprowski's work with a reasonable amount of experimentation.

Another tricky issue is sure to be the exact date that the *Nature* disclosure is considered to have taken place. The magazine would have been available in *Nature*'s London office on 26 April, and would have been posted to subscribers on that day. The Japanese law defines disclosure to have occurred when the publication is "distributed", a term not yet tested in court.

The report referred to (*Nature* **272**, 751; 1978) is by Dr Elizabeth Simpson of the Medical Research Council's National Institute of Medical Research at Mill Hill, London, and describes the proceedings of a workshop organized by M. Potter and F. Melchers at the National Institutes of Health on 3–5 April 1978. Simpson refers to the production of monoclonal antibodies against "Differentiation and tumour antigens" by "several groups", and says that Koprowski, using human cancer tissues as a source of antigens, had been able to produce monoclonal antibodies which reacted with human cells so as to "suggest that some of them may be against tumour-specific antigens".

If the challenges are upheld, Wistar would lose the Japanese market, which is the second largest pharmaceutical market in the world. Patents in North America and West Germany would not be affected.

The British patent covered the work by Koprowski, Croce and Walter Gerhard on monoclonal antibodies specific for viral antigens. The Patent Office had earlier ruled that the work was an obvious application of the basic procedures for producing monoclonal antibodies developed several years earlier but never patented by César Milstein and Georges Kohler of the Medical Research Council's Laboratory of Molecular Biology. The Patent Office noted in particular an article that had appeared in *The Lancet* — before the filing of the Wistar patent — that suggested a number of possible uses for monoclonal antibodies, including the detection of viral antigens. Wistar, which had little difficulty obtaining broad patent protection in the United States and several European countries for the viral antibodies, appealed against the rejection; late last year the patent court ruled in favour of the Patent Office.

Stephen Budiansky

European forests

Pollution, pathogens and pests

FORESTS in the Federal Republic of Germany (FRG) and many other parts of Central Europe are deteriorating rapidly. It is estimated that in West Germany 34 per cent of all forest, which covers 27 per cent of the country's total land area, is already damaged. The city of Augsburg has created a special nature park to inform its citizens about the advancing disaster and the Christmas tree business has languished. Whereas in 1982 damage to conifers was estimated in Bavaria at 10-30 per cent, the official figure is now 60 per cent for Scots pine (*Pinus sylvestris*) and 80 per cent for fir (*Abies*). Furthermore, 44 per cent of the beech forest is showing signs of distress.

The situation in Switzerland, where the first symptoms were reported at the Federal Institute of Forest Science in early 1983, has deteriorated rapidly, exacerbated by heavy snowfalls and storms in the two previous winters. The first signs of distress were shown by *Abies alba* in the eastern and northern plateau that includes Berne, the Bodensee and the most heavily populated areas. Three months ago, reports came in from the southern Alps of damage to spruce (*Picea excelsa*), the dominant species, between 1,000 and 1,800 m altitude and in November of massive damage in some localities.

In Czechoslovakia, 11 per cent of the forest is said to be destroyed and a further 11 per cent dying. A recent ecological report by the Czech Academy of Sciences, which was not published in Czechoslovakia but a copy of which reached the French newspaper *Le Monde* through the human rights organization Entraide et Action, draws a grim environmental picture that forecasts that 45–60 per cent of the nation's forest will be degraded by the end of the century.

Although most of the damage is caused by "acid rain", two serious forest pests are adding to the toll. In Switzerland, winter weather damage was followed by an exceptionally hot summer in 1983. Attacks by the bark beetle *Ips typographus* L. reached serious levels. This beetle normally produces two generations per summer, but in 1983 it produced three. This winter so far has been exceptionally mild and the beetle will overwinter in serious numbers if the normal cold fails to occur. In the stressed environments at high altitudes, regeneration of forest is slow and the Swiss institute is seriously concerned that the threatening deafforestation will lead to erosion and avalanches.

In Poland, the moth *Lymanthia monacha* (the black arches moth) known in German as *Die Nonne* (the nun) is said to be the major cause of damage. Historical accounts exist of this moth occurring in devastating swarms from Europe through Central Asia to China and Japan, killing large areas of both conifer and broad-leaved forest, until it eventually succumbs to its own disease.

Sarah Tooze

0028-0836/84/070097-01$01.00

© 1984 Macmillan Journals Ltd

French research support

More clout for CNRS

FRENCH university scientists should be bracing themselves for a radical new year. Last week, the Council of Ministers, the French cabinet, considered the programme of the Centre National de la Recherche Scientifique (CNRS), the body that supports most basic science in France, and has publicly approved of it.

Why should the council do that? To give CNRS political weight in dealing with ministries other than the ministry of research and industry, to which it belongs, says Pierre Papon, CNRS director-general. Among those ministries is the ministry of national education (MEN), responsible for the universities.

CNRS will be restructuring its relationship with the universities in 1984, says Papon. The 75 universities of France are "too many", which has led to a "balkanization" of university politics. According to Papon, CNRS will in future no longer be able to support all subjects in all universities. All this is further evidence of a tightening grip by CNRS of the distribution of its money in the university system and of an increasing emphasis on its own laboratories. But the picture is not all black for university researchers supported by CNRS. Papon insists that the total value of support will not fall — it will just be distributed differently.

In the past, CNRS guidelines for university support have centred on "excellence". Exactly what the research was about was not of great concern. Now, excellence will be necessary but not sufficient, says Papon; "coherence" with CNRS policy will also be expected. This year, for example, those policies include a regional dimension — a wish to improve support in the north and west, relatively undeveloped scientifically; an emphasis on interdisciplinary research, the barriers between disciplines being felt to be particularly strong in France; and special support for life sciences.

Clearly, this marks an increase in "directive" rather than "responsive" support for science, a matter of debate in many other countries. But France is to go still further. CNRS is to sign — possibly this week — an outline agreement with the "Direction de la Recherche", which with MEN is responsible for the universities. This will mark a new degree of cooperation between the two bodies. Some will fear this development as a device for extending CNRS policy now coloured strongly by its exposure in the ministry of industry and research, and by the aim of the present government of achieving economic growth through science and technology — into university research.

Both MEN and CNRS insist, however, that there will be room for independent policies. There is, however, considerable unease in the universities about the way in which CNRS support and influence will develop, particularly among those who do not see their work as of particular economic relevance. And it has been exacerbated by the removal of "individual aids" — a system whereby CNRS supported individuals with two or three technicians, as a step to becoming an "associated laboratory" of CNRS shared between university and the centre. There are not enough technicians to go round, says CNRS. So such early support is to be limited to a few selected "young groups", while individuals will apply for "free contracts" — two-year grants without technicians that are not designed to lead to associated status.

Robert Walgate

Concerted selectivity

IN its unusual declaration, the French cabinet has offered its support to CNRS in pursuing a policy of "selectivity and concentration", which echoes the policy of a certain Science and Engineering Research Council across the Channel. In outline, the declaration announces that CNRS will increase cooperation with "all involved in research", including:

- **The universities, with which CNRS will sign individual and collective agreements. The declaration foresees "decisions, which will allow the creation of new associations [joint research groups, the principal form of CNRS support] or the closure of others, taken in liaison with the universities and the minister of education, and taking into account the quality of the research, its relevance to national scientific priorities and the balance between regions".**
- **Other research councils. The declaration singles out INRIA (informatics), CNET (telecommunications), and CNEXO (ocean science).**
- **Industry, where the declaration foresees more collaboration agreements between CNRS and industry.**
- **"Social partners", a neologism for trade unions, involving increased research on the impact of new technologies.**
- **And the regions ("states"), which will see more conventions lasting several years such as those signed with Nord-Pas-de-Calais and Provence-Côte d'Azur, relating CNRS policy to local needs perceived by the (political) regional councils.**

The declaration also states that "voluntary mobility of researchers will be encouraged". And this could indeed be an important step for CNRS, for it would allow, for example, a CNRS scientist to leave his or her post for up to 3 years to work in industry, freeing that person's salary and position for the period. This could dramatically increase the fluidity of CNRS laboratories. Robert Walgate

Unesco

US scientists hit by withdrawal

Washington

THE United States' decision to withdraw from the United Nations Educational, Scientific and Cultural Organization (Unesco) by the end of this year has dismayed many US scientists who have worked closely with the agency. They say that Unesco's scientific programmes have generally remained free from the political posturing that has tainted the agency's other activities, and warn that withdrawal may exclude US scientists from valuable international data-sharing agreements.

Formal notice of the decision was delivered to Unesco at the end of December. Under United Nations regulations, the withdrawal will not take effect until 31 December 1984, and the United States will therefore continue to pay its $50 million contribution (about a quarter of Unesco's budget) for the remainder of this year. Explaining the American decision to pull out, a State Department spokesman said Unesco had politized virtually every activity it conducted, mismanaged its budget and routinely adopted policies hostile to the institutions of a free society.

Both the National Academy of Sciences and the National Science Foundation (NSF) told the State Department at the end of last year that a decision to pull out could have damaging repercussions for US science. A letter from the academy characterized science as one of Unesco's success stories and pointed out that no alternative machinery existed to service international collaboration concerned with such subjects as the oceans, climate, the solid Earth and the biosphere.

NSF has made a detailed study of Unesco's scientific value for the State Department but its findings have not yet been published. The general view of scientists with knowledge of Unesco, however, is that the benefits of staying in outweigh those of leaving:

- Dr Della Laura, chief of international hydrology at the US Geological Survey, says Unesco's International Hydrology Programme has an excellent record and has successfully steered clear of political issues. If US scientists are unable to continue their participation, she says, the United States will "greatly miss" an arena in which global information of hydrological problems is rapidly exchanged.
- Unesco's activities in the Earth sciences have received similar praise from Dr Lin Hoover, deputy chief of international scientific programmes at the Geological Survey. About 300 American geologists a year take part in research under the Unesco-sponsored International Geological Correlation Programme and their exclusion will force the United States to negotiate countless bilateral agreements

0028-0836/84/020098-01$01.00

© 1984 Macmillan Journals Ltd

and will reduce opportunities for US geologists to visit foreign sites in the company of international experts.

Dr Roger Revelle, professor of science and public policy at the University of California, San Diego, says the United States has been deeply involved in Unesco's Intergovernmental Oceanographic Commission and two major projects it is helping to coordinate — Tropical Oceans and Global Atmosphere (TOGA) and the World Ocean Circulation Experiment (WOCE). He says the intergovernmental machinery established by Unesco is essential to gain permission to set up tide gauges and other equipment in territorial waters.

The State Department has hinted during informal conversations with the scientific community that some of the money now given to Unesco could be diverted to the apolitical and, some believe, more efficient International Council of Scientific Unions (ICSU). But the suggestion has been dismissed by the National Academy of Sciences, which insists that only a governmental-level organization can command the prestige and resources necessary to coordinate global research programmes.

The academy is not, however, wholly uncritical of Unesco. Its letter to the State Department acknowledges that its management of scientific programmes could be improved. Now that the United States has announced its firm intention to leave, US scientists who work with Unesco hope that they will nevertheless be allowed to participate in individual programmes of obvious benefit to the United States. The United States intends, for example, to remain within the copyright convention and the International Programme for the Development of Communication. The State Department has left the door open, but it remains to be seen whether Unesco itself, jilted by its biggest paymaster, will agree.

Peter David

Solar energy

Sahara's power for Europe?

Rehovot

A SCHEME that may one day permit Sahara sunshine to power the factories of Europe was discussed this week by participants in an International Workshop at the Weizmann Institute of Science in Rehovot and at Ein Bokek on the shores of the Dead Sea.

The idea is to collect solar energy in a desert area (say Israel's Negev, the south-western part of the United States or the Sahara) and to transform it into energy-rich chemicals for piping to industrial areas further to the north where, in yet another catalytic transformation, this energy would be released as heat for use in manufacturing processes.

Professor Israel Dostrovsky of the Weizmann Institute, organizer of the workshop, points out that "solar heat has generally been used close to the site where it is collected. This is because transforming it into electricity and then back to heat (if that is what the customer needs) entails a loss of about 75 per cent of the energy." He hopes, however, that the "thermochemical pipeline" will make it possible to deliver solar heat to a remote customer with much smaller losses and, simultaneously, provide a convenient way to store solar energy for night-time use.

Participating in the workshop were US and West German scientists who have studied the transformation of surplus nuclear energy into chemical energy with much the same goals in mind. Indeed, the Germans have even built a successful 10-megawatt pilot plant at Jülich, near Cologne. But solar energy presents an additional challenge because, unlike nuclear energy, it does not come in a steady flow.

A one-megawatt pilot plant to test the "thermochemical pipeline" concept will be built on the Weizmann campus with energy from a solar tower.

While institute researchers have no delusions about solar sources supplying most of the world's energy needs, they think the new scheme is worthy of support — and particularly from the oil sheikhs of Saudia Arabia and the Gulf States. "If we're successful", Professor Dostrovsky observes, "their vast stretches of sunbaked sand will still be profitable after their oil wells run dry."

Another original Israeli development in the solar sphere won international recognition two weeks ago when a contract was signed between the Edison Company in Southern California and Ormat Turbines of Yavne, south of Tel Aviv. The Israeli firm will be responsible for the construction and operation of a solar-pond power plant, expected to supply 12,000 kilowatts of power to California Edison customers by the end of 1985, and 48,000 kilowatts in 1987 (with the addition of three more ponds).

Ormat operates two solar ponds at the Dead Sea; the first, inaugurated in 1979, produces 150 kilowatts, and the second, opened last year, produces 5 megawatts. They exploit the fact that in a standing pond of salt water a salinity gradient is created, one which they have learned to maintain artificially. Temperatures at the very salty bottom reach up to 90°C, not very hot compared with the 500°C steam used in conventional turbines but hot enough to drive the special Ormat turbine generators in which fluid with a low boiling point is substituted for steam.

Here, as elsewhere, scientists and politicians now speak frequently about what they expect to be happening by the year 2000. By then, Professor Dostrovsky hopes that his "solar pipeline" will be contributing significantly to a solution of energy problems, and the Israeli Ministry of Energy predicts that 11 per cent of the country's total energy needs will be met from solar sources, divided in the following way: 12 per cent from "passive" energy (direct absorption not using collectors), 30 per cent from flat-plate collectors, 30 per cent from concentrating collectors producing industrial process heat, 16 per cent from photovoltaic systems producing electricity directly, and 12 per cent from solar ponds.

In another 16 years, it will be possible to know whether this extremely optimistic prediction proves to be correct.

Nechemia Meyers

A place in the Sun

THE multitude of solar energy projects in Israel, were reviewed in a report recently presented to the Food and Agricultural Organization of the United Nations by Israel's Agricultural Research Organization. The authors, D. Groves and I. Segal, point out that more than 700,000 Israeli households now have their water heated by solar energy, thus accounting for 65 per cent of all domestic water heating. The result they say, is a saving of 6 per cent in electricity generation and 2 per cent in all primary energy supply. "These figures", they add, "make Israel the world's largest *per capita* solar energy user."

Almost all this water is heated by flat-plate collectors, developed in the 1950s by Dr Harry Tabor and now manufactured by no fewer than 130 individual companies.

Some of Israel's most interesting solar energy work is being done at the Desert Research Institute of Ben-Gurion University of the Negev in Sdeh Boker, the isolated place at which solar enthusiast Ben-Gurion made his home after retiring from the premiership. At Sdeh Boker, one finds, for example, a special adobe house — constructed by Michael Kaplan and Eli Levin-Epshtein — which requires only US$12 for total winter heating as against an average of $1,000 for neighbouring homes.

Solar energy is also of interest to Israeli farmers. It is, for example, being used for heating greenhouses from which vegetables and flowers are exported to Europe during the winter months. All systems under development take advantage of the fact that even though Israeli winter nights tend to be cold, there is usually excess heat available for collection during the day. Several researchers call for the collection of this heat in water, which can be recirculated during the evening hours, while others favour its collection in special salt hydrates, which are placed inside double-layered pastic sheets from which the roofs and walls are constructed. The salts change from liquid to solid and back at about 20°C, at the same time releasing large amounts of heat.

Nechemia Meyers

© 1984 Macmillan Journals Ltd

Indian science congress

Pursuit of quality endorsed

Mesra, Bihar

STRINGENT control of higher degrees and special schools for highly gifted children were among the recommendations of the 71st Indian National Science Congress, which met at the Birla Institute of Technology in Mesra last week.

The congress, which resembles a cross between the British Association and a political rally has, since 1976, had a "keynote theme", on which it presents recommendations to the relevant government bodies, and on which it receives an implementation report in the following session. This year's theme was "Quality science in India — ends and means".

This slogan was interpreted in different ways, Professor M.G.K. Menon made an impassioned plea on the serendipity value of basic research. The Prime Minister, Mrs Indira Gandhi, who opened the congress, argued that, since "quality of science" presupposed "quality of life", it implied a joint campaign of scientists against the threat of nuclear war and the subsequent ecological disaster. By the second day, however, the keynote theme had crystallized into how to overcome the prevailing mediocrity of Indian science.

India, as a number of speakers noted, can claim the world's third largest "scientific work-force" in the sense of employed persons with at least a first degree in science. In establishing this human resource, which is vital for the development of Indian agriculture and industry, there has been a concentration on quantity rather than quality of graduates. Furthermore, the low remuneration of young research fellows (around 600 rupees — £40 — a month, or about half the salary of a young graduate entering business or the civil service) means an inevitable wastage on financial grounds. The congress president, Professor R.P. Bambah of Punjab University, maintained that unless research fellows immediately have their stipends doubled, there is no long-term hope for Indian science.

This utopian suggestion did not find its way into the draft "recommendations" which this year seem aimed as much at the scientific community itself as at the government. On the subject of pay, the "recommendations" firmly reject the new scheme of promotion of lecturers to readers and readers to professors on the basis of length of service. This scheme has recently been introduced, under trade union pressure, at a few leading universities, including Delhi, in place of the existing system of promotion by merit as and when a vacancy occurs. The new system, many argued, is a blueprint for apathy and complacency.

One practical suggestion for improving scientific "quality" was that the titles and abstracts of PhD theses should be published together with the names of the adjudicators awarding the degree. This is intended to counteract the frequently reported slackness and even downright dishonesty in the degree process. At the same time, criteria for higher degrees should be standardized because several universities have acquired a reputation for "easy" PhDs. Government agencies which award research fellowships should establish a joint selection examination, and awards should be made on the basis of merit only — ending the present "positive discrimination" in favour of the disadvantaged. The civil service requirement that certain posts must be filled by graduates should be revoked, thereby reducing the pressure on the universities.

In general, Indian education was felt to be too examination-oriented — and Professor Bambah's suggestion of "pace-setting" schools for the highly gifted was based on the concept that such young people could better develop their potential in a peer group in which they would learn to work together as a team.

The congress itself had a competitive aspect — competitive awards to young postgraduate scientists selected by the Indian National Academy of Sciences and by the congress. Ironically, the first award announced was for work on RNA. Yet, although it recently hosted a major genetics conference and in spite of the importance of the subject for agriculture, India has been able to establish virtually no university departments of genetics — apparently because of a preponderance of classical botanists and zoologists in the upper echelons of the life sciences.

Vera Rich

Educational computing

Academics gain from competition

Pasadena, California

COMPETITION in the computer industry is nothing new, but computer companies are now competing for an unusual prize — the privilege of giving away millions of dollars of equipment to universities. In recent months the California Institute of Technology (Caltech) has received computers worth some $1.4 million from Hewlett-Packard and Data General at a 75 per cent discount, Massachusetts Institute of Technology (MIT) has closed a deal with IBM and Digital Equipment Corporation (DEC) under which those companies will donate $50 million in equipment and support over five years and IBM has announced a programme of grants to 20 universities totalling $50 million in hardware for the support of manufacturing engineering education. Many other companies are also offering large numbers of small computers to universities at much reduced cost.

The companies' motives are varied. Those offering bulk deals on work-stations may well be hoping to lock in a future market in personal and business computers. The Caltech and MIT deals, however, appear to reflect the companies' need to tap a body of expertise that they themselves lack, particularly in developing educational software and in assembling large integrated computer networks. Charitable donations to universities also yield tax benefits to the companies that substantially reduce their net cost. Educational software will probably be the most tangible benefit to the companies. MIT has agreed to offer IBM and DEC non-exclusive licences for any software developed with their equipment.

The present dearth of serious educational software is universally bemoaned. "The state of the art in educational uses of computers is a disaster", says Steven Lerman, an MIT engineering professor who directs the programme there. Most educational software is little more than electronic workbooks, which is "the biggest waste of hardware, software, and people's time ever invented". Lerman foresees the use of computers in a variety of truly novel ways that will take advantage of their unique capabilities. "Dry laboratory" experiments could demonstrate chemical titration, thermodynamic principles and the like with the help of sophisticated graphics — and without wasting valuable laboratory time and space.

MIT is itself raising $20 million for the programme. IBM and DEC, besides donating the machines, are providing software and maintenance support and are lending MIT several full-time personnel.

Here at Caltech, the emphasis is also on educational software development. The university is placing three of the four VAX-class Data General machines it has acquired in the hands of undergraduate students. Eventually, these machines are expected to become the central computers in a network that will include multiple work-stations. Geoffrey Fox, a theoretical physicist who was recently named Caltech's first dean for educational computing, believes it is a mistake, however, to have one computer to each student. Fox says that to get students to use computers and to stimulate the much needed software development requires a diversity of machines with capabilities beyond the "lowest common denominator" that inevitably determines the selection of a personal computer that will be issued to every student. Fox points to one US university that acquired an IBM personal computer for every freshman, only to find that the average computer was used for one hour per day for games and for one hour as a terminal for the university's main computer.

Stephen Budiansky

0028-0836/84/020100-01$01.00

© 1984 Macmillan

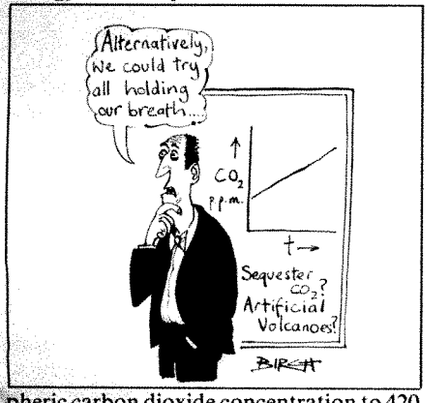
Greenhouse effect

Energy efficiency may help

Washington

ALTHOUGH a global warming due to a build-up of atmospheric carbon dioxide is inevitable, it can be postponed significantly by improved energy efficiency, according to a new study prepared for the National Science Foundation*. The report, by engineers at Massachusetts Institute of Technology (MIT) and Stanford University, breaks with recent studies by the Environmental Protection Agency (EPA) and the National Academy of Sciences (NAS) in suggesting that practical and effective steps can be taken at once to soften the blow of a warming induced by carbon dioxide. By drawing out to several centuries the time it will take for the atmospheric carbon dioxide concentration to double, society will buy time to develop new energy technologies and to enable agriculture and population distributions to adjust to climatic shifts.

The study found that "realistic energy strategies" based on improvements in energy efficiency could hold the atmospheric carbon dioxide concentration to 420 p.p.m. by the year 2050, up from 340 p.p.m. at present. Based on the recent experience of the United States and other industrialized countries, the report concludes that energy use per real dollar of gross national product can be expected to fall by 1 per cent per year.



"It makes sense to develop strategies for reducing fossil fuel carbon emissions, rather than relying solely on research to narrow uncertainties and/or ameliorative measures such as building dikes and developing new strains of 'greenhouse-resistant' crops", the report says. EPA last year suggested that there was little point in trying to halt the carbon dioxide build-up; the NAS report suggested that uncertainties in model predictions (which point to an average global warming of a few degrees centigrade for a doubling of atmospheric carbon dioxide) preclude any immediate steps to halt the build-up.

The NSF study, however, noted that increased energy efficiency, apart from being the only practical route to slowing the build-up of carbon dioxide, is economically attractive in its own right.

The NSF report found that solar and renewable energy technologies are not likely to displace fossil fuels to a sufficient extent to make a significant difference in carbon dioxide emissions in the short run. Nuclear fission, because it will probably be considerably cheaper than coal (especially if acid rain prompts new emission controls on sulphur), is seen as the only energy technology besides improved efficiency with a major short-term effect on atmospheric carbon dioxide.

The study discounted strategies to extract carbon dioxide from fossil fuel combustion and sequester it, for example, in deep ocean waters. This is only one of several half serious ideas for technical fixes lately advanced. One scheme would add sulphuric acid to the atmosphere — an "artificial volcano" that would cool the Earth, offsetting the carbon dioxide-induced warming — but the price is estimated to be $50,000 million per year. Another would establish mirrors in Earth orbit to increase sunlight available to high-latitude ocean waters; the increased light would permit the ocean biota to utilize the nutrient excess in these waters, increasing biological uptake of carbon. The capital cost for this venture is estimated very roughly at $100,000 million.

Stephen Budiansky

**Global Energy Futures and CO_2-Induced Climate Change* (by D. Rose, M. Miller & C. Agnew) MIT Energy Laboratory Report No. 83-015, prepared for the Division of Policy Research and Analysis, NSF.

Acid rain

Uncertainty persists in Europe

A REPORT on acid deposition in Britain prepared by the Warren Spring Laboratory for the UK Department of the Environment highlights the need for a long-term programme of measurement on acid precipitation. Asked to examine the distribution in Britain and time trends of acidity in precipitation in Britain and northern Europe, the authors were able to consider only a part of Britain's land surface for lack of measurement stations between which comparisons could be made.

The report therefore calls for a commitment to long-term support for measurement, especially at high altitudes and in urban areas. More use should be made of wet-only collectors (which are not susceptible to contamination by gases and particulate matter) and frequent comparisons between institutions should be made to eliminate differences due to measurement protocols.

The authors, under the chairmanship of C. F. Barrett of the Warren Spring Laboratory, were unable to reach firm conclusions about recent trends in deposited sulphate, chiefly because of climatic variations from year to year, although nitrate concentrations do appear to have increased since 1957. Looking back further, to the beginning of the century, however, there is evidence that wet depositions of both sulphate and nitrate have increased in approximate agreement with emissions.

From the few sites at which daily analyses are made, the frequency distribution of deposition of acidity seems highly episodic, with typically 30 per cent of annual acidity being deposited in 5 or 6 days. Although acidity cannot be uniquely apportioned between anions, statistical correlations suggest that at most sites studied, sulphuric acid is responsible for about 70 per cent of acidity and nitric acid for 30 per cent. The largest predicted depositions of hydrogen ions occur in Cumbria and parts of Scotland, where levels are of the same order as parts of Scandinavia and North America. Dry deposition of sulphur, however, is a more local phenomenon related to local sources of sulphur dioxide.

Meanwhile, the European Economic community's Council of Ministers has been unable to reach agreement on a proposal from the Commission of limiting air pollution from industrial plant. The proposal, which is largely procedural, ran aground over an article on emission standards which entailed the eventual possible use of community-wide emission standards, to be introduced by a two-thirds majority. The Commission was prepared to drop the offending article, but the Netherlands objected: Britain and Ireland, in contrast, could not accept the use of such standards in any circumstances. A second much more specific Commission proposal on limiting air pollution from combustion plants, which would have important consequences for power stations, specifies targets for reductions in emissions from both old and new plant, but leaves the options open to governments on how it should be implemented. This proposal has not yet been formally discussed by the council, but is likely to generate an unusual amount of heat.

Tim Beardsley

Scientific correspondence

Careful readers will have noted that correspondence is now published under two headings — the familiar general correspondence (see pages 103 and 104) and "Scientific Correspondence" (see pages 115 and 116). The objective is to provide a means by which readers can raise points of a rather technical character which are not provoked by articles or letters previously published (where "Matters Arising" remains appropriate).

Contributions to Scientific Correspondence will not usually be formally refereed. □

© 1984 Macmillan Journals Ltd

UK research councils

Astronomers' future undecided

CONTROVERSIAL recommendations are expected from the Manpower and Site Review Panel of the UK Science and Engineering Research Council (SERC), whose proposals have now been sent to SERC's Astronomy Space and Radio (ASR) board for discussion at its meeting later this month. The panel was instructed to find ways of saving more than £1.5 million from the ASR board's budget for the next financial year (1984–85). Whether this has been done by recommending radical changes or by piecemeal cuts, the board will face some painful decisions.

The panel, under the chairmanship of Professor A.P. Willmore, was asked to review the need for each of three establishments — the Rutherford Appleton Laboratory, the Royal Greenwich Observatory (RGO) and the Royal Observatory in Edinburgh (ROE) — taking into account the fact that much of the United Kingdom's gound-based astronomy will in future be carried out by remote control at facilities in Hawaii (Mauna Kea) in the Canaries (La Palma).

The terms of reference were given considerable sting by the added injunction that the ASR board must save £1.5 million or so. (The board spent about £6.5 million in grants in 1982–83.) RGO had already taken a battering last year in a review of SERC's institutions by a cost-cutting unit of the British Government under Sir Derek Rayner. That report, hotly disputed by RGO staff, recommended that Herstmonceux Castle — a major part of the RGO site — should be sold off (see *Nature* **304**, 5; 1983) and that a merger of RGO and ROE should be considered.

When the Willmore panel was set up, it was widely thought by the UK astronomical community that SERC was indeed intending to merge RGO and ROE. The matter is variously seen as a battle between satellite and ground-based astronomers over the distribution of funds, between SERC's head office and its institutions, or between SERC establishments and universities. Still others fear that the geophysical research supported by the ASR board (mainly concerned with the upper atmosphere and the magnetosphere) will be sacrificed to preserve major astronomical projects such as the millimetre-wave telescope on Mauna Kea and ROSAT, an X-ray satellite supported mainly by West Germany but with UK participation.

Not surprisingly, SERC is keeping its cards close to its chest. Indeed, the proceedings of the Willmore panel have been so secret that its members have not been allowed to remove documents from SERC's offices. The committee's members hold strong and divergent views and it is unlikely that they will make a unanimous radical recommendation. But whatever the panel has decided, the ASR board may choose to ignore it, or instruct the panel to think again.

Philip Campbell

Biotechnology

Gamma interferon race begins

Boston, Massachusetts

GENENTECH has won the race to begin US clinical trials of gamma interferon synthesized in recombinant bacteria. The trials of the drug produced by the California biotechnology company began last month under the direction of a pioneer in clinical interferon research, Dr Jordan Gutterman, a haematologist at the M.D. Anderson Hospital and Tumor Institute in Houston, Texas, one of the largest cancer centres in the United States.

Several biotechnology and pharmaceutical companies have a big stake in the success of gamma interferon, which they hope will be an anticancer drug. The competition is fierce and close: Biogen has been carrying out clinical trials of gamma interferon in Europe and Japan since September 1983. As well as demonstrating gamma interferon's antiviral action, preclinical trials by several companies have demonstrated significant antitumour activity, and greater stimulation of the immune system than by other interferons. So gamma interferon could make a good partner for other immunosuppressive chemotherapy drugs.

Gutterman has been treating patients with natural alpha interferon for the past six years, and began trials with cloned alpha interferon three years ago. He and his co-workers reported last week in the *New England Journal of Medicine* (**310**, 15; 1984) that each of seven patients suffering from hairy cell leukaemia — a slow-growing B cell neoplasm for which there is no consistently effective treatment — were brought into complete or partial remission by injections of natural alpha interferon; Gutterman's group has recently begun extending these experiments with cloned alpha.

The Texans are looking forward to rapid progress with their tests of gamma interferon; their experience over the past year with the scarce and expensive natural product is expected to help them rapidly to determine the best way to administer the drug — an important point, because intramuscular injections of natural gamma interferon have been found to be cleared rapidly from the body. Gutterman's group has laboriously had to determine the proper procedure for administering the drug by a prolonged intravenous infusion.

Gutterman is very excited about the possibility that gamma interferon may potentiate the effects of alpha. Despite his success with hairy cell leukaemia, he has found that only a quarter of the patients with melanoma or breast cancer respond to alpha interferon treatment. Substantial *in vitro* and *in vivo* data using mouse and human interferons have shown synergistic antiviral and antitumour effects, and Gutterman hopes that human malignancies may respond similarly. Combined trials may begin in a year or so, once the pharmacology of gamma interferon is worked out.

Christopher Earl

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 30 Dec.	Change
23¼	9¾	**Biogen (Switzerland)**	12	10¾	−1¼
6¼	1⅝	**Bio-Logicals (Canada)**	2	1⅝	−⅜
16⅛	7¼	**Bio-Response (USA)**	11¾	10	−1¾
19	10½	**Cetus (USA)**	12⅜	10⅞	−1½
15½	6½	**Collaborative Research (USA)**	8	6¾	−1¼
39⅞	15	**Damon (USA)**	17⅝	15⅜	−2¼
34¼	16⅜	**Enzo-Biochem (USA)**	24¼	25	+¾
18⅞	8⅝	**Flow General (USA)**	11¼	9½	−1¾
49¾	25⅞	**Genentech (USA)**	36½	34½	−2
17¾	7⅞	**Genetic Systems (USA)**	10⅝	8⅞	−1¾
23¼	12½	**Genex (USA)**	15	13	−2
31	18¾	**Hybritech (USA)**	22½	18¾	−3¾
22¼	10⅞	**Molecular Genetics (USA)**	12⅞	12	−⅞
23¼	10½	**Monoclonal Antibodies (USA)**	14¾	12	−2¾
73½	42	**Novo Industri A/S (Denmark)**	65	56	−9
30¼	13⅝	**Pharmacia (Sweden)**	21	19⅜	−1⅝

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature*'s weighted index of biotechnology stocks stood at **175** on 30 December, compared with **196** a month earlier. Data from E.F. Hutton, Inc.

0028-0836/84/020102-01$01.00

Chile under Pinochet

SIR — I have read (*Nature* **306**, 11–12; 1983) that Chilean universities have not only survived Pinochet's dictatorship but, due to a mixture of despotic benevolence, the existence of a United Nations Regional Program for Post-Graduate Training and the quality of young scientists nurtured by their seniors, Chilean science has in fact been revived. The article exudes an aura of optimism for the future, ending with a reflective statement about the necessity for pragmatic objectives and the dismissal of "over-ambitious utopian dreams and violence".

I read the article with dismay. Certainly, history has taught me that tyrants have at times been generous patrons of the arts and conspicuous builders, but my naive heart would like to believe that tyranny kills all it touches. Furthermore, the article is disturbing because its anecdotal account of hardship fals short of picturing the excesses of Pinochet's murderous tyranny, and because the reported relaxation of the regime is hard to reconcile with reports from Amnesty International of repression, torture and assassination still rampant after 10 years of dictatorship. "Between 1973 and 1979, hundreds . . . disappeared after being arrested by the security forces . . . coordinated by the Direccion de Inteligencia National (DINA). Disappearances and killings . . . remain officially unexplained. Since 1977, a number of alleged members of banned political parties and organizations . . . have died in the custody of the CNI (Central National de Informaciones, Chile's secret police), in circumstances which indicate that they may have died after torture or . . . [been] killed by other means." (*Political Killings by Governments, An Amnesty International Report*, 20–21; 1983).

Let us put the survival issue into perspective. By the time the medical school community was taken to the soccer field, the university had already paid a grievous price — the disappearance of faculty members who were underground, exiled, or already dead. Later on, economic constraints brought the "ousting of many people and early retirements". The survivors were those silenced by fear and various collaborators — those people with deep-rooted rightist convictions and those pragmatic enough to accommodate their principles to the establishment. On the one hand, there was repression and on the other, compliance and consensus. How much consent is hard to evaluate, but guidelines for suppression and removal of academic personnel to be effective after the coup were clearly spelled out in a document signed by a pediatrics professor of the University of Chile.

The subject of the "open and constructive discussion" in Chilean universities which are more open and constructive than those that occur "in other countries respected for their democratic governments" is not spelled out. I must then conclude that despotism has consented not to be too despotic for people to discuss — allow me to guess — the sodium pump, quantum statistics, the cost of paper clips, promotions and fund allocations. I strongly believe in academic freedom but the liberty that we are told Chilean scientists enjoy occurs simultaneously with hardship upon the Chileans that cannot be silenced nor should it be forgotten. It consists of 50 per cent unemployment in the shanty towns, it consists of the hunger of the poor, desperate citizens fighting with stones against police tear gas and bullets, people still being tortured and in prison; it consists of exiled people, it consists of the dead.

I hope that Chilean transition to democracy will occur soon and that it will be obtained without further violence. However, as long as the aims of a few are placed above the elemental rights of the enslaved many, the germ of violence will be there. The preservation of an ivory tower for a score of people cannot be the constraint to justice and equality. Justice and equality must not be utopian dreams.

ELSA BELLO-REUSS

Department of Physiology and Biophysics, Washington University School of Medicine, St Louis, Missouri 63110, USA

Creation or evolution

SIR — In the article "Creation versus evolution: no middle way" (*Nature* **305**, 571–574; 1983) George M. Marsden presents an interesting historical review of the roots of the creationism/evolution controversy in the United States, but slights its most important aspect: the science education issue. Because of pressure from antievolutionists, the amount of evolution covered in pre-college science books is documentably declining, or, even worse, is being accompanied by "theories" that the world was produced in six 24-hour days about 10,000 years ago.

There is value in Marsden's reminder that scientific creationism is more than a scientific issue. Proponents are genuinely worried that civilization and the moral order are threatened by modern antisupernaturalism. Because they define creationism narrowly, as Marsden documents, they see evolution as creationism's antithesis. Opposition to evolution thus becomes a moral charge. Textbook censors like the Gablers in Texas discourage discussions of the philosophic implications of various world views because they lead to "relativism", "humanism" and other nasty ideas. They therefore demand that creationism be presented in *science*, not history, social studies or philosophy classes. Unfortunately, there is no scientific evidence for (and much against) ideas such as obligate carnivores being former vegetarians (in order to have lived compatibly on Noah's Ark).

Whether or not evolution is "a key mythological element in a philosophy that functions as a virtual religion" (antisupernaturalism) is beside the point. Indeed, evolution *can* be the basis of a philosophical world view, but conceivably so can heliocentrism, or even photosynthesis. Evolution, heliocentrism and photosynthesis can be presented without philosophical trappings — it is difficult to imagine the latter two being taught otherwise. The facts are that photosynthesis occurs, the Sun is the centre of the Solar System, and change through time (evolution) has occurred. Children need and deserve to be taught this. One can be aware of the role of history and culture in science and still insist that science be taught in the science classroom.

EUGENIE C. SCOTT

Department of Epidemiology and International Health, University of California, San Francisco, California 94143, USA

Research Corporation

SIR — Research Corporation takes exception to the article published in *Nature* on 13 October, 1983 (p.569) which alleges mistakes in the handling of a non-isotopic method for detecting gene sequences invented by David Ward of Yale University. That Yale did not "bungle" the technology transfer process is demonstrated by that institution's successful programme of licensing it to industry for development and commercial use.

Research Corporation, a nonprofit foundation that evaluates, patents and licenses inventions for a number of colleges and universities, was erroneously identified as having performed a review of Dr Ward's discovery. In fact, Yale did not submit the invention to Research Corporation, and this organization did not evaluate it or offer any opinion as to its worth.

As a further comment, and in contradiction to writer Budiansky's assertions, Yale University continues to maintain an invention administration agreement with Research Corporation, which now bases the larger part of its Invention Administration Program in Tucson, Arizona (not New York).

JOHN P. SCHAEFER

Research Corporation, 6840 East Broadway Boulevard, Tucson, Arizona 85710, USA

● We apologize for the error. Contrary to what was implied by Yale officials, Research Corporation did not see the original invention. Dr Schaefer is also correct in saying that an arrangement between Yale and Research Corporation continues; it is, however, non-exclusive, and Yale expects largely to assume these functions itself with the establishment of its own office — Editor, *Nature*.

Paying for high-energy physics

SIR — Your article "Paying for high-energy physics" (*Nature* 1 December 1983, p.421) gives numerous false impressions.

The argument that it was not really necessary to observe the $W^{\pm}$ and Z° bosons, because "the [Salam–Weinberg] theory [of weak interactions] . . . may . . . have been held to have been confirmed by what was learned (at CERN) in the 1970s about neutral currents" is wrong. The neutral current data, in common with all earlier information about weak interactions, are consistent with a force of zero range. It is true that those data are also consistent with the Glashow–Weinberg–Salam (GWS) theory, in which the weak interaction is due to the exchange of the W and Z bosons, thereby having finite range $\hbar/m_Z c \sim 2 \times 10^{-16}$ cm. However, the existence of such bosons was only a speculation until they were discovered at CERN earlier this year. The theoretical arguments for the existence of some structure in weak interactions at short distances were rather compelling but the GWS theory was not unique in fitting the earlier data. There were rival theories of the same type with additional bosons, and also very different theories which predicted W and Z bosons but as composite rather than fundamental objects.

The fact that the masses of the W and Z agree with the GWS predictions, within the errors, is certainly not sufficient to establish their true nature. Indeed, the experiments have reported some tentative evidence for the decay $Z^0 \rightarrow e^+ e^- \gamma$, which cannot be explained by the GWS model. If confirmed, this radiative Z^0 decay might perhaps be accommodated by models in which the W and Z are composite. In any case, these anomalous events may indicate quite new physics to come. The fact that the GWS model is still potentially so vulnerable shows that we are still at the beginning of this chapter of the story.

Next, the 2,500 university physicists who use CERN facilities each year go there because it is their deep wish to be involved. There would be many more if there were funds to support them. It must also be remembered that this subject holds a very deep fascination both for the general public and especially for the young scientist still at school, as becomes very clear from interviewing for university entrance. What is going on at CERN is one of the most exhilarating enterprises mankind has known, a great flight of the creative human spirit to determine in full detail what a strange world we live in, and it is not surprising that so many physicists wish so profoundly to take part in this demanding work. You ask what they would be doing if CERN were not there: the answer is simply — "something less significant and much less interesting".

The insinuation that the advanced techniques used at CERN are just borrowed from American laboratories is simply false. The proposal to transform the existing CERN Super Proton Synchrotron into the Proton-Antiproton Collider, with which the W and Z were produced, provides the most obvious counter-example. The crucial technique of stochastic cooling of the antiproton bunches — the breakthrough which made this step to much higher energies possible — was proposed and developed at CERN: it has now been taken up by the Americans for their Tevatron collider under construction at Fermilab, near Chicago. CERN also proposed and built the first proton–proton collider (ISR). Furthermore, CERN is notable for its development of new experimental methods; the neutrino horn, multiwire proportional drift chambers, the development and use of large liquid argon and/or uranium calorimeters, the ring-imaging Cerenkov detector and the ISIS detector (a trend-setter in the problem field of particle identification at high energies) were all pioneered at CERN, to mention just a few outstanding items. Under the pressure of experimental needs, substantial technical developments have been made at CERN which have proved beneficial outside in fields as diverse as electronics and shipbuilding. It would be healthy for this country if more UK engineers were to gain practical experience by taking part in the advanced engineering work going on at CERN all the time.

We agree that the time may be ripe for intercontinental collaboration in the 1990s. For the present, the Americans feel they may be able to build a superconducting collider on their own. However, we cannot anticipate *any* time when experiments will be unnecessary; "cheap theory" alone will not be able to account with certainty for the microscopic structure of the world in which we live, without the aid of experiment.

R. H. DALITZ
C. H. LLEWELLYN SMITH
Department of Theoretical Physics,
University of Oxford,
Oxford OX1 3NP, UK

SIR — The "News and Views" item "Paying for high-energy physics" (*Nature* 1 December 1983, p.421) contains so many inaccuracies that it is difficult to know how to reply adequately at reasonable length, but perhaps I may attempt to correct some of its more misleading assertions.

Comparing the SF 680 million CERN annual budget with the materials cost of the new electron–positron collider, LEP, is not comparing like with like. The annual budget is divided almost equally between staff costs — and many of the staff are engaged in building LEP at present — and materials costs. The materials budget this year is being spent on running five accelerators (one of which operates in two very different modes), and in building LEP. Electricity consumption is appreciable, but not outrageous for a large scientific laboratory whose facilities operate round the clock, seven days per week for much of the year. Indeed, it has been estimated recently that stopping all of CERN's accelerators for a complete year would save a total of just SF 25 million in electricity charges — 3.7 per cent of the annual budget.

The super proton synchrotron will not be closed when LEP is completed. It is required as an injector for LEP and, more importantly, is expected to be used in the LEP era by up to half of the European high energy physics community for both fixed target and colliding beam experiments.

It is both scientifically unsound and unnecessarily churlish to attempt to run down CERN's recent triumphs in detecting the W and Z bosons. Maintaining that the observation of neutral currents by CERN a decade ago was a sufficient test of weak interaction theory is as sensible as claiming that the Venus de Milo may be adequately appreciated by studying the shadows cast on the floor by the spotlights that illuminate it. Continuing the analogy, the recent experiments have provided us with a clear silhouette, in remarkable agreement with the descriptions furnished by those distinguished intellectual tourists, Glashow, Salam and Weinberg. We are almost persuaded that they have been telling us the whole truth, but LEP should provide the detailed colour photographs that will settle the issue beyond doubt.

The article poses the question of what CERN's 2,500 visiting scientists would be doing if CERN did not exist. The most plausible answer is: "Working around the world in groups too small to be effective, lacking contact with their peers and the stimulation of direct competition, performing second-rate research with inadequate facilities". D. C. IMRIE

Chairman,
SERC Sub-Committee on CERN,
Department of Physics and Astronomy,
University College London,
London WC1E 6BT, UK

No thanks — Anon

SIR — Here is a formal "You're welcome" to all those authors who have recently included in the Acknowledgements section of their published papers a line thanking me, an Anonymous Reviewer, for critical comments on their manuscript. I suggest, however, a moratorium on this practice, since thanking me in print is a not particularly informative use of expensive journal space. If authors really feel that they must show their appreciation for an exceptionally helpful anonymous review, they can send me a personal note of thanks via journal editors. Then at least I'll know that it was my comments, and not those of some other Anonymous Reviewer, that were so appreciated. *Name withheld by request*

● *Nature*'s policy is not to thank Anonymous Reviewers — when the misdeed is spotted.

Antarctic mining regime at risk

John Maddox

In Washington next week Antarctic Treaty members will wrestle with mineral exploitation. Their task would be easier if territorial claims were subsumed in a wider framework.

LAST week, it was announced from New Delhi that an Indian expedition of 83 people had reached the coast of Antarctica in a chartered Finnish ice-breaker. Next week, in Washington, there will be a special meeting of the Antarctic Treaty powers to resume the drafting of a convention on the exploitation of mineral resources in Antarctica. The two events are strictly independent, but in the minds of delegates gathering in Washington, there will seem to be an important connection. For the accession of India to the Antarctic Treaty last September (together with Brazil) seems to have reminded the parties to the treaty that change is close at hand.

Anxiety about the future of the treaty has been mounting for several years, and certainly since the Law of the Sea Conference reached its abortive conclusion in 1982. The principle that the sea-bed should be regarded as the "common heritage of mankind" was rejected by several governments, that of the United States chief among them, so that the status of the resulting treaty is in doubt. But the earnest advocacy of the doctrine of common heritage by the governments of many developing countries, and its obvious applicability to the Antarctic, has inevitably cast doubt upon the durability of the arrangement under which the Antarctic Treaty vests responsibility for the management of a whole continent in the governments that agreed to give themselves that responsibility at the end of 1959.

In its essentials, the difficulty springs not from the Law of the Sea but from the reasons why the negotiation of that treaty was frustrated—the general unwillingness of newly formed governments to fall in with international arrangements previously agreed. Uppishness of this kind has become a familiar irritant of established governments, in the United Nations and in other international gatherings. But if the Law of the Sea were less like a dead letter, it would present the Antarctic powers with a further and more particular difficulty, for the sea-bed provisions of the Law of the Sea Treaty defined as the common heritage of mankind even those parts of the oceanic sea-bed lying south of 60°S, the region in which the Antarctic Treaty applies.

Mercifully, this potential conflict between the two treaty regimes remains an academic matter. So, superficially, the Antarctic Treaty itself may seem to be. What can be the value of an international treaty to regulate the uses made of a tract of territory whose practical value is, for the time being, only marginal? That is too detached a view. The benefits of the Antarctic Treaty far transcend its immediate utility. First, the treaty how nations may be persuaded to bury conflicting interests in the pursuit of a more general common interest, for that reason, is a valuable precedent for further international agreements on more urgent matters, arms control for example. Second, the treaty does serve to regulate matters of great importance to one section of the international community, for it has assured access to Antarctica for those wishing to make scientific observations in the region. Third, and more recently, the treaty has provided a framework within which it has been possible to arrange for the orderly exploitation of marine resources. The ambition among the treaty powers to conclude a similar agreement on mineral resources is natural enough, and laudable as well, but more contentious.

To the scientific community, the origins of the treaty are also important because the treaty is a tangible legacy of the International Geophysical Year (IGY), the eighteen-month-long period of coordinated geophysical observation from 1 July 1957 to 31 December 1958. Antarctic exploitation was inevitably a conspicuous part of that cross between a planned research programme and a public relations campaign. In the climate of the late 1950s, with the cold war dragging to its end, the notion that the only substantial part of the Earth's solid surface free from weapons of any kind should be permanently demilitarized won ready consent.

In reality, there was little choice. Open access to the Antarctic by scientific expeditions during IGY had been agreed by the states with territorial claims on Antarctica but the declaration by the United States and the Soviet Union that they would continue with Antarctic research after IGY put the claimants in a fix, as did the continuing disputes among those with overlapping claims.

All with an interest in the Antarctic discovered a common interest in postponing a resolution of these difficulties, and a common fear that if they failed to agree among themselves, matters would be taken out of their hands. The result is that the negotiation of the Antarctic Treaty, on the initiative of the United States, was accomplished in just over eighteen months.

The treaty itself is a simple document. It declares that "in the interests of all mankind", Antarctica shall "continue forever to be used exclusively for peaceful purposes" and that international cooperation and "freedom of scientific investigation in Antarctica" typified by experience during IGY should also continue. Its provisions forbid the establishment of military bases in Antarctica, the testing of weapons (including nuclear weapons) and even the disposal of radioactive waste. Access to all parts of Antarctica is assured, but the twelve original parties to the treaty are free to inspect activities undertaken by other governments — a right exercised almost exclusively by the United States. Nothing is said about the duration of the agreement except that after thirty years from ratification (completed on 23 June 1961), full parties should be free to ask for a conference to review the operation of the treaty and, by implication, to consider its future.

The twelve founder members were a motley crew. They included the two superpowers of the 1950s, Khruschev's Soviet Union and Eisenhower's United States, a group of European states with traditional interests in Antarctic exploration (Belgium, France, Norway and the United Kingdom), Japan, and a group of states in the Southern Hemisphere including three former British dominions (Australia, New Zealand and South Africa) and Argentina and Chile, neighbours in South America and rivals in Antarctica.

Both the United States and the Soviet Union have eschewed claims of sovereignty in Antarctica. Britain and its former dominions consider, however, that they have legitimate claims on parts of the continent, as do Argentina and Chile. The Norwegian claim (predating Amundsen's trans-Antarctic journey) seems not to be a live issue, but Britain, Argentina and Chile claim sectors of Antarctica which overlap with each of the other two. It is not clear whether France has made a valid claim to sovereignty or has merely reserved the right to do so, but the issue has been put firmly in abeyance by the Antarctic Treaty, under which claimants and non-claimants agree that claims to sovereignty will be neither advanced nor liquidated.

But if the moratorium on the issue of Antarctic sovereignty has not made that contentious issue go away. For one thing, the treaty partly owes its existence to the fear, in the late 1950s, that disputes about

© 1984 Macmillan Journals Ltd

Antarctic sovereignty might get out of hand. Second, the structure of the treaty guarantees continuing precedence for the original signatories.

This is the central issue on which the discontents of other governments have focused. Briefly, the treaty distinguishes between three groups of adherents — the twelve original signatories which are automatically consultative parties, signatories which have qualified as consultative parties by their demonstration of "substantial scientific research activity" in Antarctica and other signatories which have not thus qualified. West Germany and Poland became consultative parties in 1979 and Brazil and India (rather hurriedly) last September. Ordinary signatories of the treaty, which now include the People's Republic of China, number more than a dozen. East Germany, Romania and Uruguay formally accompanied their adherence with a protest at the discriminatory provisions of the treaty. Malaysia, not a signatory of the treaty, has gone further by raising the issue of Antarctica at last year's general assembly of the United Nations.

In the circumstances, the charge that the Antarctic Treaty is a club of self-interested governments cannot easily be resisted. This, after all, is what the treaty implies. The charge that the treaty powers are an exclusive club is, however, unfair. The fact that India was able to qualify last year as a consultative party on the basis of expeditions to Antarctica in two successive summers is a sign that, in the present international climate at least, the consultative parties are not concerned like robins to keep others out. Indeed, the consultative parties have now taken to saying that complainants against the alleged exclusiveness of the treaty have a simple remedy — sign the treaty, mount a couple of expeditions to Antarctica and then become full members. This does not, however, satisfy those who insist that they have no obligation to abide by rules in whose development they played no part.

The broader challenge to the treaty, from states such as Malaysia, based on the doctrine of common heritage and the argument that all members of the United Nations should participate in the administration of Antarctica, will be resisted by many of the consultative parties on more pragmatic grounds. Antarctica, they will insist, has been well managed since 1961, and is likely to be badly managed under a different regime.

On the question of management, the consultative parties are on sure ground. Since 1961, access to Antarctica by scientific expeditions has indeed been open. From the outset, oversight of exploration and of the treaty's requirement that the results of scientific investigations should be freely exchanged has been the responsibility of the Scientific Committee on Antarctic Research (SCAR), a committee established by the International Council of Scientific Unions which predates the Antarctic Treaty itself. This arrangement, whereby a group of governments has turned over to a non-governmental body such an important scientific advisory function, may well be unique.

SCAR has played an important part in the principal developments within the framework of the treaty since it first entered into force, culminating in the Canberra Convention for the Conservation of Antarctic Marine Resources, which was signed in 1978 and which entered into force in 1980. This had been preceded by two other measures concerned with the conservation of Antarctic flora and fauna (promulgated in 1964) and Antarctic seals, signed in 1972 and ratified six years later.

Like the Antarctic Treaty itself, these measures seek to reconcile conflicting interests by verbal ingenuity. Thus the convention on marine resources does not include the word "exploitation" but "rational use" is implicitly defined as a part of conservation. The result is that the convention on marine resources may be understood as a fisheries convention by those who wish to hunt for krill (chiefly Japan and the Soviet Union). Exploitation is not yet on such a scale as to call in question the restrictions that may in due course be recommended. Meanwhile, SCAR (and its companion committee SCOR, where "O" means oceans) is mounting a detailed investigation of the ecological balance between krill and marine animals in Antarctica.

The exploitation of mineral resources is bound to be more contentious because it touches directly on the issue of sovereignty. The need for an agreement of some kind has been a recurrent theme at consultative meetings since the 1970s. The meeting next week in Washington will be seeking to build on what has been agreed so far — that the need for an understanding is urgent not so much for economic reasons (for nobody suggests that mining anything would be practicable in the near future) but to defend the Antarctic Treaty regime against its critics and that the fragile Antarctic environment must at all costs be safeguarded. Paradoxically, even the non-claimant members of the treaty acknowledge that one of the best defences against the argument that Antarctica should be thrown open to allcomers is that some of them have claims to sovereignty.

The crucial question to be decided is the mechanism by which the exploration and exploitation of Antarctic minerals should be licensed. The consultative parties seem to have agreed that there must be an "external accommodation" in the sense that outsiders should be free to participate provided that they demonstrate practical activity, not merely academic interest, in the discovery of minerals and agree to abide by the rules.

The complementary "internal" accommodation, intended to reconcile the interests of claimants and non-claimants, is causing more trouble. The intention at present seems to be that there should be some central body with responsibility for mineral exploitation in Antarctica as a whole together with a number of sub-authorities The hope seems to be that claimant governments would continue to regard involvement with the management of Antarctic resources, perhaps with special responsibility in areas on which their claims have been lodged, as a sufficient recompense for not flying their national flags too ostentatiously.

Legally, there is an important precedent for such an arrangement — the treaty signed in the 1920s under which Spitsbergen (Svalbard), east of Greenland, was acknowledged to be the sovereign territory of Norway, which required that Spitsbergen be demilitarized and which provided for the exploitation of mineral resources (chiefly coal) by other parties. Poland and the Soviet Union pay a modest royalty on what they mine to cover the cost of administration.

Politically, were it not for the overlapping claims of Argentina, Chile and the United Kingdom, the transfer of such a system to Antarctica would be relatively straightforward. Non-claimants could join with the claimants in the detailed administration of sectors of Antarctica, thus satisfying the *amour propre* of all parties and postponing the issue of sovereignty. The snag is that the three overlapping claimants are in no mood to settle on a basis for joint administration of any part of Antarctica — Argentina and Chile cannot agree even about the disputed part of Tierra del Fuego, Argentina and Britain have the Falklands war behind them.

This is why the circumstances call for a new departure. The Antarctic Treaty powers are right to calculate that an external accommodation will not be possible while they cannot agree among themselves on how to regulate their affairs in Antarctica. Against the background of the past three decades of successful co-operation in Antarctica, and with the knowledge that however vast may be the resources hidden beneath the ice-covered continent, the economic value of Antarctic minerals will always be marginal, there is a need for a face-saving form of words.

The ideal would be that the claimants should abandon their claims, but that is too much to ask. A second best is that the claimants should agree that, for some specified period (say a century), their territories should be committed to the trusteeship of others — the twelve original signatories of the Antarctic Treaty for example. That, after all, is the reality of what has been happening since 1961. It would be infuriating if the rewards of two decades of constructive work should now be put in hazard because the claimants, none of which could hope to exercise its sovereignty, cannot swallow such a meagre mouthful of pride. □

From Santorini to Armageddon

North American tree-ring data confirm that volcanic eruptions can cause climatic change. So would nuclear war, but to a degree not yet calculable.

THE general concern with cataclysmic happenings on the surface of the Earth requires no special explanation. In a more or less orderly world, people are naturally disquieted by the notion that their lives may be disrupted (or even ended) by events over which they have no control. For if the collision of the Earth with an asteroid at the end of the Cretaceous may have extinguished the dinosaurs and many other kinds of species, may not less spectacular but more probable events abruptly change the course of history without warning, breaking the comfortable expectation of continuity with the future that we now enjoy?

That, no doubt, is part of the reason why there is such widespread alarm about the risk of nuclear war. The same applies to the more recent general preoccupation with the results of purported recalculations of the long-term consequences of a nuclear war, those for example represented by the summary of a calculation now published by Professor Carl Sagan and a group of associates (*Science* **222**, 1283; 1983). They assert that after a nuclear war, the Earth's atmosphere would be so laden with dust that solar insolation of the surface would be drastically reduced, thus also reducing surface temperatures and perhaps even reducing the rate of photosynthesis to below that required for survival.

The issue is not as academic as it might be made to seem by the remark "We'll all be too dead to care". That might be true of the populations of the principal combatants and their neighbours, but populations in the other hemisphere, while damaged by fall-out, would still have a vital interest in the feasibility of survival. So if there are long-term climatic consequences of nuclear war, it is important to know what they are. Those wishing to follow the argument might prudently begin not with the article by Sagan *et al.* but with that of Lamarche and Hirschboek from the University of Arizona in this issue of *Nature* (page 121).

That rich tale, fascinating in its own right, also has a bearing on the question raised by Sagan *et al.* What Lamarche and Hirschboek have done is to correlate with the known occurrences of major volcanoes the occurrences of frost damage rings in bristlecone pines, the long-lived species by means of which dendrochronology in eastern California has been extended back for nearly 5,500 years. The results are striking. Frost-rings in bristlecone pines do indeed correlate well in time with the occurrence of major volcanoes, among which Krakatoa (1883) is, so to speak, the type-specimen.

Within the past century, during which historical records approach in accuracy the tree-ring calendar, the data show that frost-rings follow eruptions more frequently than if the two kinds of events were independent. But during this period, there were summer cold snaps in the western United States not attributable to eruptions (1941 and 1886 for example) and, in the earlier period, many major eruptions that appear not to have caused frost damage.

That is what would be expected. The veil of dust from a volcanic eruption is only one of many causes of climatic variation, while eruptions differ markedly in character, some yielding only short-lived dust clouds.

Several features of the study now described are nevertheless especially pleasing. First, the notion that major eruptions can cause detectable climatic events is modestly confirmed. Second, the work further supports the view that one of the meteorological consequences of volcanic dust in the atmosphere is (in the Northern Hemisphere) an expansion of the circumpolar vortex in the mid-troposphere and a tendency for the pattern of the stratospheric jet-stream to consist of a more exaggerated sequence of lunges towards low latitudes. That should enhance the confidence of model-builders and support those who have been suggesting that the cold spring and autumn in the Rocky Mountains last year (1983) may indeed have been a consequence of the El Chicón eruption in 1982. Finally, a valuable bonus, there is an objective date (1626 BC) for the volcanic explosion of virtually all of the island of Santorini (then called Thera), one of the traumatic events of late Minoan history.

The bearing of this careful study on the question raised by Sagan *et al.* is only indirect. It does, however, qualitatively confirm one of the chief predictions of the consequences of nuclear war — that dust in the atmosphere can have long-term climatic consequencs. But many of the events suggested by the tree-ring study seem to have been exceedingly short-lived. Thus the frost-rings found in California, Utah and Nevada in 1884, the year after the eruption at Krakatoa, seem to correspond with a southerly incursion of cold air from Canada on 9 and 10 September (after a summer that had admittedly been unusually cool).

By contrast, Sagan *et al.* predict much lowered temperatures — on some pictures of the course of a nuclear war, perhaps 20 per cent below normal for 3–6 months afterwards. The chief reason for this huge discrepancy between the consequences of a nuclear war and a volcanic eruption stems from the assumptions made about the quantities of dust carried into the stratosphere by the different events. But everybody, Sagan *et al.* included, agrees that this field is shot through with uncertainty.

The assumptions made about nuclear dust appear, however, to be chiefly responsible for the differences between the new and earlier calculations, that of the 1975 report from the National Academy of Sciences for example. In what is called the "baseline scenario" of a nuclear war (10,000 nuclear explosions with a total yield of 5,000 megatonnes), it is supposed that 1,000 million tonnes of dust would be created, that 80 per cent of that would find its way into the stratosphere and that 8.6 per cent of that would consist of particles smaller than 1 micrometre radius. These assumptions would load the atmosphere with more fine dust than previous calculations have assumed. The new assumptions (and the use made of the one-dimensional model for predicting the attenuation of the obscuring effects of stratospheric dust) are, unfortunately, to be discussed only in a paper still "in preparation". Until that appears, those familiar with the well documented difficulties of measuring the composition and the size distribution of dust even from well studied volcanoes such as Mount St Helens (see for example Brazier, S. *et al. Nature* **301**, 115; 1983) will be wondering where the apparently much more precise data about nuclear dust has come from.

None of this implies that such calculations should wait on accurate parameters for all possible variables. It would probably also be common ground between the authors of the latest calculation and their more quizzical readers that the use of one-dimensional models is likely to yield upper limits for the effects calculated. What might, however, reasonably be asked of the authors is that their conclusions, derived as they are from necessarily crude models and uncertain data, should be plainly stamped with the label QUALITATIVE for fear that their apparent precision may prove spurious.

John Maddox

© 1984 Macmillan Journals Ltd

Genetic engineering

Prospects for transforming monocot crop plants

from Richard Flavell and Raymond Mathias

THE past year has at long last seen the successful genetic modification of plants by the insertion of DNA from the test tube — although as yet only tobacco and a few closely related species have been transformed with new active genes[1-4]. Success has come by exploiting the components of the natural DNA transfer system of the soil bacterium *Agrobacterium tumefaciens*, a pathogen of many dicotyledonous species (dicots). During the infection process, the bacterium transfers a part of one of its plasmids, the Ti plasmid, into the host plant chromosomes[5]. What the genetic engineers have done is to place new genes into an appropriate segment of this plasmid, using a combination of *in vitro* recombinant DNA techniques and *in vivo* bacterial genetics. When the bacterium carrying the engineered plasmid is used to infect plant cells, the plasmid segment carrying the new genes is transferred into the plant chromosomes.

The excitement that has rightly accompanied these successes has been tempered by the knowledge that the natural host range of *A. tumefaciens* is restricted only to dicots. So an efficient technique for DNA transfer cannot be used to transform monocotyledonous species (monocots). Yet the genetic transformation of monocots remains a most important goal in plant genetic engineering, for many of the world's major food crops are monocots — rice, barley, maize, wheat and sorghum, for example. The many claims that 'transformed' monocot plants have been obtained after the microinjection or uptake of DNA into immature or mature seeds[6-8] have been based on the observation of altered plant phenotypes, but have not been supported by the more definite proofs that can be gained using molecular biology. Much effort is now being directed towards giving such proof.

The successful transformation of tobacco involves the incorporation of DNA into single cells, the selection and culture of transformed cell lines and the regeneration of whole plants therefrom. Few plant species can, however, be taken from single cells through the tissue culture steps as readily as can tobacco and the important monocot crop species have proved particularly recalcitrant in this respect[9]. But while a reproducible single-cell system capable of efficient regeneration into whole plants may still be some way away for the important monocot species, recent developments suggest that excessive pessimism is unwarranted.

In maize, for example, certain cell culture lines produce protoplasts which readily regenerate to the callus stage[10,11], while other lines can be maintained as cell suspension cultures in which aggregates of 20–50 cells can be regenerated to callus and plants with high efficiency[12]. Protoplasts from embryogenic cell suspension cultures of pearl millet have been reported to undergo sustained divisions that give rise to calli, embryoids and whole plants[13]. Also protoplasts of barley, rice, sorghum and diploid wheat have been proliferated to the callus stage but without plant formation[14]. These developments do not necessarily imply that it will soon be possible to insert DNA into single monocot cells and to regenerate transformed plants efficiently from them, but it may be now worthwhile attempting the microinjection of DNA into the separate cells of a cell cluster capable of regenerating into a transformed plant.

As the regeneration of whole plants from single cells is likely to remain a severe problem for many monocots, other routes for plant transformation are now being explored. These approaches seek to avoid the problems of cell culture altogether by making the gametes the recipients of introduced DNA. The pollen of many species can be germinated *in vitro*[15] and the sperm nucleus can be made a target for the microinjection of DNA as it passes down the pollen tube. If the injected genes are maintained in the nucleus after transfer of the germinated pollen to an ovary, they may be incorporated into the zygote nucleus by the natural fertilization process, resulting in a transformed embryo and potentially a transformed plant. In those species in which the pollen remains viable for only a short period or in which pollen germination *in vitro* is not yet possible, microinjection directly into the egg cell is being explored as an alternative. The principal difficulty in this approach is that the egg is buried in the maternal ovary tissue and is difficult to locate but may be less serious in those few species whose fertilized ovules can be cultured and microinjected after excision.

Little is as yet known, in plants, of the influence of the structure of the potentially transforming DNA which, in principle, can influence the survival of the DNA, the efficiency with which it is integrated into a host replicon and whether it behaves as a replicon. It may be that any piece of DNA introduced into a nucleus has a high probability of being incorporated into a chromosome, as found in mammalian cells[16]. The dramatic progress in transforming *Drosophila* by the microinjection of P-elements into suitable recipient genotypes is a striking illustration of the value of using vectors with special properties[17-19]. Complete P-elements encode genes which facilitate their autonomous integration into the chromosomes of *Drosophila* lines not carrying specific repressors of P-element activity. Novel genes spliced into these elements disrupt the essential 'mobility' gene functions, but when such 'inactive' engineered elements are microinjected into *Drosophila* embryos along with unmodified P-elements, the 'trans' acting gene products of the unmodified elements facilitate the chromosomal integration of the engineered element with their new genes.

Transposable elements were first recognized in maize[20], and new examples have now been isolated from maize chromosomes by DNA cloning techniques[21-23] (see, for example, this issue, page 127). These elements are therefore for use in experiments analogous to those which have been so successful for the incorporation of genes into *Drosophila*. The segment of plasmid DNA that is transferred to dicotyledonous chromosomes by *Agrobacterium* is a type of transposable element. Many laboratories are therefore beginning experiments to see if its transposition can be induced in monocot cells when the Ti plasmid is introduced into cells by means other than natural infection by *Agrobacterium*.

The recognition of transformed cells, tissues or plants, is of critical importance in transformation studies, for successful events may occur only at low frequencies. Consideration must therefore be given to the availability of selectable marker genes for incorporation into vectors. Suitably engineered antibiotic resistance genes from prokaryotes have already been used successfully to select transformants from the *Agrobacterium* transformation system and these genes[1-4], with their proven efficiency, will no doubt be incorporated into monocot vectors. An interesting selection scheme has been developed for transforming cell lines lacking functional alcohol dehydrogenase (ADH) genes: the vector genes will carry a functional ADH gene[23], putative transformants will be cultured under anaerobic conditions where selection operates in favour of cells containing ADH[24] and the presence of functional ADH detected easily by a simple staining reaction[25].

Taken together, these developments have clearly demonstrated the feasibility of plant transformation in dicots. The progress in monocot cell culture and the expanding interest in the development of alternative methods of DNA delivery, coupled with the availability of Ti-plasmid, monocot transposable elements and selectable markers, suggest that the transformation of at least some monocot species cannot be far off. The need to combine the

Richard Flavell and Raymond Mathias are in the Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ.

0028-0836/84/020108-01$01.00

right techniques with the right recipient cells and a suitable DNA delivery system may still keep us waiting a little while longer. We must remember too, that for agriculture, the ability efficiently to insert genes into one monocot crop species is not enough. We must learn how to manipulate all the major crop species. □

1. Herrera-Estrella L. *et al EMBO J.* **2**, 987 (1983).
2. Herrera-Estrella, L., Depider, A., Van Montague, M. & Schell, J. *Nature* **303** 209 (1983).
3. Fraley, R.T. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4803 (1983).
4. Bevan, M., Flavell, R.B. & Chilton, M.D. *Nature* **304**, 184 (1983).
5. Bevan, M.W. & Chilton, M.D. *A. Rev. Genet.* **16**, 357 (1982).
6. Ledoux, L. & Huart, R. *J. molec. Biol.* **43**, 243 (1969).
7. Soyfer, V.N. *et al. Mutat. Res.* **36**, 303 (1976).
8. Korohoda, J. & Strzalka, K.Z. *Pflanzenphysiol.* **94**, 95 (1979).
9. Potrykus, I. *Advances in Protoplast Research* (eds Ferenxzy, L., Farkas, G.L. & Lazar, G.) (Akademiari Kiado, Budapest, 1980).
10. Potrykus, I., Harms, C.T. & Lorz, H. *Theor. appl. Genet.* **54**, 209 (1979).
11. Chourey, P.S., Zurawski, D.B. *Theor. appl. Genet.* **59**, 341 (1981).
12. Green, C.E., Armstrong, C.L. & Anderson, P.C. *Miami Winter Symp.* **20**, (1983).
13. Vasil, V. & Vasil, I.K. *Theor. appl. Genet.* **56**, 97 (1980).
14. Vasil, I.K. in *Plant Improvement and Somatic Cell Genetics* (eds Vasil, I.K., Scowcroft, W.R. & Frey, W.J.) 179–204 (Academic Press, 1982).
15. Raman, K., Walden, D.B. & Greyson, R.I. J. *Heredity* **71**, 311 (1980).
16. Wigler, M. *et al. Cell* **16**, 777 (1979).
17. Spradling, A.C. & Rubin, G.M. *Science* **218**, 341 (1982).
18. Rubin, G.M. & Spradling, A.C. *Science* **218**, 348 (1982).
19. Spradling, A.C. & Rubin, G.M. *Cell* **34**, 47 (1983).
20. McClinlock, B. *Cold Spring Harb. Symp. quant. Biol.* **16**, 13 (1951).
21. Federoff, N., Wessler, S. & Shure, M. *Cell* (in the press).
22. Döring, H.P., Tillmann, E. & Starlinger, P. *Nature* **307**, 127–130 (1983).
23. Peacock, W.J. *et al. Miami Winter Symp.* **20**, (1983).
24. Shimamoto, K. & King, P.J. *Molec. gen. Genet.* **191**, 271 (1983).
25. Freeling, M. *Genetics* **83**, 70 (1976).

Genetics

The origins of men with two X chromosomes

from Paul Burgoyne

APPROXIMATELY one in 20,000 men have a female (XX) sex-chromosome constitution. The occurrence of XX males is of great interest because it raises the question of whether testes can develop in the absence of the testis-determining locus located on the Y chromosome. Recent work has done much to support the view that XX males have a testis-determining fragment of the Y chromosome attached to one X chromosome, but a group of three related XX men do not fit this explanation. As is often the case, these exceptions are potentially the most informative.

In 1966 Ferguson-Smith[1] suggested that the paternally derived X of XX males may harbour a testis-determining fragment of the Y. He envisaged that the fragment might have been transferred to the X by 'accidental crossing-over' between the X and the Y in the father and referred to the event as an X–Y interchange. Originally the idea of X–Y interchange was founded on the observation that some XX men fail to inherit their father's allele for the X-linked red cell antigen Xg^a. The interchange model explains this by assuming that the *Xg* locus, which is located at the tip of the short arm of the X chromosome, is lost from the paternal X when it acquires part of the Y short arm containing the testis-determining locus.

In 1979 another dimension was added to the X–Y interchange model when Evans *et al.*[2] showed a difference in length between the short arms of the two X chromosomes of some but (as others have emphasized[3]) not all XX men. This was taken as evidence for the addition of Y-chromosomal material to the short arm of the paternal X, and in terms of the interchange model this implied that the accidental crossing-over could be unequal with more Y-chromosomal material being gained than X-chromosomal material lost. Polani[4] considers that the organization of the X and Y pairing segments may predispose them to unequal exchanges.

Two recent reports provide further genetic evidence of X–Y interchange. The first[5] indicates that the steroid sulphatase locus (*STS*), which, like *Xg*, is near the tip of the X short arm and escapes X-inactivation, can likewise be missing from the paternal X in some XX males. While most XX men have high levels of STS activity befitting their XX status, in the case described not only was STS activity low but one of the Xs failed to express STS when incorporated in a human–mouse somatic cell hybrid line. The second report[6], published in this issue of *Nature*, describes an XX male who failed to inherit his father's *Xg* allele but did inherit his father's Y-linked 12E7 ('*Yg*')[7] allele. This is the first genetic demonstration of both the loss and gain of material by the paternally derived X of an XX male.

In view of the weight of evidence for X–Y interchange in the aetiology of XX maleness, it was reassuring to hear a report by Bishop, Fellous and Weisenbach at a recent meeting in Oxford that by using Y-specific probes[8] they have identified Y-specific sequences in the DNA from all three XX males they have examined (and see this issue of *Nature*)[9].

The case for X–Y interchange thus seems to be proven, but is this the whole story? Albert de la Chapelle[3] has long argued for a heterogeneous aetiology for XX males, and when the extremely rare familial cases are considered there is certainly food for thought. Let us consider two pedigrees; that described by Kasdan and colleagues[10] where there is an autosomal dominant pattern of inheritance, and that of de la Chapelle[3] in which transmission through females formally rules out dominance. The autosomal dominant pedigree has a direct counterpart in the XX sex-reversal mutation *Sxr* in the mouse. Although inherited in autosomal fashion *Sxr* has turned out to be a fragment of the Y which is regularly transferred to the X during meiosis in carrier males[11,12]. This intriguing finding was discussed in an earlier article[13]. The important point in the present context is that an apparently autosomal pattern of inheritance can be produced if crossing-over is obligatory in the pairing segment of the X and Y[14]. Thus the Kasdan pedigree may, like *Sxr*, be explained by X–Y interchange.

The de la Chapelle pedigree cannot be dismissed so easily. First, the recessive inheritance in my view argues strongly against the involvement of the Y-borne testis-determining locus. More important, in one of the three XX males in this pedigree both X chromosomes appear to be maternal, since he does not express the paternal alleles for *Xg* (located at the tip of the X short arm) or *Xm* (located near the end of the X long arm). I believe this unlikely anomaly of X inheritance suggests that this XX male has received from his mother two X chromosomes bearing a recessive 'testis-determining' mutant. Wolf[15] has put forward a model for testis determination in which testis differentiation is prevented in XX individuals by an X-borne repressor locus. When a Y chromosome is present this repressor locus is in some way neutralized so allowing testes to develop. This model allows for the development of testes in XX individuals if both copies of the X-borne repressor locus are inoperative through mutation or deletion. Such an X-borne recessive effect is consistent with the de la Chapelle pedigree, provided that the X-borne repressor locus is inherited in a pseudoautosomal fashion (like *Sxr*). I have previously suggested pseudoautosomal inheritance of this repressor locus in order to explain recessive XX sex reversal in goats[14].

The XX males from these two pedigrees are a must for investigation using Y-specific probes. The XX males from the Kasdan pedigree may well reveal Y-chromosomal sequences, but I for one shall be very surprised if this is also true of de la Chapelle's XX males. □

Paul Burgoyne is at the MRC Mammalian Development Unit, 4 Stephenson Way, London NW1 2HE.

1. Ferguson-Smith, M.A. *Lancet* 27 August, 475 (1966).
2. Evans, H.J. *et al. Hum. Genet.* **49**, 11 (1979).
3. de la Chapelle, A. *Hum. Genet.* **58**, 105 (1981).
4. Polani, P.E. *Hum. Genet.* **60**, 207 (1982).
5. Wieacker, P. *et al. Cytogenet. Cell Genet.* **35**, 71 (1983).
6. de la Chapelle, A. *et al. Nature* **307**, 170–171 (1983).
7. Goodfellow, P.N. & Tippett, P. *Nature* **289**, 404 (1981).
8. Bishop, C.E. *et al. Nature* **303**, 831 (1983).
9. Guettaen, G. *et al. Nature* **307**, 172–173 (1983).
10. Kasdan, R. *et al. New Engl. J. Med.* **288**, 539 (1973).
11. Singh, L. & Jones, K. W. *Cell* **28**, 205 (1982).
12. Evans, E. P. Burtenshaw, M. & Cattanach, B.M. *Nature* **300**, 443 (1982).
13. Simpson, E. *Nature* **300**, 404 (1982).
14. Burgoyne, P.S. *Hum. Genet.* **61**, 85 (1982).
15. Wolf, U. in *Development and Function of Reproductive Organs*, 260 (Excerpta Medica, Amsterdam).

Applied mathematics

Scattering by fractal objects

from Eric Jakeman

"A FRACTAL is a mathematical set or object whose form is extremely irregular and/or fragmented at all scales." So runs Mandelbrot's definition of the term he coined in an essay first published[1] in French in 1975. He points out that classical mathematics has on the whole been concerned with Euclidean geometry based on the notion of smooth shapes and with the continuous evolving dynamics of Newton, whereas most natural phenomena, objects and patterns are abruptly changing, with structure on many scales. Thus the mathematical description of a whole range of familiar objects ranging from coastlines to trees presents great difficulties. In his essay, Mendelbrot shows how these can be resolved by the concepts of fractal geometry[2].

To reduce the totality of fragmented objects to a class requiring minimal parameterization, it is necessary to introduce the notion of 'scaling', which refers to the similarity in appearance of an object when it is subject to arbitrary magnification. Regular scaling fractals can be generated by following simple iterative construction procedures. The best known object of this kind is the Von-Koch 'snowflake' curve[3] obtained by repeatedly replacing the middle third of each element of the construct by two vertices of the appropriate equilateral triangle (Fig. 1). This leads to an object of finite area but infinite perimeter. Its bounding curve is evidently continuous but does not have a locally well-defined direction (slope or derivative).

Although a number of these strange but regular geometrical constructs are known, the vast majority of natural objects are fragmented in an irregular way and it is in the description of these randomly varying structures that the concepts of fractal geometry are proving most valuable. An early investigation of one such structure is attributed to L.F. Richardson, who found that the length of land frontiers and coastlines appears to increase as the resolution of the measurement improves[2]. Thus, if the length of the coastline of Britain is determined using a 100-km measuring stick, the answer will be smaller than that obtained using a 10-km stick, which will resolve and therefore have to negotiate the longer distance round smaller scale structures such as river estuaries and inlets.

This observation leads quite naturally to the principle parameter characterizing a scaling fractal curve, for it is found that if the length of the measuring stick is l then the apparent length of such a curve is proportional to l^{1-D}, where D is a number greater than unity, but not necessarily integral, which uniquely characterizes the scaling behaviour of the curve. The value of D is in fact a measure of the density with which the curve fills the space (surface, volume) in which it is embedded and can be interpreted as an anomalous or fractal dimension.

Although Mandelbrot's ideas have diffused rapidly, the most striking applications of his work remain the numerical simulations of random fractal objects such as islands and landscapes which, in spite of being entirely artificially contrived, acquire an uncannily familiar appearance when supplemented by suitable shadowing effects[2]. The ability to recognize the familiar in this context is dependent, of course, on information being carried to the eye by light scattered from the object and there is indeed increasing interest in the properties not only of light but of other frequencies of the electromagnetic spectrum as well as of sound waves which have been scattered by fractal objects.

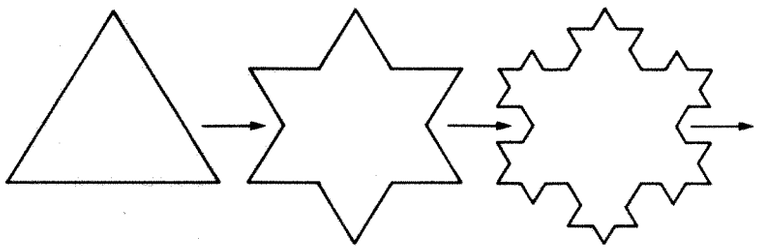
In fact there is a long history of interest in one multi-scale scattering system — the turbulent medium. Although not referred to as such, a fractal description involving the Kolmogorov spectrum has commonly been employed since the pioneering work of Tatarski in the early 1960s[4]. The full implications of using models of this type have only become clear recently, however, through the quantitative investigation of scattering by simple models of fractal surfaces[5]. Since the slope of a fractal surface is ill-defined, ray or geometrical optics do not apply and the scattering is of an entirely diffractive nature. This results in relatively low contrast intensity (brightness) patterns[6] that are very different from those generated by smoothly varying surfaces whose lens-like behaviour produces bright geometrical features of the kind which can often be seen on the floor of a swimming pool.

In the case of pulsed scattering, the fractal dimension of the surface is represented in the temporal decay of the tail of the return pulse, so that this kind of surface should in principle be easily recognizable and capable of characterization by remote sensing techniques[7]. In practice variations of reflectivity may confuse such measurements, while real surfaces will also have smallest (inner) and largest (outer) scale sizes with a hierarchical or fractal structure in between. To investigate the latter regime, the wavelength of the probing radiation has to be much greater than the height fluctuations over inner-scale sized elements of the surface (so that their scattering effect can be neglected by comparison with that of the larger scales) and the receiving optics must be designed to collect radiation from an area of the surface which is smaller than the outer scale size.

Although many solid surfaces of both microscopic and geophysical proportions appear to be fractal[8], at least over some limited range of scale sizes, there is evidence that certain fluid systems are better described as objects with fractal slope. The sea surface is one such system, for example. Ray or geometrical optics effects do play an important role in determining the characteristics of the scattered intensity pattern in this case, leading to strong variations in brightness. Since the local surface curvature remains ill-defined, however, lens-like behaviour does not occur and a new kind of geometrical optics or short-wave limit is found which is free from the catastrophes (caustics, focusing points) associated with smoothly varying surfaces. The statistical properties of the scattered intensity remain finite in this limit and can again be related to the fractal dimension[9].

The full implications of current work on fractal scattering cannot yet be properly assessed but will surely extend beyond the immediate practical objectives of the investigations. Certainly many new and sometimes surprising results are emerging by recognizing the role of fractals in well-established areas of science as well as in the more exotic frontier areas such as dynamical systems and chaos. □

Eric Jakeman is in the Royal Signals and Radar Establishment, St Andrews Road, Great Malvern, WR14 3PS.



The Von-Koch snowflake curve

1. Mandelbrot, B.B., *Les objects fractals: forme, hasard et dimension (Flammarion, Paris, 1975).*
2. Mandelbrot, B.B., *The fractal geometry of nature* (Freeman, San Francisco 1982).
3. Cundy, H.M. & Rollet, A.P., *Mathematical models* (Clarendon, Oxford 1954).
4. Tatarski, V.I., *Wave propagation in a turbulent medium* (McGraw-Hill, New York, 1961).
5. Berry, M.V., *J. Phys. A 12* 781 (1979).
6. Jordan D.L., Hollins, R.C. & Jakeman, E. *Appl. Phys. B31* 179 (1983).
7. Berry, M.V. & Blackwell, T.M., *J. Phys. A 14*, 3101 (1981).
8. Sayles, R.S. & Thomas, T.R. *Nature* 271 431 (1978).
9. Jakeman, E. *J. Opt. Soc. Am.* 72, 1034 (1982).

0028-0836/84/020110-01$01.00

© 1984 Macmillan

Galaxy structure

Two kinds of halo gas identified

from Joel Bregman

ALTHOUGH most of the cool gas in our galaxy lies in the galactic disk, it is not entirely confined there and the study of gas above and below the disk (halo gas) has been an especially vigorous area of research during the past few years. Unfortunately, it has frequently been difficult to discriminate between disk and halo gas, for it is not possible to determine distances to gas clouds and we can only look at the halo gas through the disk. In a recent paper C. E. Albert has used a new approach[1], a study of low ionization gas within 8,000 light years of the disk (the lower galactic halo). She has conclusively shown that a considerable amount of neutral (cool) gas exists in the lower galactic halo and that there are two kinematically distinct types of halo gas.

Gas is believed to exist in the halo in several different forms[2]. Weak radio continuum emission has been attributed to synchrotron emission from cosmic ray electrons that may extend up to 30,000 light years from the disk[3,4]. Plasma at temperatures of millions of degrees may also permeate the halo and be responsible for part of the diffuse X-ray background (0.1–2 keV)[5]. Virtually nothing is known about the vertical distribution of this gas except that it is unlikely to extend beyond 30,000 light years from the disk. Radio telescope surveys have revealed the presence of neutral hydrogen gas at a few hundred degrees kelvin but with unusual velocities[6,7]; this gas is presumed to lie in the halo as well. Finally, absorption line spectroscopy in the optical and ultraviolet bands has been used to demonstrate the presence of halo gas with a temperature in the range 10^3–10^5 K[8–10].

Optical and ultraviolet spectroscopy have been powerful tools for analysing the properties of halo gas. By detecting absorption lines caused by gas intervening between the Sun and a background source (a star or quasar), one is able to estimate the column density, vertical distribution, chemical abundance and temperature of the gas. Strong absorption is commonly detected for the ultraviolet resonance lines of C IV and Si IV, which is interpreted as evidence for warm gas (10^4–10^5 K) lying between 1,500 and 10,000 light years above the disk[9–11]. For neutral gas (< 10^3 K), a more precise study of the vertical distribution has been made by comparing the total column density (from the 21 cm HI emission line) with the column density between the Sun and a halo star (using the Ly α line of neutral hydrogen)[12].

There is a vast amount of gas above the thin disk of the galaxy (half-thickness about 300 light-years) and about one-third of the total column density of neutral gas is beyond a height of 3,000 light years. However, little neutral gas exists further than 6,000 light years from the disk. Albert has added to our understanding by comparing the difference in absorption for pairs of stars, one of which is just above the galactic disk and the other in the halo. She shows that the halo is rich in gas and distinguishes between two types of neutral halo gas: a layer of gas similar to disk gas that has a scale height of about 3,000 light years, and higher velocity gas richer in certain heavy elements (Ti, Ca, and Na) that is found only above the disk. Whether or not there is a true difference between the distribution of warm and neutral gas and how these components are related to the million degree gas and the cosmic rays remains to be seen.

The absorption-line gas is rotating, but not precisely with the galactic disk. This has been clearly shown by Albert and others[13] who demonstrated that the velocities at which absorption against halo stars is observed are considerably different from these expected for corotating gas. Evidently, these gas clouds have large peculiar velocities with respect to a corotating halo. The data are qualitatively consistent with features of a corona that rotates more slowly than the disk and of galactic fountain models. More data are needed before the various models can be tested critically and more studies along the lines of Albert's would be valuable. □

Joel Bregman is in the department of physics at New York University.

1. Albert, C.E. *Astrophys. J.* **272**, 509 (1983).
2. York, D. G., *Ann. Rev. Astr. Ap.* **20**, 221 (1982).
3. Webster, A. *Mon. Not. R. astr. Soc.* **185**, 507 (1978).
4. Higdon, J.C. *Astrophys. J.* **232**, 113 (1979).
5. Nousek, J. A., Fried, P. M., Sanders, W. T. Kraushaar, W.L. *Astrophys. J.* **258**, 83 (1982).
6. Giovanelli, R. *Astr. J.* **85**, 1155 (1980).
7. Schwarz, U. J. & Oort, J.H. *Astrophys.* **101**, 305 (1981).
8. Songaila, A. & York, D.G. *Astrophys. J.* **242**, 976 (1980).
9. Savage, B.D., & de Boer, K.S. *Astrophys. J.* **243**, 460 (1981).
10. Pettini, M. & West, K. A. *Astrophys. J.* **260**, 561 (1982).
11. York, D. G., Blades, J. C., Cowie, L. L., Morton, D.C., Songaila, A. & Wu, C.C. *Astrophys. J.* **255**, 467 (1982).
12. Hobbs, L.M., Morgan, W.W., Albert, C.E. & Lockman, F.J., *Astrophys. J.* **263**, 690 (1982).
13. deBoer, K.S. & Savage, B.D. *Astrophys. J.* **265**, 210 (1983).

Palaeontology

Small companions for early dinosaurs

from Michael Benton

FOSSILIZED cave systems around Bristol, England, have yielded an important fauna of small reptiles dating from the times of the early dinosaurs, about 200 million years ago. Until recently, these were thought to be specialized upland faunas because the animals were rather different from those of the typical lowland dinosaur beds. Strong evidence has now, however, been presented against this view, and new information on the dating and palaeoecology of these remarkable faunas has also been given[1–6].

Fossil mammal teeth were first found in the Bristol fissures by Charles Moore over 100 years ago[7], and the first reptile remains were described[8] in 1939. These consist of the bones of a small lizard-like animal (*Clevosaurus*), very like the unique living tuatara, *Sphenodon,* from New Zealand. Many more important mammal and reptile fossils have been collected since then from dozens of fossil caves in South Wales and around Bristol. The reptiles include a remarkable small gliding animal called *Kuehneosaurus,* isolated bones of the carnivorous crocodile-shaped *Rileya* and the dinosaur *Thecodontosaurus,* a crocodile, and various as yet undescribed sphenodontids and small archosaurs (crocodiles/dinosaurs/thecodontians). The latest new forms to be described have been *Gephyrosaurus*[5,6], a 40-cm long lizard-like animal, and *Planocephalosaurus,* a small sphenodontid[3].

The dating of the fissures has always been a problem. Some may be late Triassic (Norian) in age, others Rhaetic, and others early Jurassic (between about 190 and 225 million years ago). Some of the South Wales fissures have been dated as Rhaetic or early Jurassic on the basis of plants, spores and gastropods[9]. One of the fissures near Bristol has yielded more abundant spores which suggest a Rhaetic age[1]. The main fissure sites, and the reptiles that they have produced are described in references 1, 4, 9–11.

At the end of the Triassic period, the scenery around Bristol consisted of limestone hills in which deep fissures and caves formed. The climate was subtropical with wet and dry seasons, and the vertebrate fauna consisted of dinosaurs, small lizard-shaped reptiles and early mammals. Occasionally, these animals fell into the fissures, or were washed in by flash floods with mud and sand. The habitat was assumed to have been a rocky 'upland',

Michael Benton is in the University Museum, Parkes Road, Oxford, OX1 3PW.

© 1984 Macmillan Journals Ltd

well away from the lush pastured dinosaur country below. However, there is now strong evidence for marine influence in at least one fissure (Tytherington); marine microfossils (acritarchs and dinoflagellates) occur, kerogen derived from marine algae is abundant[1] and a glauconitic clay mineral has been found which could indicate brackish conditions[2]. This fissure at least must have been located near the coast with its base below sea-level. This would permit mixing of the meteoric waters that washed the debris into the cave with saline waters from the sea.

We must now view the fissure animals as representing a sample of a lowland fauna rather than a peculiar upland fauna. Very large animals are excluded — like the five-metre long dinosaur *Plateosaurus* which is found widely in Europe at the same time. However, many single bones of its smaller relative *Thecodontosaurus* have been found in various fissures. The most abundant animals, however, are lizard-sized, from 10–50 cm in total body length. A detailed study of the ecology of an assemblage of these small animals from Slickstones Quarry has just been published[4], and it gives us a unique impression of the fauna scuttling around beneath the shadow of the dinosaurs.

The Slickstones fauna consists of 12 or more forms, and includes abundant remains of six different sphenodontids. Most of these probably ate insects and other invertebrates, as does the living *Sphenodon*, but one form at least may have been herbivorous — a conclusion suggested by patterns of tooth wear. The authors go on to suggest that differences in the composition of the 'faunas' found in different fissures were caused by interspecific competition for food resources[4]. This seems to be stretching the evidence a little far, unless we assume that the beasts actually lived in the caves. The sphenodontids were preyed upon by dinosaurs and other archosaurs which are represented in the fissure fossils by a variety of jaws and isolated sharp knife-like teeth.

The Bristol fissures should produce a great deal more important information. Many of the fissure reptiles have yet to be described and the careful collecting techniques being used should also yield much new information on their palaeoecology. There is a rare satisfaction in finding out about some of the *small* reptiles that have now been shown to have lived during the Age of the Dinosaurs. □

1. Marshall, J.E.A. & Whiteside, D.I. *Nature* **287**, 627 (1980).
2. Whiteside, D.I. & Robinson, D. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **41**, 81 (1983).
3. Fraser, N.C. *Palaeontology* **25**, 709 (1982); (in the press).
4. Fraser, N.C. & Walkden, G.M. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **42**, 341 (1983).
5. Evans, S.E. *Zool. J. Linn. Soc.* **70**, 203 (1980).
6. Evans, S.E. *Zool. J. Linn. Soc.* **73**, 81 (1981).
7. Owen, R. *Monogr. Palaeontogr. Soc.* **24** (1871).
8. Swinton, W.E. *Ann. Mag. nat. Hist.* (11) 4, 591 (1939).
9. Kermack, K.A., Mussett, F. & Rigney, H.W. *Zool. J. Linn. Soc.* **53**, 87 (1973).
10. Robinson, P.L. *J. Linn. Soc. (Zool.)* **43**, 260 (1957).
11. Halstead, L.B. & Nicoll, P.G. *Stud. Speleol.* **2**, 93 (1971).

Archaeology

Stonehenge comes alive

From Julian Richards

THE antiquarians of the seventeenth and eighteenth centuries, the barrow diggers of the nineteenth, and the excavators, fieldworkers and astronomers of the twentieth have all been drawn to the Stonehenge area to observe, to dig and to speculate. To many it has long been apparent that the visible remains, barrows, henges and cursus monuments, while evidence of intensive and consistent activity during the Neolithic and Bronze Ages, provided only a partial picture of prehistoric society. Ideology and death left their enduring traces, but if we could see the dead, then where were the living?

"Stonehenge and Its Environs" (Edinburgh University Press) is the title of the survey carried out by the Royal Commission on Historic Monuments which has started to provide clues in the hunt for the living. Traces of now flattened prehistoric fields, small enclosures and boundary ditches point to an organization of the landscape beyond ritual and burial and provide an invaluable framework on which the next phase of investigation could be based. In 1980, the Trust for Wessex Archaeology began a programme of survey in the area of some 2,500 hectares studied by the Royal Commission. The overall brief of this Stonehenge Environs Project, commissioned by the Inspectorate of Ancient Monuments, was the location and evaluation of areas of prehistoric activity and the provision, by sample excavation, of firm indications of preservation for specific monuments.

With the exception of rare pockets of old grassland and woodland, and areas recently returned to grass by the National Trust, the arable landscape immediately dictated the most suitable field survey approach: surface collection 'field-walking'. The systematic collection of artefacts from the weathered surface of ploughed fields is becoming increasingly recognized as a major field survey technique. The use of flint and similar lithic material for tools throughout prehistory provides one enduring component of a total artefact assemblage that may also have included bone, metal and pottery.

The two-stage surface collection strategy used in this and other Trust for Wessex Archaeology projects aims initially to locate widespread patterns and anomalous areas. These are assessed in the light of localized topography and soil changes. Collection is initially by means of transects within hectare squares based on the national grid and provides an initial surface sample of approximately 8–10 per cent. The second stage of more detailed gridded collection tends to be reserved as an intensive pre-excavation sampling technique when it is carried out in conjunction with geophysical and geochemical surface surveys.

Over the past three winters more than 1,000 hectares have been examined and patterns of prehistoric activity have been recorded. Far from being a 'ritual landscape', devoid of inhabitants, the recent survey has shown areas of intensive domestic and industrial activity contemporary with the major ceremonial centres. Even within the relatively restricted study area, the patterns represented by the surviving monuments can be seen reflected in shifting zones of domestic activity culminating in the developed agricultural and territorial landscape of the later Bronze Age, *c.* 1000 BC.

Survey and sample excavation have thus provided a spatial, functional and chronological framework for prehistoric activity around Stonehenge, the only missing component being evidence for environmental change. The potential of upland chalk areas to provide environmental evidence is limited largely to land snails within protected contexts, particularly in buried soils and within ditch deposits. The palaeoenvironmental potential of the Stonehenge area, with its barrow mounds, ditches and other potentially dateable earth works, is thus extremely high and has been exploited in tandem with the main project. Research funding from the Society of Antiquaries, the British Academy and the Prehistoric Society has facilitated the investigation and analysis of a number of overlapping dateable environmental sequences.

The field aspects of the project are now almost complete, with only a short season of surface collection and recording of field monuments remaining. The project will then progress to a phase of full-time analytical work intended to produce both management strategies and a full synthetic publication of the projected results.

It is hoped to continue research in the area, particularly on the aspects of the prehistoric environment, but this will now depend on the availability of research funds other than from the UK Department of the Environment.

It is only by moving beyond empirical observations that we can hope to comprehend the societies in which Stonehenge was built. These must be understood if we are to preserve in a sensible manner their tangible, if ephemeral, remains. It is hoped that the Stonehenge Environs Project will not only have added to our understanding of the area, but also provided the means by which it can be both preserved and explained. □

Julian Richards is at the Trust for Wessex Archaeology, The Archaeological Centre, 65, The Close, Salisbury, SP1 2EN.

0028-0836/84/020112-01$01.00

Neurogenesis in *Drosophila*

Notch mapping function obscure

from Michael Akam

WHY does the *Notch* locus of *Drosophila melanogaster* attract so much attention, when the phenotype for which it is named is merely the generation of notches in the tips of the wings? The locus has for example now been cloned by two independent groups[1-3].

There are good reasons for this interest. Complete lack of function of the *Notch* locus results in a much more drastic developmental defect than notching of the wings — great increase in the proportion of ectodermal cells which become neuroblasts[4-7], consequentially an extensive hypertrophy of the nervous system at the expense of the epidermis and, eventually, the death of the embryo.

The notching phenotype is the dominant, but developmentally trivial, effect of heterozygosity for such a null mutation. Moreover, *Notch* is one of the best characterized of the genetically complex loci in *Drosophila*[5,8-12]. In addition to the dominant alleles, there are many recessive alleles which fail to complement *Notch* null mutations so that by conventional criteria they lie in the *Notch* gene. The phenotypes of these recessive mutations are varied. They include lethal mutations which have a range of lethal phases *(1(1)Notch)*, homozygous viable mutations which affect the morphology of the adult eye and bristles (*facet* and *split*) and *notchoid* mutations which, when homozygous, mimic the dominant phenotype of null alleles but have little or no effect on the nervous system. Mutations within any one of these classes generally fail to complement one another, but recessive mutations with different phenotypes often show total complementation. Thus by this test, each phenotypic class defines a different gene.

There are also *Abruptex* mutations. These have a dominant wing phenotype distinguishable from that of the null alleles; some are recessive lethals, some homozygous viable; in *trans* with other *Notch* complex mutations they may either enhance or suppress the *Notch* phenotypes.

Many of these mutations have been mapped by genetic recombination[8,9,11,12]. Recessive alleles and *Abruptex* mutations are interspersed with the null alleles, ruling out any possibility that the latter are deletions of several functional units. The genetic unit which they define is extremely large — 0.13 map units, or 19 times the genetic length of the xanthine dehydrogenase gene at the *rosy* locus[2].

The aims of the molecular studies at *Notch* are twofold: to understand the molecular basis of the genetic complexity and to identify the *Notch* products, and their role in neurogenesis.

The isolation and delimitation of the *Notch* complex was much facilitated by the numerous deletion and inversion mutations accumulated during decades of genetic analysis. Artavanis-Tsakonas and his colleagues noted that one deficiency[11] juxtaposed the *Notch* locus and a segment of the X chromosome which they had already cloned[1]. Thus it was one small step to hop into the complex. Both Artavanis-Tsakonas *et al.*[1,3] and Kidd *et al.*[2] report extensive chromosomal walks from this starting point. Both also map the location of chromosome breakpoints associated with *Notch* complex mutations, and regions of the complex homologous to transcripts. Kidd *et al.* report further the extensive analysis of the major *Notch* transcipt by two dimensional S1 mapping[2], and Artavanis-Tsakonas *et al.*[3] the isolation of cDNA clones derived from this major transcript.

Their results indicate that the *Notch* locus, like other complex loci in *Drosophila*[13], contains at least one long transcription unit with multiple introns, in this case spanning 35–40 kb. The genetic complexity reflects the complex organization of this transcription unit, but appears also to depend upon the particular characteristics of those mutations which result from the insertion of transposable elements.

There is a close correspondence between genetic and molecular maps. At *Notch*, one map unit corresponds to approximately 280kb of DNA, and *Notch* null mutations span 34kb. A spliced transcript longer than 10kb (ref. 1) and shorter than 11.5kb (ref. 2) is encoded throughout the region spanned by these *Notch* mutations. Kidd *et al.* have shown that it contains at least 8 introns ranging in size from < 1 to 20 kb, and find some heterogeneity at its 3′ ends[2]. One region at the 3′ end of this transcript contains a repetitive sequence which is homologous to other RNA species[3].

In several cases, mutations in the complex are correlated with the insertion of transposable elements into the locus. Four insertions which have given rise to *Notch* null mutations all lie in or very close to the exons of the major transcript[2]. In contrast, four recessive *facet* alleles and one recessive *Notch* lethal carry insertions within the major intron of this transcript[2,3]. Kidd and his colleagues speculate that stage or tissue specific transcription of the transposable elements in these mutations may result in the regulated inactivation of the *Notch* locus, and thus in the localized phenotype of the recessive mutations.

Shellenbarger and Mohler[10] suggested in 1975 that the diversity of the *Notch* complex recessive mutations results from the inactivation of a single *Notch* product at specific stages in development. This model, originally proposed to account for the interactions between temperature sensitive *Notch* mutations and the recessive alleles, accomodates remarkably well the molecular explanation now proposed.

By no means all *Notch* locus mutations result from insertions or gross rearrangements. The majority of both irradiation and chemically induced mutations were not detectably rearranged[1-3]. Most of these have however been mapped by recombination; using the correlation between genetic and molecular maps established for the insertion mutants, the majority of *Notch* null alleles can be assigned to the major exon at the 3′ end of the *Notch* transcription unit[2,3]. Thus they may be point mutations in a *Notch* protein.

From these data it seems that there is no necessity to postulate a multiplicity of products to explain the genetic complexity of the *Notch* locus. However, alternate splicing or processing pathways may yield a family of products from the major transcription unit, and that closely adjacent transcription units may be related to the function of *Notch*[1,3].

A very different molecular approach leads Thorig *et al.*[14,15] to suggest that the *Notch* complex is a multifunctional locus encoding a group of mitochondrial flavoprotein enzymes — NADH oxidase, NADH dehydrogenase, succinate dehydrogenase and glycerophosphate dehydrogenase. The activities of these enzymes in flies are dependent on the dosage of *Notch* genes, but the data indicating that *Notch* itself encodes these enzymes are not yet compelling. However, as Thorig *et al.* point out, the *rudimentary* locus of *Drosophila* exhibits a range of mutant phenotypes and complex complementation behaviour, and this locus is now known to be the structural gene for a polypeptide carrying three enzymes of pyrimidine biosynthesis[16].

In what sense then is *Notch* a gene controlling neurogenesis? Wright[5] suggested that the *Notch* product may be a repressor of neurogenesis — in the current parlance of *Drosophila* development, between epidermal and neurogenic pathways. Recent series papers make it clear that the situation is more complex. In a systematic search for mutations with embryonic phenotypes similar to that of *Notch*, Campos-Ortega and his colleagues[6,7,17,18] have identified six more 'neurogenic loci'. Lack of any one of these gene functions results in essentially the same developmental switch as that seen in *Notch* embryos — in extreme cases, all the cells in a large region of the ectoderm develop as neuroblasts, and no ventral cuticle is formed.

Michael Akam is an MRC Senior fellow in the Department of Genetics, Downing Street, Cambridge, CB2 3EH and a fellow of Kings College, Cambridge.

0028-0836/84/020113-01$01.00

© 1984 Macmillan Journals Ltd

If indeed *Notch* does encode mitochondrial enzymes, then one factor affecting the determination of neuroblasts in insects may prove to be a critical balance in some metabolic state of the ectodermal cells. Most of the seven 'neurogenic loci' might then be genes whose products affect this balance. It is perhaps relevant that efforts to find the 'real' neural inductor in amphibians led to the conclusion that the ectoderm is finely balanced between epidermal and neural differentiation, and that many non-physiological stimuli could provide the trigger for neural induction[19].

If however, the products of the 'neurogenic genes' do encode such housekeeping products as flavoprotein enzymes, why is it that so many tissues of the *Drosophila* embryo differentiate apparently normally in their absence?

Whether or not the *Notch* gene makes an 'interesting' product, it is interesting to study the effects of mutation on this complex transcription unit. And lurking among the set of neurogenic loci that Campos-Ortega has identified, there may yet be a selector gene which monitors the state or position of each ectodermal cell, and mediates its developmental commitment. It remains to be seen whether the genetic and molecular analysis of these genes will elucidate their role in neurogenesis. □

1. Artavanis-Tsakonas, S., Muscavitch, M.A.T. & Yedvobnick, B. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1977 (1983).
2. Kidd, S., Lockett, T.J. & Young, M.W. *Cell* **34**, 421 (1983).
3. Artavanis-Tsakonas, S. *et al. Developmental Genetics* (in the press).
4. Poulson, D.F. *Am. Nat.* **79**, 340 (1945).
5. Wright, T.R.F. *Adv. Genet.* **15**, 261 (1970).
6. Jimenez, F. & Campos-Ortega, J.A. *Wilhelm Roux Arch.* **191**, 191 (1982).
7. Lehmann, R., Jimenez, F., Dietrich, U. & Campos-Ortega, J.A. *Whilhelm Roux Arch.* **192**, 62 (1983).
8. Welshons, W.J. *Science* **150**, 1122 (1965).
9. Welshons, W.J. *Genetics* **76**, 775 (1974).
10. Schellenbarger, D.L. & Mohler, J.D. *Genetics* **81**, 143 (1978).
11. Keppy, D.O. & Welshons, W.J. *Chromosoma* **76**, 191 (1980).
12. Foster, G.G. *Genetics* **81**, 99 (1975).
13. North, G. *Nature* **303**, 134 (1983).
14. Thorig, G.E.W., Heinstra, P.W.H. & Scharloo, W. *Mol. Gen. Genet.* **182**, 31 (1981).
15. Thorig, G.E.W., Heinstra, P.W.H. & Scharloo, W. *Genetics* **99**, 65 (1981).
16. Segraves, W.A., Louis, C., Schedl, P., & Jarry, B.P. *Mol. Gen. Genet* **189**, 34 (1983).
17. Lehman, R., Dietrich, U., Jimenez, F. & Campos-Ortega, J.A. *Wilhelm Roux Arch.* **190**, 226 (1981).
18. Campos-Ortega, J.A. *Wilhelm Roux Arch.* **192**, 317 (1983).
19. Slacke, J.M.W. *From egg to embryo* Ch.3. (Cambridge University Press).

Geochemistry

Chemical inhomogeneity of mantle above 670 km transition

from Don L. Anderson

WHAT chemical composition the mantle has and on what scale mantle convection takes place are related questions which require data from both seismology and petrology to answer. The mantle, which represents about 68 per cent of the Earth's mass, is usually considered, especially by petrologists, to be mostly homogeneous in composition. Discontinuities within it are generally assumed to represent isochemical phase changes in an olivine-rich material rather than chemical boundaries. Thus, the 400 km and 670 km boundaries detected by seismic means are taken to represent the 'olivine-spinel' and 'spinel-post-spinel' phase changes, respectively. The 670 km discontinuity is the more significant, for seismic velocities increase greatly at this depth. This boundary must be very sharp as it is both a good reflector of short-period seismic waves and is the lower boundary of earthquake activity — no reliably-located earthquake has ever been found below it. These observations can be used to argue that the '670' is a chemical interface that provides a barrier to convection.

A.C. Lees, M.S.T. Bukowinski and R. Jeanloz (*J. geophys. Res.* **88**, 8145; 1983) have recently computed the reflection coefficient for a variety of plausible phase changes and conclude that mineralogical and seismic data are compatible with a two-layered mantle that has a discontinuous change in both phase and composition at 670 km, and that the change in material properties must occur over a region less than 3 km thick. Phase changes are typically spread out over a much greater depth interval and give reflection coefficients more than an order of magnitude less than those observed. This conclusion is not new but it serves to keep up the pressure on those who advocate that the mantle is chemically uniform and is involved in convection as a whole. Although the nature of the 670 km discontinuity is important, Lees and colleagues' results may produce a yet more significant conclusion.

They calculate the velocity in the transition region for olivine, for orthopyroxene and olivine and for garnet mineral assemblages, including the effects of phase changes. The velocities in all these minerals and assemblages are much greater than the seismic velocities in the transition region and there is no similarity in shape or depth between the mineralogical models and the 400 and 670 km mantle discontinuities. Taken at face value, these results would seem to rule out peridotite, pyrolite or any olivine-orthopyroxene rich assemblage for the 400–670 km depth interval and the olivine-spinel-post-spinel explanations for mantle discontinuities. Although the temperature and temperature gradient can be adjusted to give a fair match of olivine-rich aggregate densities with the seismically-determined density, the much more accurately determined velocities are poorly matched. The velocity and density gradients of the transition region are greater than calculated for the peridotite minerals. A spread-out phase change or a compositional gradient is implied.

Lees and colleagues do not pursue these discrepancies in the region above 650 km. They restrict themselves to conventional mineralogies involving $(Mg,Fe)_2SiO_4$, $(Mg,Fe)SiO_3$ and $(Mg,Fe)_3Al_2Si_3O_{12}$. They assume that the MgO, FeO and SiO_2 contents of mantle silicates control the physical properties and the locations of phase boundaries, but, as usual, the effects of CaO, Al_2O_3 and Na_2O are ignored. These are minor components of peridotite and pyrolite minerals, the presumed dominant minerals of the mantle. Eclogite, an alternative mineralogy for the transition region, is dominantly clinopyroxene (diopside and jadeite) and garnet, transforming gradually to garnetite ($CaO–Al_2O_3–Na_2O$-rich garnet solid solution) over the pressure interval of the transition region. Unfortunately, velocities in an eclogite transition region, including phase changes, are not treated, in spite of the obvious discrepancies with the peridotite minerals.

The idea of a peridotite mantle dominated by olivine is deeply entrenched in geology. Olivine and ortho-pyroxene are dominant minerals in xenoliths brought to the surface in alkali basalts and kimberlites and in upthrusted segments of the mantle exposed in fold belts and oceanic fracture zones. The most abundant rocks emerging from the mantle, however, are basalts. At high pressure, these rocks are composed mainly of garnet and clinopyroxene — they are eclogites. At one time it was thought that the upper mantle and the source region of basalts were primarily eclogite. More recently, it has been assumed that the basalt-eclogite fraction of the mantle is dispersed and is liberated by 20–30 per cent partial melting, leaving behind a refractory olivine-orthopyroxene-rich residue. The fact that olivine-orthopyroxene aggregates cannot explain the 400 and 670 km discontinuities, and the velocity and velocity gradients in the transition region, will force a reexamination of the basalt source region, the chemical uniformity of the mantle, and the composition of this region of the mantle.

It is also important to look at clinopyroxene-garnet-rich assemblages. Lees *et al's* inability to match the compressional velocity is particularly significant because theirs is not an *ab initio* calculation. They used the seismic data themselves to constrain the velocity-bulk modulus ratio. Those who would argue for a chemically uniform mantle and an olivine-rich transition region are now faced with a formidable array of contrary evidence. □

Don L. Anderson is in the Seismological Laboratory, California Institute of Technology, Pasadena, California 91125.

0028-0836/84/020114-01$01.00

© 1984 Macmillan Journals Ltd

Living plant fossils

SIR — The article "Seeds of thought for plant conservationists" by P.D. Moore[1] is of great interest but it should be added that seeds are not the only plant reproductive parts that remain viable over a considerable period of time in datable contexts. The same is true of fungal mycelia and spores[2], bacteria[3,4], apparently not viruses[5] but probably algae, which can survive as cysts.

Moore[2] isolated 61 microfungi from two peat profiles collected in the Dublin-Wicklow mountains, Ireland, using Warcup's soil plate method. More viable remains occurred at the surface but the number of viable taxa (6 or 7) was constant with depth. Unfortunately the peats have not been dated by radiocarbon methods.

Bacteria of the thermophilic actinomycete genus *Thermoactinomyces* are relatively easily isolated and identified[5]. Their resistant endospores survive for longer than 1,500 years in anaerobic lake muds[3] and have been cultured using occupation debris from the Roman fort site of Vindolanda, Northumberland[4]. The concentration of colonies grown varied with sediment type. The microbiology of grain stored in Iron Age-type pits has also been studied[7] as part of an experimental project on agricultural practices.

Conservation should not be confined solely to higher plants and the medical value of *Penicillium* is a good indication why. Soils that are totally anaerobic or clay-rich (holding water tightly in the clay-humus bond) may serve as storehouses of viable remains of lower plants in addition to seed banks, as Moore suggests.

Definition of extinction in lower plants may be even more difficult than for angiosperms, given the possible longevity of spores.

The palaeoecological importance of these findings requires stressing. While little research on fossil Holocene microfungal remains has so far been carried out in Europe, interest may grow if it can be demonstrated that data which add to interpretations of vegetation history based on pollen analysis may thereby be acquired. Microfungi are impossible to identify taxonomically using mycelial remains preserved in peats, and accurate determination using the various spore types is very difficult[8], but if viable remains occur the prospects are much better.

The various spore types of *Ceratocystis ulmi* (Dutch elm disease) are extremely small[9] and are probably not identifiable by light microscopy. Finds of fossil elm bark beetles would provide no proof that they were carrying the disease. However, if *C. ulmi* spores remained viable for 5,000–5,200 years, the theory that the elm decline in the British Isles was due wholly or partly to this disease could be properly tested. It has already been shown[6] that *Thermomycetes vulgaris* (actinomyces) spores are plentiful in samples from Seamere, Norfolk, which have cereal and associated weed pollen and *Thermomycetes spp.* occur today in association with cereal cultivation and storage.

Finally, it is of note that Seaward *et al.*[4] found viable endospores of *T. vulgaris* and *T. dichotomica* at Vindolanda differing from those regarded as typical of currently recognized species. So resuscitation can add potentially to genetic diversity.

B.K. MALONEY
Department of Geography and Palaeoecology Centre, Queen's University, Belfast BT7 1NN, Northern Ireland

1. Moore, P.D. *Nature* **303**, 572 (1983).
2. Moore, J.J. *Scient. Proc., R.D.S.*, **26**, 379–415 (1954).
3. Cross, T., Attwell, R.W., in *Spore Research 1973* (eds Barker, A.N., Gould, G.W. & Wolf, J) (Academic, London, 1974).
4. Seaward, M.R.D., Cross, T. & Unsworth, B.A. *Nature*, **261**, 407–408 (1976).
5. Shackley, M. *Environmental Archaeology* (Allen and Unwin, London, 1981).
6. Unsworth, B.A., Cross, T., Seawald, M.R.D. & Sims, R.E. *J. appl. Bact.*, **42**, 45–52 (1977).
7. Lacey, J. *Stored Prod. Res.* **8**, 151–154 (1972).
8. Van Geel, B. *thesis*, Univ. Amsterdam (1976).
9. McNeel, D.J., Kulkarni R.K. & Nickerson K.W. *Can. J. Bot.* **61**, 1349–1352 (1983).

Plants from culture

SIR — Recently there has been an increasing amount of research on the inheritance of characters in the progeny of tissue culture-derived plants. Genetic analyses of this type are likely to become even more common as the techniques for generating (somaclonal) genetic variants and transformants via tissue culture become more widely applied to crop species. In discussing results of this type of research, it has come to our attention that there is a potential source of confusion in the nomenclature used.

It is common practice to self-pollinate plants regenerated from culture and to analyse the resulting progeny for the inheritance of novel traits. The progeny of such selfed regenerated plants have been referred to variously as the F_1, F_2, R_1, R_2, RF_1, RF_2, SC_2, S_1 and G_1 generation[1–9]. It is therefore apparent that a consistent nomenclature is needed to clarify the nature of the material being described.

Ideally any system adopted should be logical, easy to use and be compatible with established plant breeding nomenclature. We believe that these criteria can be met by strict adherence to the use of letters to designate the process, followed by numbers to show how many times the process has been performed. Thus the original regenerated plants are to be referred to as the R_1 generation. It follows that successive cycles of cell culture and plant regeneration, without a sexual cycle, would produce R_2, R_3, etc. generations. If the regenerated plants from the R_1 generation are selfed, their progeny become the R_1S_1 generation. Repeated selfing to eventually produce homozygous inbreds would progress through the R_1S_2, R_1S_3, R_1S_4, etc. generations. Back-crossing to non-cultured material would similarly be described as R_1BC_1, R_1BC_2, R_1BC_3, and so on. By using nomenclature that is compatible with current plant breeding conventions, the same system can easily be extended to more complex breeding programmes. For example, using maize inbreds, (Mo17 × B73 R_1S_1) BC_2 would represent the second backcross generation derived from the cross of Mo17 by an S_1 progeny of a plant regenerated from a B73 culture. Although such complex situations would not be met by most researchers, we feel that any system adopted should be able to cope with all eventualities.

N.P. EVERETT
S.M. FLASHMAN
Stauffer Chemical Company, 1200 S 47th Street, Richmond, California 94804, USA

1. Wullems, G.J., Molendijk, L., Ooms, G. & Schilperoort, R.A. *Cell* **24**, 719-727 (1981).
2. Bourgin, J.-P. *Molec. gen. Genet.* **161**, 225-230 (1978)
3. Hibberd, K.A. & Green, C.E. *Proc. natn. Acad. Sci. U.S.A.* **79**, 559-563 (1982).
4. Lörtz, H. & Scowcroft, W.R. *Theor. appl. Genet.* **66**, 67-75 (1983).
5. Edallo, S., Zucchinali, C., Perenzin, M. & Salami, F. *Maydica* **26**, 39-56 (1981).
6. Gengenbach, B.G,, Connelly, J.A., Pring, D.R. & Conde, M.F. *Theor. appl. Genet.* **59**, 161-167 (1981).
7. Otten, L. *et al. Molec. gen. Genet.* **183**, 209-213 (1981).
8. Straub, J. & Schell, J. *Molec. gen. Genet.* **183**, 209-213 (1981).
9. Beckert, M., Pollacsek, M. & Caenen, M. *Agronomie* **3**, 9-18 (1983).

Species named

SIR —It is surely of fundamental importance in research work in the areas of basic biology and genetics that the organisms being studied should be precisely identified. I find it extraordinary, therefore, that in the interesting article by Bernards *et al.* on the growth of chromosome ends in trypanosomes (*Nature* **303**, 592; 1983), that neither the genus nor the species of the trypanosomes studied was given.

DAVID WALLIKER
Institute of Animal Genetics, University of Edinburgh, Edinburgh EH9 3JN, UK

BERNARDS AND BORST REPLY — Our experiments were done with *Trypanosoma brucei brucei*, strain 427. We expect, however, that the growth and contraction of chromosome ends will be a property of chromosomes in all African trypanosome species, and possibly in all organisms. We apologize that this expectation has inadvertently crept into our paper and we hope that other readers have found the missing information in our earlier papers, amply quoted in the *Nature* article.

A. BERNARDS
P. BORST
Antoni van Leeuwenhoekhuis, Het Nederlands Kankerinstituut, 1066 CX Amsterdam, The Netherlands

What is the solution?

SIR — The centenary of Sydney Ringer's important work[1] on the ionic requirements for maintenance of viability of living tissues *in vitro* has, astonishingly, been unremarked; it was not even mentioned at the recent 29th International Congress of the International Union of Physiological Sciences in Sydney, Australia. In the same field we are approaching the 75th anniversary of Tyrode's contribution[2] and have just passed the 50th anniversary of the advance made by Krebs and Henseleit[3]. The use of Ringer's, Tyrode's and Krebs' solutions in biomedical research must be among the most common techniques of science, perhaps even rivalling Student's *t*-test. I estimate that Krebs' solution alone features in more than 10,000 scientific papers a year.

These solutions, and their better nourished relatives, the tissue culture media, represent a veritable scientific Ganges. They have become holy waters, with all of the associated mysticism, dogma, priesthoods and devout acolytes. Nostrums from the ancient scriptures, frequently mistranslated to include idiosyncratic errors, are being perpetuated unquestioningly through generations of disciples. There have even been attempts at minor deification with the eponyms "Ringer" and "Krebs" being applied, respectively, to almost any such solution and to bicarbonate/CO_2 buffered solutions in particular. It has been forgotten that the tissue sustaining properties of normal saline were known before Sydney Ringer and that bicarbonate/CO_2 buffered salines were in use before Krebs and Henseleit.

But none of these solutions was designed to simulate the natural bathing fluid of tissue cells, the *milieu intérieur* of Claude Bernard. All were based on what was known of blood plasma composition and were adjusted empirically to produce a desired response. This has resulted in the use of solutions which maintain grossly abnormal *p*H, electrical potential and osmotic equilibrium in the isolated tissues they support. Because such phenomena as cellular excitability, drug–receptor interaction and enzyme activity may be profoundly affected, why do these solutions continue to be used?

Certainly, there are occasional glimmers of awareness, as a few authors each year notice some of the unphysiological characteristics of Krebs', Tyrode's and Ringer's solutions. These continual rediscoveries have resulted in a multiplicity of slightly "modified" solutions. The fact that each of these is capable of maintaining tissues in a reasonably functional state should not be surprising. Living cells have to cope with the fluctuations produced by water intoxication or dehydration, high or low salt intake, labile *p*H and such ionic imbalances as result from muscle exercise during marathon running, for example. Nor should it be surprising that some cellular functions are best demonstrated in solutions with defined, even highly abnormal, compositions. But, if our objective is to gain insight into *in vivo* function, we should expect normal cellular function only in cells coping with environmental conditions close to normal.

Is there a fundamentalist conspiracy promoting a scotomatical attitude among biomedical scientists so that even those who are in the forefront of the giga-ohm-laser-dye-coupled-computerized-tomographic-recombinant-DNA style of cellular research continue to use string and sealing wax technology for their "physiological bathing solutions"?

I espouse heresy. In 1969, I devised a solution based quantitatively upon the ionic composition of a representative mammalian interstitial fluid[4]. This I termed synthetic interstitial fluid (SIF) and it has been used with great success in many laboratories, notably in experiments on isolated human and other mammalian muscles. SIF may not be the ultimate in physiologically balanced salt solutions but it certainly surpasses those now being used.

A. H. BRETAG

School of Pharmacy,
South Australian Institute of Technology,
North Terrace,
Adelaide, South Australia 5000

1. Ringer, S.L., *J. Physiol., Lond.* **4**, 222–225 (1883).
2. Tyrode, M.V. *Archs int. Pharmacodyn. Ther.* **20**, 205–223 (1910).
3. Krebs, H.A. & Henseleit, K. *Hoppe-Seyler's Z. physiol. Chem.* **210**, 33–66 (1932).
4. Bretag, A.H. *Life Sci.* **8**, 319–329 (1969).

ITP nomenclature

SIR — The interesting recent letter *Nature* (3 November, 1983 p.67–69) and News and Views article (3 November, p.16–17) on the possible second messenger function of inositol 1,4,5-trisphosphate may presage a surge of interest in the function and metabolism of inositol phosphates. It would therefore be helpful immediately to establish acceptable and unambiguous abbreviations of these compounds. Streb *et al.* did to a large degree achieve this aim; for example, they used Ins 1,4,5P_3 to indicate inositol 1,4,5-trisphosphate. However, they unfortunately chose cyclic IMP as their abbreviation for inositol 1,2-cyclic phosphate: their own logic would demand the abbreviation Ins1,2-cyclicP, and cyclic IMP would normally be taken to mean inosine 3, 5-cyclic phosphate.

Hesketh's News and Views item was less careful: the inositol lipids were referred to by the outdated PI and PI-4,4-bisphosphate terminology, rather than as PtdIns and PtdIns4,5P_2, and Ins1,4,5P_3 appeared as ITP, which is internationally accepted as an abbreviation for inosine triphosphate. Although the abbreviations in which inositol appears as Ins are more cumbersome, they are unambiguous, and this is a great virtue in a field into which many new workers are moving.

Another flaw in the published material was the incorrect citation of our recent paper (ref. 5 of the News and Views item): the correct citation is *Biochem. J.* **212**, 733–747; 1983).

R. H. MICHELL

Department of Biochemistry,
University of Birmingham,
PO Box 363, Birmingham B15 2TT, UK

1. Rubenstein, E., Bonner, W.A., Noyes, H.P. & Brown, G.S. *Nature* **306**, 118 (1983).
2. Landstreet, J.D. & Angel, J.R.P. *Nature* **230**, 103 (1971).
3. Martin, P.G., Illing, R. & Angel, J.R.P. *Mon. Not. R. astr. Soc.* **159**, 191–201 (1972).
4. Korchak, A.A. & Syrovatskii, S.I. *Astr. Zh.* **38**, 885–897 (1961); *Soviet Astr. -AJ* **5**, 678–686 (1962).
5. Legg, M.P.C. & Westfold, K.C. *Astrophys. J.* **154**, 499–514 (1968).
6. Epstein, R.I. *Astrophys. J.* **183**, 593–610 (1973).
7. Chanmugam, G. & Dulk, G.A. *Astrophys. J.* **244**, 569–578 (1981).

Familial cancers

SIR — Several reports have recently appeared showing that familial retinoblastoma is due to mutations in each of the two copies of an autosomal gene. In a review of this work in *Nature*[1] your reviewer, like most people writing about the genetics of retinoblastoma, implies that Alfred Knudson was the originator of the idea that certain familial cancers such as retinoblastoma could be due to the combination of an inherited mutation and a somatic mutation. Actually this hypothesis was proposed by Robert de Mars in a symposium organized by the University of Texas M.D. Anderson Hospital[2], two years before Knudson's first paper on the subject.

Until then, familial retinoblastoma had been classified as due to an autosomal dominant mutation, but de Mars made the following point: ". . . I suggest that many of the pedigrees that are labelled as autosomal dominants . . . could actually be interpreted as autosomal recessives. We must relate the terms dominant and recessive not only to the level of the individual as a whole . . . but also to the level of the individual cell, or cells, which are involved. I think many pedigrees are consistent with the notion that one of the parents in these families might be heterozygous for a recessive and that the neoplasms appear as a result of subsequent somatic mutations in which individual cells become homozygous for a recessive neoplasm-causing gene."

JOHN CAIRNS

Department of Cancer Biology,
Harvard School of Public Health,
665 Huntingdon Avenue,
Boston, Massachusetts 02115, USA

1. Gilbert, F. *Nature* **305**, 761–762 (1983).
2. de Mars, R. in *23rd an. Symp. Fundamental Cancer Research, 1969*, 105–106 (Williams and Wilkins, Baltimore, Maryland, 1970).

Aspherical heterogeneity of the mantle from phase velocities of mantle waves

Ichiro Nakanishi & Don L. Anderson

Seismological Laboratory, California Institute of Technology, Pasadena, California 91125, USA

Long-period surface waves are used to map the lateral heterogeneity of the upper 100–600 km of the mantle. There is good correlation of velocity with surface tectonics and heat flow. Convergence regions are generally slow for Love waves and fast for Rayleigh waves. Back arc basins have slower than average shallow mantle. Some island arcs show evidence of fast material at greater depth. Deep-seated slow anomalies underlie the Red Sea–Afar region of north-east Africa, western North American–northern East Pacific Rise, Indian Ocean triple junction and the Tasman Sea–Campbell Plateau regions. The fastest regions are in the north central Australia–New Guinea and the South Atlantic.

SURFACE waves or, equivalently, short-period free oscillations are sensitive to the seismic velocities and density of the upper mantle and provide a powerful method for mapping lateral heterogeneity of this part of the Earth.

We have measured surface wave phase velocities over about 300 paths by applying a single-station method[1] to long-period fundamental-mode Love (G) and Rayleigh (R) waves from 13 large earthquakes. We assume that surface waves propagate along the great circle path connecting an earthquake and a station and that they can be expressed as travelling waves generated from a double-couple point source. In this study we measured apparent phase velocities of G_2, G_3, R_2 and R_3, and thus have information for both long and short arcs.

We expand the measured velocities in spherical harmonics to avoid an *a priori* association with surface tectonics. A motivation of this study and our previous study[2] is that the commonly used great circle velocity measurement method[3], or, equivalently, the free oscillation method[4] gives only the even-order terms of a spherical harmonic description of the Earth's lateral heterogeneity[5,6]. This information, of course, is not sufficient to describe adequately the heterogeneity. The single-station observations provide the missing information, although the measurement accuracy is inferior to that of the great circle observations. To suppress the instability due to the poorly determined odd harmonics we apply a filtering procedure to the inverted spherical harmonic coefficients[7,8]. Finally, we correlate the resulting phase velocity distribution with surface tectonics, heat flow and the geoid. A detailed description of our single-station velocity measurements, filtering procedure and interpretation, will be presented elsewhere (ref. 7 and C. H. Nataf, I.N. and D.L.A., in preparation).

Single-station velocity measurement

We processed long-period fundamental-mode surface waves generated from 13 large shallow earthquakes recorded on long-period digital networks (IDA and GDSN). The transversely rotated horizontal and vertical component seismographs are used for analysing Love and Rayleigh waves, respectively. The number of individual paths (short arc plus long arc) is larger than about 300 for both waves. The great circle paths for Rayleigh waves are shown in Fig. 1*b*. Coverage is less complete for Love waves.

For the single-station method we have to incorporate information about the source process. In this study we correct the source effect by using source mechanisms and source process times obtained by Nakanishi and Kanamori[9]. The directional part of the source finiteness is ignored. Epicentral locations and origin times are from the National Earthquake Information Service.

To calculate the distance along the great circle path we assume a geometric flattening $fg = 1/297.001$ and a mean radius $R_0 =$ 6371.211 km. We do not correct for the frequency dependent ellipticity[10–12]. This correction does not affect the conclusions of the present article[8].

Geographical variation

We expand the phase slowness $1/C(\Omega, \omega)$ in terms of spherical harmonics:

$$1/C(\Omega, \omega) = \sum_{l=0}^{\infty} \sum_{m=-l}^{m=l} s_{lm} Y_{lm}(\Omega) \quad (1)$$

where $\Omega = (\theta, \phi)$, ω is the angular frequency, and $Y_{lm}(\Omega)$ are the fully-normalized spherical harmonics. We truncate equation (1) at $l = l_{max}$ and write the observed phase delay along the propagation path

$$t_{ES} = \sum_{l=0}^{l_{max}} \sum_{m=-l}^{m=l} S_{lm} \int_E^S Y_{lm}(\Omega)\, ds \quad (2)$$

If the number of observations is much larger than the number of coefficients s_{lm}, and the surface wave paths are well distributed over the Earth's surface, we can determine the coefficients s_{lm} by solving equation (2) in a least-squares sense and can synthesize $C(\Omega, \omega)$ by equation (1).

We processed G_2, G_3, R_2 and R_3. G_2 and R_2 travel the long arc between source and receiver. G_3 and R_3 travel in the opposite direction and travel the short arc and one complete additional orbit. To calculate the path integral of Y_{lm} in equation (2) for these waves, we used Backus' solution[5] of the great circle integral of spherical harmonics and numerical evaluation of the integral for G_1 and R_1.

We solved equation (2) and determined s_{lm}. Since $l_{max} = 8$ inversion does not appreciably improve the fit over the $l_{max} = 6$ inversion[7], we discuss the solutions for $l_{max} = 6$. We could synthesize $C(\Omega, \omega)$ by straightforward application of the sum formula (1). The coefficients of $l =$ odd, however, have larger uncertainties than those of $l =$ even harmonics. To suppress the contribution of the poorly constrained odd harmonics we apply a filter based on the method of Whaler and Gubbins[13].

Figure 2 shows the filtered velocity contour maps for Love waves at for 100, 153, 200 and 250 s. The overall pattern of the Love wave phase velocity variations shows a general correlation with surface tectonics. These waves are most sensitive to the S_H velocity in the upper 200 km (153 s) to 300 km (250 s) of the mantle.

The lowest velocity regions are located in the southeastern Pacific, western North America, north-east Africa centred on the Afar, the central Atlantic, the central Indian Ocean, and the marginal seas in the western Pacific. A high-velocity region is located in the north central Pacific, centred near the Hawaiian swell. Love wave phase velocities are low along portions of the

© 1984 Macmillan Journals Ltd

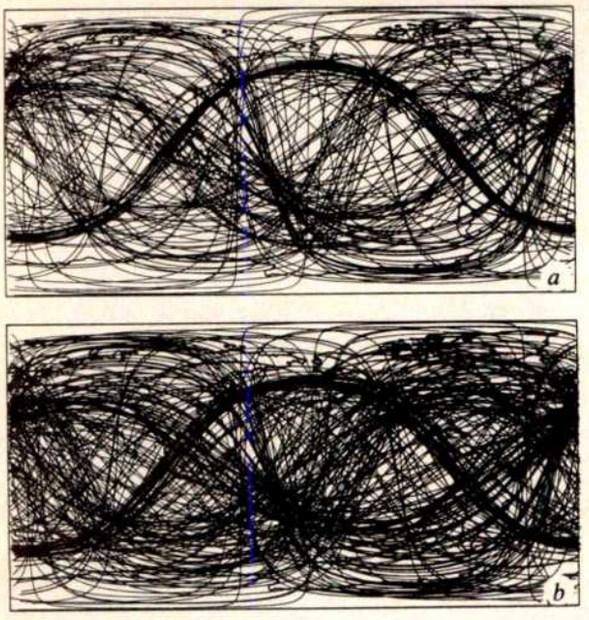


Fig. 1 Great circle paths for Love wave (*a*), Raleigh wave (*b*) observations. The same map projection as Figs. 2, 3, 4 and 6.

Mid-Atlantic Ridge, especially near the triple junctions in the north and south Atlantic.

High-velocity regions in continents generally coincide with Precambrian shields and Phanerozoic platforms (northwestern Eurasia, western and southern parts of Africa, eastern parts of North and South America, and Antarctica). Tectonically active regions, such as the Middle East centred on the Red Sea, eastern and southern Eurasia, eastern Australia and western North America are slow as are island arcs, such as the southern Alaskan margin, the Aleutian, Kurile, Japanese, Izu–Bonin, Mariana, Ryukyu, Philippine, Fiji, Tonga, Kermadec and New Zealand arcs.

Rayleigh wave phase velocities are presented in Fig. 3. Rayleigh wave phase velocity also correlates well with surface tectonics, particularly at short periods. The periods considered in this study are most sensitive to S_V velocity between about 100 and 400 km (153 s) to 600 km (250 s) and thus sample deeper than the Love waves.

The slowest regions, for Rayleigh waves, are centred on the Red Sea–Afar, Kerguelen–Indian Ocean triple junction, western North America centred on the Gulf of California, the north-east Atlantic, and Tasman Sea–New Zealand–Campbell Plateau. The fastest regions are the western Pacific, New Guinea–western Australia–eastern Indian Ocean, west Africa, northern Europe and the Southern Atlantic.

One difference between Love waves and Rayleigh waves is evident in the island arcs along the northwestern margins of the Pacific Ocean, such as the Aleutian, Kurite, Japanese, Izu–Bonin–Mariana, Ryuku and Philippine regions. These regions are characterized by the high velocity of the Rayleigh waves. An exception is the Aleutian region for a period of 250 s. In the contour map for 250 s (Fig. 3*d*) a high-velocity region occurs near the Izu–Bonin–Mariana trenches. The difference between Love and Rayleigh wave results may be explained by the difference in penetration depth. The Love wave results indicate that the shallow mantle, in the vicinity of island arcs and back-arc basins, is slow. Rayleigh waves sample the fast material which has subducted beneath the island arcs. Because of the large wavelengths, the lateral extent of the fast material must be considerable. Anisotropy may also be involved. In regions of convergence and divergence the mantle flow can be expected to be generally vertical. In the centres of convection cells, the flow is mainly horizontal. The sense of anisotropy will change

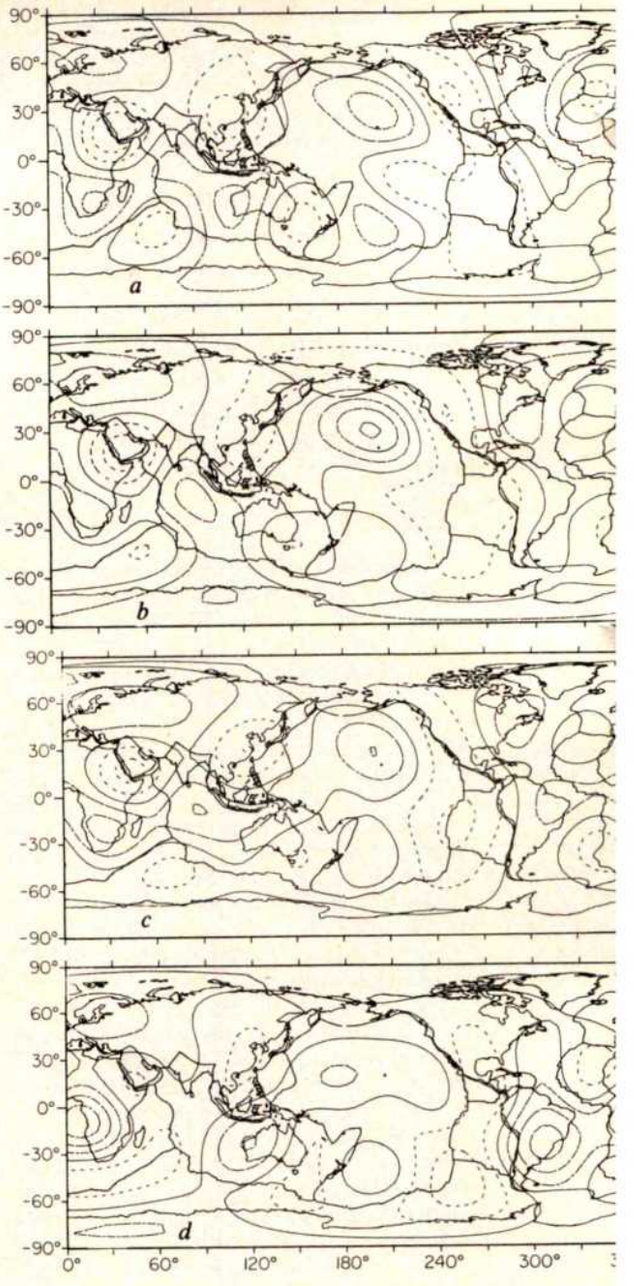


Fig. 2 Love wave phase velocity distributions synthesized by usi
$l = 1$–6 terms. The solid, chain and broken lines indicate spheri
average, higher than average and lower than average velocity. T
contour interval is 0.05 km s^{-1}. *a*, 100 s; *b*, 153 s; *c*, 200 s; *d*, 250

at ridges and subduction zones. This point will be analyse
detail elsewhere.

For Rayleigh waves the Atlantic Ocean is generally
velocity except for the northern part. The low velocities as
ated with the mid-oceanic ridge system, which appear in
Love wave maps, are not evident in the long-period Ray
wave maps. This may indicate that many ridge segments
passive or that $S_V > S_H$ in regions of ascending flow.

Heat flow and geoid

Heat flow and geoid maps have been presented in term
spherical harmonics and comparison with our surface
results is straightforward. For this purpose we use the heat
distribution of Chapman and Pollack[14] and the GEM 8 ge
corrected for the hydrostatic figure of the Earth[16]. Chap
and Pollack used observed heat flow supplemented by a tec

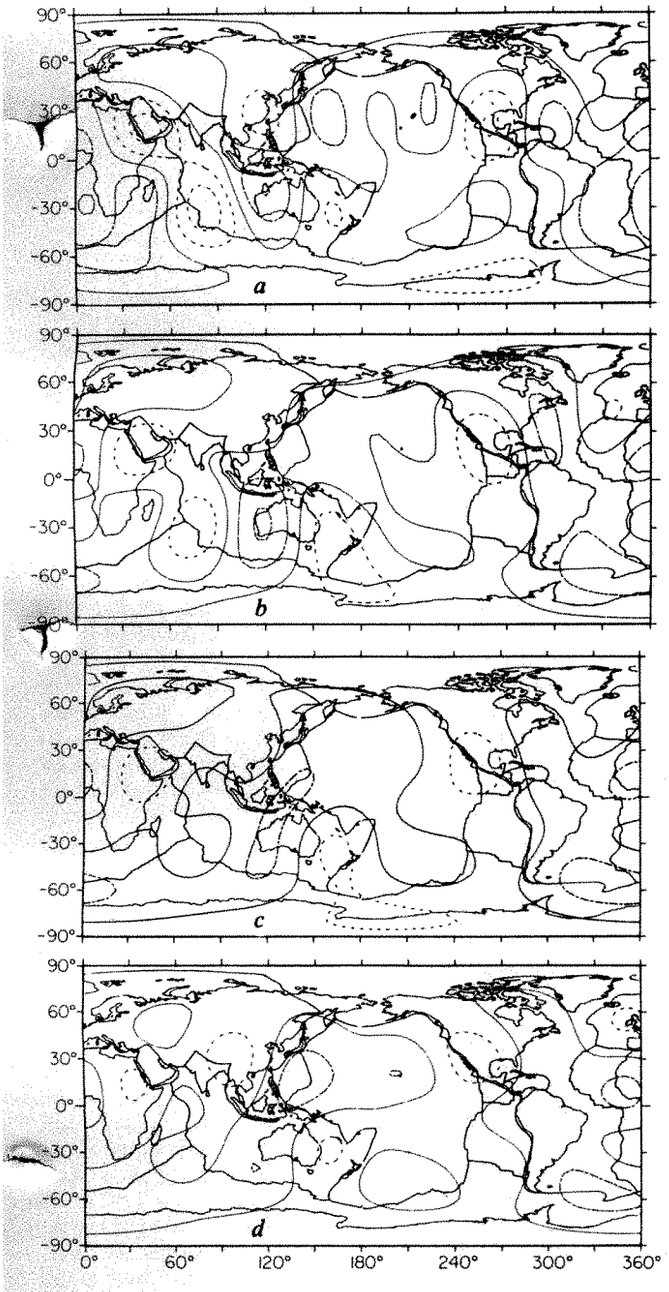


Fig. 3 Rayleigh wave phase velocity distributions synthesized by using $l = 1$–6 terms. The symbol convections are the same as in Fig. 2. *a*, 100 *s*; *b*, 153 s; *c*, 200 s; *d*, 250 s.

predictor. Figure 4*a* and *b* show the heat flow and geoid maps truncated at $l = 6$.

Figures 2–4 show that the phase velocities, especially Love waves, are well correlated with heat flow and, therefore, surface tectonics. The geoid has a weaker correlation with the surface wave velocities.

We compute the degree correlation coefficients γl for $l = 1$ to 6 by

$$\gamma_l = \frac{\sum_{m=-l}^{m=l} S_{lm} t_{lm}}{\left(\sum_{m=-l}^{m=l} S_{lm}^2\right)^{1/2} \left(\sum_{m=-l}^{m=l} t_{lm}^2\right)^{1/2}} \quad (3)$$

where S_{lm} and t_{lm} are coefficients for surface waves and for heat flow or geoid. The results are shown in Fig. 5. The heat flow

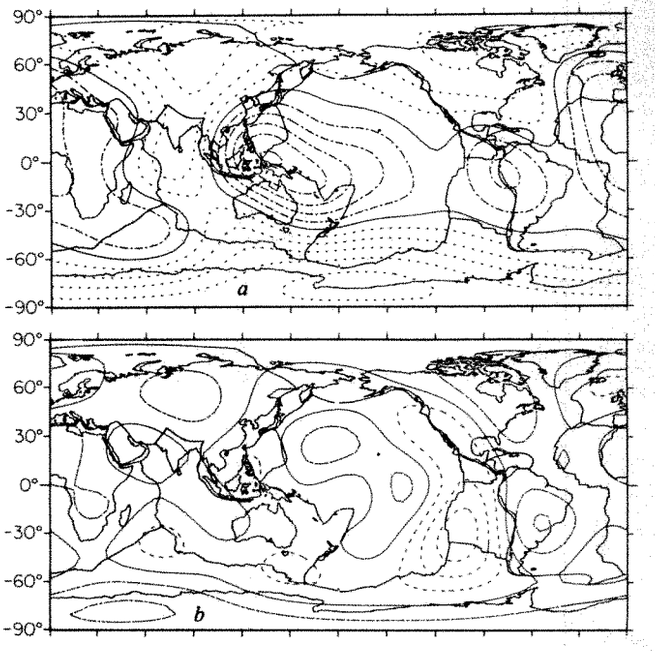


Fig. 4 Contour maps of heat flow and the geoid. *a*, Non-hydrostatic geoid (GEM 8) of Wagner *et al.*[15] and Nakiboglu[16]. The contour interval is 20 m. $l = 2$–6. The solid, chain and dashed lines indicate the spherical average, higher and lower geoid. *b*, Heat flow of Chapman and Pollack[14]. The contour interval is 10 mW m^{-2}. $l = 1$–6. The solid, chain and dashed lines represent the spherical average, lower and higher heat flow, respectively.

has positive correlation with phase slowness for degrees from 1 to 6. The geoid shows negative correlation at $l = 2$ (Love and Rayleigh) and 3 (Rayleigh), and positive correlation at $l = 4$ to 6.

The correlation of heat flow with Love wave velocities can also be seen by comparing Figs 2 and 4. Because the heat flow map is based on tectonics, as well as heat flow data, this comparison indicates that upper mantle velocities correlate with surface tectonics. Shields are low heat flow areas and exhibit high Love wave velocities, in spite of the thick low-velocity crust. From our Love wave map we would predict that south-east Asia and the Afar region are characterized by more extensive heat flow highs than predicted by Fig. 4*b*. These regions have few heat flow observations. The Canadian Shield and Siberian Platform anomalies are less evident in the Love wave maps than in the heat flow map.

The correlation between surface wave velocities and the geoid is weak. Geoid highs are associated with both subduction and hotspots. Ridges are not evident in either the long wavelength geoid or Rayleigh wave maps. The $l = 2$ correlation indicates that the long wavelength part of the geoid has a negative correlation with slowness, that is $l = 2$ geoid highs correlate with $l = 2$ velocity highs. For shorter wavelength features the reverse is true.

Discussion

Love waves are sensitive to the S_H velocity of the shallow mantle, above about 300 km for the periods considered here. The slowest regions are at plate boundaries, particularly triple junctions. Slow velocities extend around the Pacific plate and include the East Pacific Rise, western North America, Alaska–Aleutian arcs, south-east Asia and the Pacific–Antarctic Rise. Parts of the Mid-Atlantic Rise and Indian Ocean Rise are also slow. The Red Sea–Gulf of Aden–East African Rift (Afar triple junction) is one of the slowest regions. The upper mantle velocity anomaly in this slowly spreading region is as pronounced as under the rapidly spreading East Pacific Rise. Because it also shows up

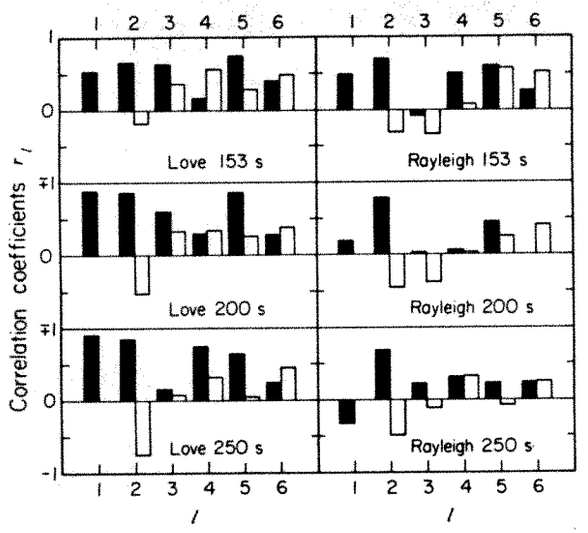


Fig. 5 Degree correlations of surface wave slowness with heat flow and geoid. Solid bars are correlations with heat flow. Open bars are correlations with geoid.

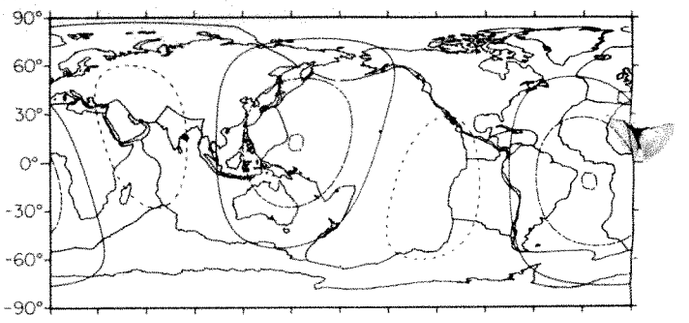


Fig. 6 $l=2$ map of 250-s Rayleigh wave phase velocity. The symbols are the same as in Fig. 2. Rayleigh waves of this period are most sensitive to S_V velocities between depths of 150 and 600 km.

for long-period Rayleigh waves, this is a substantial and deep-seated anomaly. Shields are generally fast, particularly the Brazilian, Australian and South African shields. The fastest oceanic regions are the north-central Pacific, centred near Hawaii, and the eastern Indian Ocean.

Rayleigh waves are sensitive to shallow P-velocities and, for the periods considered here, S_V velocity from about 100 to 600 km. The fastest regions are the western Pacific, western Africa and the South Atlantic. Western North America, the Red Sea area, south-east Asia and the north Atlantic are the slowest regions.

The Precambrian shields are generally faster than average. Silver and Joran[17] distinguish between shields and stable platforms. Okal's regionalization[18], a more suitable model for great circle Rayleigh wave phase velocities[18,19], combines these two tectonic regions and has a separate region for island arcs. If we adopt Okal's model, we obtain slower than average Rayleigh wave phase velocity at long period for the stable continental areas[8,18,19]. In Fig. 3*c* the velocities in the South Atlantic and the Philippine Sea plate are faster than shields.

Regions of convergence, or descending mantle flow, are relatively fast for long-period Rayleigh waves, particularly near New Guinea, Sumatra, western and northwestern Pacific. These regions have large positive geoid anomalies. Dense, cold material in the uppermantle can explain these results. To explain the surface wave results the fast material must be below the depth sampled by 250-s Love waves and 150-s Rayleigh waves, which is ~300 km depth, and must occupy a considerable volume of the upper mantle. Conductive cooling of the oceanic lithosphere probably extends no deeper than ~150 km. It is doubtful that the subduction of such a thin high-velocity plate could explain the present results and those of our previous regionalization study[8]. Either slabs preferentially subduct in cold regions of the mantle or they pile up in the upper mantle. At shorter periods the velocity of the mantle beneath subduction zones is average or slower than average, presumably reflecting the extensional tectonics and hot shallow mantle under back arc basins. In the tectonic regionalization study of Nakanishi and Anderson[8], it was also shown that convergent regions are faster than shields at long periods.

It has been proposed that the $l=2$ term is the dominant component of the Earth's asphericity and that it is caused by heterogeneity in the transition region, 420–670 km (ref. 4). Furthermore, it was suggested that the $l=2$ transition zone model was superior to tectonic regionalization. The tectonic model chosen[17], however, ignores convergence regions. A scheme which includes these regions[18] gives results which are similar to spherical harmonic inversions[8,20] and show, in addition, that odd harmonics are important. This study shows that there are large lateral variations in the shallow mantle. These plus variations in crustal thickness and water depth are not accounted for in the transition zone model[4] which attributes *all* of the observed variation to inhomogeneity of the 420–670 km depth range of the mantle.

A map synthesized from the $l=2$ coefficients at 250 s (ref. 8) is given in Fig. 6. This is characterized by a broad closed high-velocity region centred in the western Pacific, north-east of New Guinea, and a slow velocity region centred on the East Pacific Rise. Because of symmetry the antipodal regions are identical. This map is similar to that presented by Masters *et al.*[4] but our interpretation is quite different. Because the pattern is similar at short periods[8,20] it is the shallow mantle as well as the transition region that controls the pattern. In particular, the East Pacific Rise and the Red Sea areas control the location and shape of the lows in the $l=2$ pattern and the fast velocities in the western Pacific–Australia determine the location of the velocity highs. The $l=2$ coefficients are just part of the complete set that is required to describe fully the Earth's asphericity and need no special explanation. The $l=2$ pattern of the associated density field does have special significance because it is involved in the Chandler wobble, tidal friction, the orientation of the Earth's spin axis and polar wander, but again there is no reason to suppose that the $l=2$ pattern requires a special explanation. The tectonic model of Okal[18], for example, based on surface tectonics, has a strong $l=2$ component. The strong $l=2$ component comes from the ocean–continent configuration, the locations of island arcs, and the thermal structure of the sea floor. In particular the velocity highs in Fig. 6 encompass most of the world's old oceanic lithosphere and convergence regions and the lows include most of the young oceanic lithosphere and many of the world's hotspots. The correlation between surface wave velocity and heat flow (that is tectonics) and between velocity and geoid is relatively high for $l=2$, particularly at long periods (Fig. 5). If the rotation axis of the Earth is controlled by density anomalies in the upper mantle, and if density correlates with velocity, then the pattern in Fig. 6 would be symmetric about the Equator.

The most important results of the present study are: (1) discovery of low-velocity anomalies centred on south-east Asia, Afar and the Gulf of California and the demonstration that the latter two persist to long periods, indicating a deep upper mantle origin; (2) the association of high velocities, at long periods, with the western Pacific subduction zones, indicating a large volume of fast material in the upper mantle and; (3) the high velocities in the South Atlantic which do not have an obvious explanation in terms of present plate configurations. The South Atlantic may be underlain by ancient subducted Pacific Ocean lithosphere.

We thank Henri-Claude Nataf, Hiroo Kanamori, and Bradford Hager for suggestions. Jeffrey Given, Fumiko Tajima, and

Jeanne Sauber helped us retrieve the seismograms from the GDSN day tapes at an early stage of this study. The IDA data used in this study were made available by courtesy of the IDA project team at the Institute of Geophysics and Planetary Physics, University of California, San Diego. This research was supported by NASA grant NSG-7610 and NSF grant EAR811-5236. Contribution no. 3912, Division of Geological and Planetary Sciences, California Institute of Technology.

Received 28 June; accepted 14 September 1983.

1. Brune, J. N., Nafe, J. E. & Oliver, J. E. *J. geophys. Res.* **65**, 247–257 (1960).
2. Nakanishi, I. & Anderson, D. L. *Bull. seism. Soc. Am.* **72**, 1185–1194 (1982).
3. Sato, Y., *Bull. seism. Soc. Am.* **48**, 231–251 (1958).
4. Masters, G., Jordan, T. H., Silver, P. G. & Gilbert, F. *Nature* **298**, 609–613 (1982).
5. Backus, G., *Bull. seism. Soc. Am.* **54**, 571–610 (1964).
6. Zharkov, B. N. & Lyubimov, V. M. *Izv. Phys. Solid Earth* No. 10, 613–618 (1970).
7. Nakanishi, I. & Anderson, D. L. *Geophys. J. R. astr. Soc.* (submitted).
8. Nakanishi, I. & Anderson, D. L., *J. geophys. Res.* (in the press).
9. Nakanishi, I. & Kanamori, H. *Bull. seism. Soc. Am.* (submitted).
10. Dahlen, F. A. *J. geophys. Res.* **80**, 4895–4903 (1975).
11. Dahlen, F. A., *J. geophys. Res.* **81**, 4951–4956 (1976).
12. Dziewonski, A. M. & Sailor, R. V. *J. geophys. Res.* **81**, 4947–4950 (1976).
13. Whaler, K. A. & Gubbins, D. *Geophys. J. R. astr. Soc.* **65**, 645–693 (1981).
14. Chapman, D. S. & Pollack, H. N. *Earth planet. Sci. Lett.* **28**, 23–32 (1975).
15. Wagner, C. A., Lerch, F. J., Brownd, J. E. & Richardson, J. A. *J. geophys. Res.* **82**, 901–914 (1977).
16. Nakiboglu, S. M. *Phys. Earth planet. Inter.* **28**, 302–311 (1982).
17. Silver P. G. & Jordan, T. H. *Geophys. J. R. astr. Soc.* **64**, 605–634 (1981).
18. Okal, E. A. *Geophys. J. R. astr. Soc.* **49**, 357–370 (1977).
19. Souriau, A. & Souriau, M. *Geophys. J. R. astr. Soc.* **73**, 533–551 (1983).
20. Kawakatsu, H. *Geophys. Res. Lett.* **10**, 186–189 (1983).

Frost rings in trees as records of major volcanic eruptions

Valmore C. LaMarche Jr* & Katherine K. Hirschboeck†

* Laboratory of Tree-Ring Research and † Department of Geosciences, University of Arizona, Tucson, Arizona 85721, USA

New data about climatically-effective volcanic eruptions during the past several thousand years may be contained in frost-damage zones in the annual rings of trees. There is good agreement in the timing of frost events and recent eruptions, and the damage can be plausibly linked to climatic effects of stratospheric aerosol veils on hemispheric and global scales. The cataclysmic proto-historic eruption of Santorini (Thera), in the Aegean, is tentatively dated to 1628–26 BC from frost-ring evidence.

VERY occasionally, a major explosive eruption injects large amounts of ash and sulphur aerosols into the stratosphere and upper troposphere. In addition to causing rare atmospheric optical effects—a bluish tint to the Sun and Moon, a milky cast in the daylight sky, and lurid sunsets[1,2]—such events can have pronounced effects on weather and climate that may persist for several years. Current interest[3,4] in the climatic effects of volcanic dust and aerosols has been heightened by a series of eruptions of the Mexican volcano El Chichón in 1982 and the eruption of Mt St Helens. A new dimension has been added by the recent development of proxy records of past eruptions from measurements of acidity, chemical impurities and microparticle content of ice cores from the polar regions[5–7]. We propose here that the occurrence of frost-damage zones in accurately dated tree-ring sequences from subalpine bristlecone pines in the western USA is closely linked to climatological effects of major volcanogenic atmospheric veils, and that these frost rings represent new, independent proxy records of climatically effective eruptions during the past several thousand years.

Frost rings and meteorological events

Frost damage to the wood of mature trees is a rare phenomenon caused by temperatures well below freezing at some time during the growing season, when secondary wall thickening and lignification of immature xylem cells in the annual ring is not yet complete. Freezing promotes extracellular ice formation and dehydration which result in crushing of the outermost zone of weaker cells, leaving a permanent, anatomically distinctive record in the wood[8]. Two successive nights with temperatures reaching −5 °C and an intervening day at about the freezing level are sufficient to cause frost damage[9]. Two types of frost rings can be distinguished: earlywood frost damage in the inner part of the ring, which occurs near the beginning of the growing season and latewood frost damage, which typically involves only the dark zone of small, thick-walled cells formed near the end of the growing season (Fig. 1). If the period of cambial activity is known, observing the position of the damage in a ring often permits dating of the event to within a week or two. If daily meteorological data are available, it is usually possible to identify the specific date on which the damage took place and thus, to characterize the synoptic meteorological situation as well as the antecedent climatic conditions that may have contributed to formation of a frost ring.

Frost-damage zones have been produced in the annual rings of subalpine bristlecone pines (*Pinus longaeva* D. K. Bailey and *P. aristata* Engel.) at intervals of a few decades to a few hundred years for at least the past 4,000 yr. They are observed at localities ranging from California to Colorado, a distance of some 1,300 km. In the course of tree-ring chronology development, the presence and type of frost damage in dated annual rings from living trees[10–12] and sub-fossil wood[13,14] was routinely noted. It soon became clear that frost damage had not occurred randomly, but that characteristic frost-ring dates were common to many trees at the same site or general locality, and even to localities several hundred kilometres apart. For the period of meteorological record it was possible to identify the dates of damaging related meteorological events because the growing season of bristlecone pine is known fairly well. For example[15,16], in the White Mountains, California and the Snake Range, Nevada the frost event that produced latewood damage in AD 1884 (Fig. 1) probably took place on 9–10 September, and similar latewood damage in 1965 probably occurred on 17–19 September. In each case temperatures reached new record lows for the month at nearby meteorological stations. In 1902 an earlywood event recorded in the Snake range almost certainly occurred on 3–4 July when the minimum temperature at upper treeline is estimated to have been −10 °C or below. There is clearly a relationship between such unusual meteorological events and the formation of frost rings in subalpine bristlecone pines.

Antecedent climatic conditions may also be important. Study

0028-0836/84/070121—06$01.00

© 1984 Macmillan Journals Ltd

of two years (1884 and 1965) in which latewood frost damage took place in bristlecone pines in California and Nevada shows that these were notably cool summers in the western Great Basin[17,18]. One effect of such cool conditions is to delay both the onset and the completion of cambial activity. Bristlecone pines near upper treeline normally begin radial growth about late June, and cell maturation is complete by late August, some 3–4 weeks later than in trees near the lower forest border in the same area[19]. During a cool summer, maturation may be delayed to late September, when severely cold weather would be more likely to occur even during a normal year, and this would widen the tree's 'window of vulnerability' to damaging frost.

Climatic effects of eruptions

One important consequence of a large explosive eruption is the spread of a stratospheric veil of fine silicate ash and sulphur aerosols, with resultant surface cooling[20] that may be accentuated at high latitudes by the veil's long residence time in the Arctic stratosphere[21,22]. The degree of concentration of such a

Table 1 Notable frost-ring events in the western USA and dates of major volcanic events

Frost event	Volcanic event			
Date	Date(s)	Volcano(es)	Estimated DVI/E_{max}	Combined DVI/E_{max}
AD 595	–	–	–	–
628		(see Table 3)		
687	–	–	–	–
1200	–	–	–	–
1227	–	–	–	–
1299	–	–	–	–
1453		(see Table 3)		
1485	–	–	–	–
		(Beginning of Lamb chronology AD 1500)		
No (see Table 3)	1500	Unknown, Java	1,000	1,000
No	1586	Kelud, Java	1,000	1,000
No	1593	Ringgit, Java	1,000+	1,000+
1601	1601	Unknown	1,000+	1,000+
No	1614	Little Sunda Islands	1,000	1,000
1640	1638	Roung, Java	500	1,000
	1640	Komagatake, Japan	500	
1660	1660	Katla, Iceland	800	2,400
	1660	Omate, Peru	1,000	
	1660	Pichincha, Ecuador	300	
	1660	Teon, Banda Sea	300	
No	1673	Gamma Kunnora, Moluccas	1,000	1,000
1680	1680	Tongkoko, Celebes	1,000	1,400
	1680	Krakatoa, Sunda Strait	400	
No	1693	Hekla, Iceland	300	1,300+
	1693	Serua, Moluccas	500	
	1694	Amboina, Moluccas	250+	
	1694	Celebes	250+	
1732	No			
No	1752	Little Sunda Islands	1,000	2,500
	1754	Taal, Philippines	300	
	1755	Katla, Iceland	1,200	
1761	No			
No	1783	Eldeyjar and Jökull, Iceland	2,300	5,400
	1783	Asama Yama, Japan	600	
	1785	Vesuvius, Italy	2,500	
1805	No			
1817	1814	Mayon, Philippines	300	3,300
	1815	Tambora, Sunda Strait	3,000	
No	1821	Eyjafjallajökull	300	1,100
	1822	Vesuvius	300	
	1822	Galunggung	500	
1828	No			
1831	No			
1837	1835	Coseguina, Nicaragua	4,000	4,000+
	1837	Avachinskaya Sopka, Kamchatka	–	
1866	No			
1884	1883	Krakatoa, Sunda Strait	1,000	1,000
1902*	1902	Pelée, Martinique	100	1,000
	1902	Soufrière, St Vincent	300	
	1902	Santa Maria, Guatemala	600	
1912	1912	Katmai, Alaska	500†	500+
1941	No			
1965	1963	Agung, Bali	800†	800+

Notable frost rings are those occurring at two or more localities or in 50% or more of sampled trees in any one locality (years of occurrence in both Great Basin and Rocky Mountains are underlined). Period analysed is AD 560–1969. Major volcanic events are individual eruptions or closely spaced eruption sequences (bracketed) having a combined Dust Veil Index (DVI/E_{max}) estimated by Lamb[1] as 1,000 or more. Period analysed for volcanic events is AD 1500–1968.

* Earlywood.

† Estimated aerosol injection has been revised upward[23,27,28].



Fig. 1 Latewood frost-damage zone in 1884 annual ring, bristlecone pine, White Mountains, California. Severe frost event occurred 9–10 September 1884.

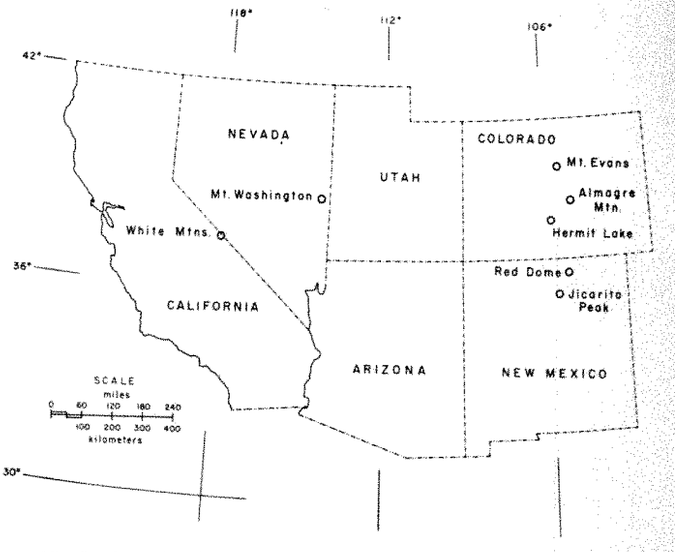


Fig. 2 Location of tree-ring sample localities in the western USA.

cooling effect, and the time lag involved depends in part on the location of the volcano and the composition of the veil[23], on the prevailing circulation, and in part on the season of the year in which the eruption occurs[1].

The effects of an aerosol veil on the large-scale atmospheric circulation have been studied by empirical methods and by modelling. Early work by Wexler[24] on the 700 mbar pattern to be expected following a major eruption suggested that a probable circulation response would be expansion of the circumpolar vortex and intensification of an upper level trough over western North America in January. He proposed a summer pattern of westerlies displaced south of normal, and unseasonable synoptic conditions, such that the July weather chart would resemble that of a normal mid-May. Wexler's basic premise seems to be supported by Lamb's observation[21] of southward displacement of the sub-polar low-pressure zone in the North Atlantic sector in the first July following a great eruption, and continuing in some cases for 3–4 yr. Flohn[25] has speculated that one effect of the intensified polar cold vortices expected to result from high-latitude cooling associated with a stratospheric veil is increased meridionalization of the mid-latitude westerlies. Such quasi-stationary, meridional flow patterns are "... characterized by large-scale standing eddies, extending with diagonal troughs near 200 mbar far into the tropics, and leading to an increased frequency of extreme and unusual weather situations..." Modelling results seem to support the general kind of circulation changes proposed by Wexler, Flohn, and others as consequences of a volcanic aerosol veil. Hunt[26], in an early attempt, incorporated volcanic debris in an experiment using a three-dimensional, multilevel general circulation model with a dynamic dust veil of Krakatoa's magnitude. In the short term, he found an overall decrease in zonal wind intensity and changes in the intensity of

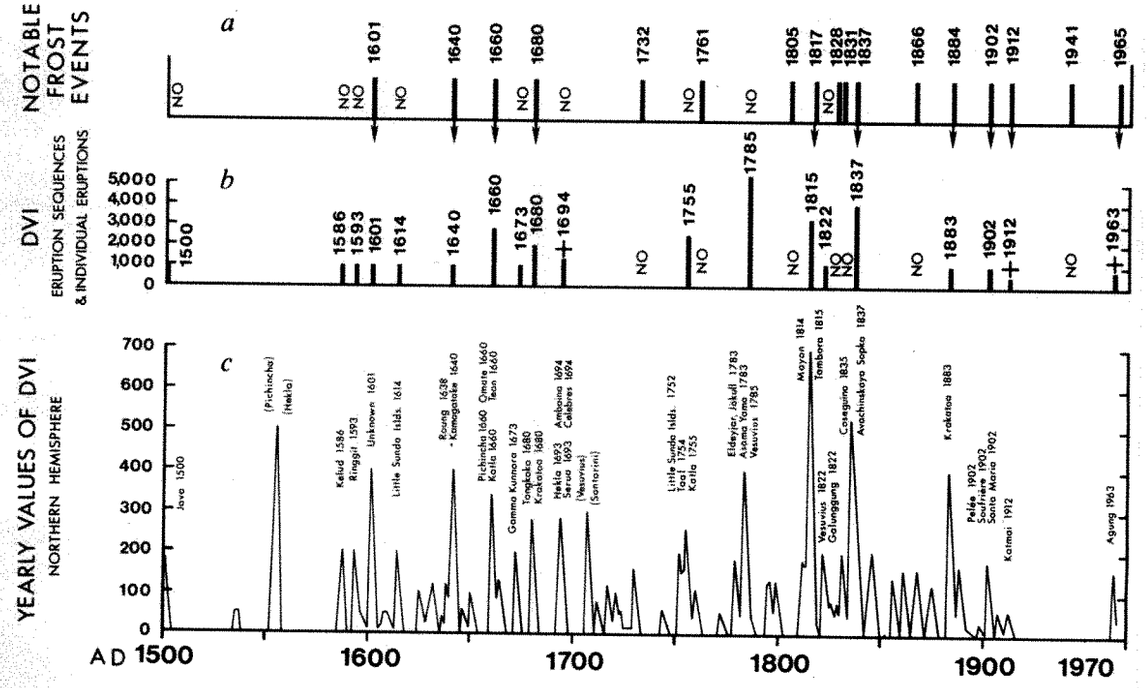


Fig. 3 Frost rings in bristlecone pines in the western USA in relation to major volcanic eruptions on global scale. *a*, Dates of notable frost-ring events. Arrow indicates associated eruption and NO indicates absence of notable frost event at time of major eruption. *b*, Dust Veil Index (DVI/E_{max}) and dates of eruptions and eruption sequences of 1,000 or greater[1]. NO indicates apparent absence of major eruption corresponding to frost-ring date shown in *a*. *c*, Integrated yearly DVI[1], with names of volcanoes and dates of major eruptions. Some other large eruptions are indicated by names of volcanoes in parentheses.

the mean meridional cells, coupled with a decrease in mean hemispheric surface temperature. Cool summers that may contribute to occurrence of frost rings in the western USA seem to be characterized by such increased meridionality, with frequent development of a deep trough at the 700-mbar level in the middle troposphere[10]. Individual spring, summer, and autumn months can be 2–4 °C cooler than normal under this synoptic regime. Bearing in mind that the climatic impact of any individual veil will be tempered by such factors as the preeruption state of the Earth–atmosphere system and the location of the volcano, we suggest that the anomalous circulation over western North America in years following great eruptions could consist of southerly displacement of the general westerly flow and/or more frequent development of an upper level trough, accompanied by occasional outbreaks of unseasonably cold air from higher latitudes. Synoptic situations more typical of winter may be expected to occur in late spring and in early autumn. Such a scenario seems to have been followed in the frost-ring year of 1884, where an examination of daily surface-pressure maps for 9 and 10 September shows a very large high-pressure area extending from northern Saskatchewan and Manitoba down through California, Nevada and Utah, probably representing an outbreak of cold Arctic air that took place unusually early, near the end of an already cool and delayed growing season. The mid-May snowstorm and late September cold-wave in 1983 in the Rocky Mountains and High Plains of the western USA could be more recent examples.

Frost-ring records

Data from bristlecone pines at seven localities in the western USA[12] were studied in this work (Fig. 2). These include three sites in New Mexico and southern Colorado, two sites in the Colorado Front Range, one site on the Nevada-Utah border, and four sites in the White Mountains of eastern California. The tree-ring records from the Rocky Mountains begin between AD 560 and 1535. The chronology from the central Great Basin begins in AD 737. Although all of the sites have potential for chronology extension based on records from dead trees and remnants, this approach has been most successful at one site (Campito Mountain) in the White Mountains, where the continuous upper treeline chronology begins in 3435 BC [11,13,16]. The chronological control provided by these cross-dated tree-ring records representing large numbers of trees ensures accurate placement in time of both the frost-damage event and any associated volcanic eruptions.

Frost rings vary considerably from one event to another in the severity of cell damage, in their frequency of occurrence at a particular site, and in their range of distribution. Branches, seedlings, and very young trees also show a high incidence of frost damage[15], but the results discussed here are based on fairly homogeneous samples of old, well-established trees. With few exceptions, such as 1902, earlywood damage in a given year is restricted to a single locality, perhaps reflecting a local meteorological event or indicating that only at this locality had the trees begun cambial activity, rendering them susceptible to frost damage. However, latewood frost damage is frequently found to have occurred at several localities in the same year, even where these are separated by hundreds of kilometres. For example, several frost events are common to the Rocky Mountains and to the Snake Range, others to the Snake Range and the White Mountains. These variations in severity and geographical extent of latewood damage provided us with a basis for the stratification of frost-ring years that we have used to identify the more important occurrences, termed 'notable frost-ring events'. The results for all of the western USA for the period represented by chronologies from two or more localities are given in Table 1. There are 25 of these notable events in a total of some 116 individual years during which frost damage occurred in at least one sampled tree somewhere in the region. A similar stratification based on less stringent criteria was used for the White Mountains record alone, covering the much longer period 3435 BC to AD 1971.

Krakatoa effect

The postulated linkage between atmospheric veil effects caused by major volcanic eruptions and the climatological and meteorological setting for severe and widespread frost damage was originally suggested by the remarkable coincidence of frost-ring dates which fell no more than 2 yr after each of the four climatically effective Northern Hemisphere or equatorial eruptions and eruption sequences of the past 100 yr. These dates are 1884 (Krakatoa, 1883), 1902 (Pelée, Soufrière, early 1902), 1912 (Katmai (Novarupta), early 1912), and 1965 (Agung, 1963). In addition to their measured effects on the intensity of the direct solar beam[21], the aerosol veils associated with most of these eruptions seem to have caused widespread surface cooling[20,23]. To provide a much longer, if less accurate data set for further evaluation, we referred to Lamb's volcanic eruption chronology[1] and to his dust-veil estimates. A better eruption catalogue is now available[28], which is longer, more complete, and probably more objective than Lamb's in its assessment of relative magnitudes, because it incorporates the Volcanic Explosivity Index (VEI) of Newhall and Self[29], which emphasizes the explosive eruptions that are most effective in injecting gas and ash into the stratosphere. However, this index does not weight eruptions by geographical location to provide a scaling of probable climatic impact, so we chose to use Lamb's geographically weighted Dust Veil Index (DVI/E_{max}) in this initial comparison. We identified as 'major volcanic events' those eruptions and/or closely spaced eruption sequences, for which Lamb had assigned DVIs of 1,000 or more. For reference purposes, note that Lamb had scaled his indices to give Krakatoa, 1883, an index of 1,000. There are 19 such events in the total period, AD 1500–1968, covered by his chronology (Table 1 and Fig. 3). In 10 cases, a notable frost event as previously defined occurred in the western USA in the same year or within 1 or 2 yr following a major eruption or eruption sequence. It is also true that in nine cases a frost event occurred without an apparent antecedent volcanic event, and seven volcanic events have no known associated frost event.

To test the statistical significance of this apparent relationship, we separated the total period of concurrent frost and volcanic events into two parts. That period from 1882 to 1968 includes the four well-documented volcanic events cited above and, moreover, is a period for which meteorological data are adequate to characterize climatically-effective eruptions through their depression of hemispheric temperatures as well as to study associated regional climatic and meteorological anomalies. Our approach was to divide the 87-yr period into 29 discrete 3-yr intervals (2–3 yr is a typical decay period for stratospheric aerosol veils[20]), or triads, and then to count the number of cases in which a notable frost event occurred in the same triad as a major volcanic event. The starting date of 1882 was chosen so that 1883–84 and 1963–65 would be respectively grouped in the same 3-yr intervals, and so that the last year of Lamb's chronology would be included. From elementary probability theory[30], the expected frequency of joint occurrences of events in two random, completely independent series is equal to the product of their individual probabilities. Thus, if we assume that these probabilities are equal to the observed average frequencies, we would expect such joint occurrences to take place with a frequency of only 0.024, less than one time in 29 triads (Table 2). The observed number is nearly six times that expected by chance (note here that neither in this nor in the following analysis does the frost-ring date precede that of the associated volcanic eruption within a triad). A similar analysis was performed for AD 1500–1880 that included 127 triads and six joint occurrences. The observed number is over four times the expected chance value. The final step in our evaluation was a contingency analysis using the χ^2 statistic calculated from the observed and expected frequencies of joint occurrence[31].

Despite the small sample size, the results suggest that for each subperiod, the observed number of joint occurrences of volcanic and frost events is very unlikely to have arisen by chance. Therefore, we can reject the null hypothesis of random association, and accept the alternative hypothesis—that major eruptions are likely to be closely followed by notable frost events—at better than the 99.9% confidence level.

The analytical results show that we can tentatively associate many notable frost events in the western USA over the past several hundred years with antecedent volcanic eruptions catalogued by Lamb. As shown in Table 3 there are other records that can be used to extend this comparison qualitatively much farther into the past, using the long tree-ring record of frost events from the White Mountains, California. However, because it is based largely on data from a single tree-ring site, this frost-event chronology offers a less complete basis for comparison with volcanic events than does the broadly based regional data set given in Table 1. There are also greater uncertainties in the volcanological records in the earlier time periods. We have used the eruption catalogue of Simkin *et al.*[28], which is much longer than Lamb's. The initial Greenland ice core data[5] also provide a long, but locally biased and perhaps less well dated and possibly less complete eruption record spanning the past several thousand years, based on acidity peaks attributed to volcanogenic aerosols. Finally, we have referred to well-dated eruptions in the late BC-early AD period that are based on careful evaluation[2] of historical and archaeological records of volcanism and atmospheric veils in the Mediterranean region.

The apparent correspondence of frost events and eruptions is not as good as in the more recent past, but some coincidences are worth noting. Severe frost damage occurred in AD 1601, and may be linked to the acidity peak described[5] as one of the strongest of the earlier signals in the Greenland ice record. The ice-core data do not reflect a peak at the time of the 1500 frost ring, but an eruption occurred that produced atmospheric optical effects in Europe and is reported by Lamb to have taken place in Java[1]. A large eruption of Mt St Helens also occurred about this time[28]. The severe frost event of 1453 could be linked to a low acidity peak about 1453[5], possibly related to a large eruption of Kelud in 1451[28]. The high acidity peak[5] at about 1258 is not represented in the frost record and, there seem to have been no great eruptions that might account for the numerous frost events from 687 to 1200. However, another strong signal in the Greenland ice is dated at about 623 and may reflect the eruption whose atmospheric veil was recorded in Europe in 626[2] and which might be related to the 628 frost event. The volcano is unknown, but could be Rabaul, in New Britain[28]. There is no documentary record of an atmospheric veil at the time of the frost event in 601, and no corresponding acidity peak. However, a major eruption took place in the White River field, Alaska, at about that time[28].

Referring to the earliest part of our record, only four notable frost-damage events are recognized in the White Mountains in the 4036-yr period before AD 601. One of these events, in AD 119, falls in a gap between the Crête and Camp Century ice records, but could be related to a major eruption of Ilopango, El Salvador, in the Second or Third Centuries[28]. Another, dated to 42 BC, might correspond to one of three peaks in the Camp Century core, approximately dated to 260, 210, and 50 BC, respectively[5]. It follows closely the great eruption of Etna, well dated to 44 BC from direct observations[2]. A third frost event, in 2035 BC, is the most severe in the entire record, as it occurs in all the trees in the sample and caused severe anatomical damage[16]. There does not appear to be a corresponding acidity

Table 2 Basic data and results of contingency analysis of frost-ring occurrence following major eruptions, AD 1500–1968

Period	1882–1968	1500–1880
No. of triads	29	127
No. of volcanic events	4	15
Observed frequency per triad	0.14	0.12
No. of frost events	5	12
Observed frequency per triad	0.17	0.09
Expected frequency of joint occurrences	0.024	0.011
Expected no. of joint occurrences	0.7	1.4
Observed no. of joint occurrences	4	6
χ^2	16.0	14.7
p	<0.0005	<0.0005

Volcanic and frost events are those listed in Table 1.
* Includes Yates correction for continuity due to small sample size[31].

Table 3 Notable frost-ring events in the White Mountains, California, and dates of possible associated eruptions

Frost events	Volcano	Volcanic events Date				
		Lamb[1]	Stothers & Rampino[2]	Hammer *et al.*[5]	Simkin *et al.*[28]	VEI
2035 BC	St Helens			—	1900?BC	5
1626	Santorini			1390 ± 50 BC	1470 ± 20	6
42	Etna		44 BC	50 ± 20	44	3+
AD 119	Ilopango		—	gap	AD 260 ± 100	6?
601	White River		—	—	525 ± 100	6
628	Rabaul?		AD 626	AD 622–623	540 ± 100	6
687	—			—	—	—
1003	—			—	—	—
1029	—			—	—	—
1077*	—			—	—	—
1099	—			—	—	—
1171	—			—	—	—
1200	—			—	—	—
1453	Kelud?	—		1453–1454	1451	3+
1500	St Helens	—		—	1500	5
	Unknown (Java?)	1500		—	—	—
1601	Unknown	—		1600–1601	—	—
1884	Krakatoa	1883		1883	1883	6

Notable frost rings occur at two or more sites, or in 20% or more of trees (years of occurrence in 50% or more of trees on any site are underlined). Period analysed is 3435 BC to AD 1971. Northern Hemisphere and equatorial eruptions were listed if they have a Volcanic Explosivity Index (VEI[29]) of 5 or greater[28], or were represented by ice core acidity peaks[5] or well documented atmospheric veils[1,2]. Source 1 covers the period from AD 1500; 2 from 700 BC to AD 630.
* Earlywood.

peak in Greenland, but the frost-ring date coincides approximately with a large radiocarbon-dated eruption of Mt St Helens[28].

Santorini connection

The remaining frost event in the BC–early AD time period, dated to 1626 BC, is especially notable, not only for its severity, but because it offers the intriguing possibility of dating precisely the cataclysmic eruption on Santorini (Thera) in the Aegean Sea in the second millennium BC. The frost-ring date had seemed to place the eruption too early in time to match the conventionally accepted date of 1500–1450 BC based on the archaeological evidence available in the early 1970s[32], but this discrepancy in dates may now be resolved.

Because the volume of ejecta has been estimated at several times that of Krakatoa, the Santorini eruption seems likely to have produced a major, climatically effective aerosol veil, which is also likely, on present evidence, to have been linked with a notable frost-ring event. Betancourt and Weinstein[33] have carefully reviewed both the archaeological and radiocarbon evidence for the dating of the beginning of the late Bronze Age in the Aegean. In particular, they focus on radiocarbon dates from material obtained during the excavation of a late Minoan settlement near the village of Akrotiri. It lies on the south side of the largest remnant of the original volcanic island, most of which was either ejected or collapsed into the central caldera following the eruption. The excavations were begun by Marinatos in 1967, and the evidence indicates that the buildings were first toppled by earthquake, and soon thereafter buried under many metres of volcanic ash from the eruption. Betancourt and Weinstein evaluate two sets of dates associated with the destruction of ancient Akrotiri, after first calibrating or correcting the raw dates for atmospheric radiocarbon variability, using the 1973 MASCA tables. [For a fuller documentation and a discussion of these samples see Michael[34], who supported the seventeenth century BC eruption date.] The first set of dates is from 'long-lived' material, mostly wood from olive, pine, and cedar that would be expected to predate the eruption considerably; the dates are older than 1700 BC. More relevant here are the dates on six short-lived samples, including charred seeds of legumes from a storage jar, and charred fragments of shrubs. The average date given for these samples is 1688 BC ± 57 yr. More recently, four additional dates have been reported on grain from storage jars from the destruction level[35]. They have a large range, but yield an average date of 1675 BC. Given the uncertainties in the individual radiocarbon determinations coupled with the uncertainty in the calibrations themselves, our proposed eruption date of 1626 BC or perhaps 1 or 2 yr earlier, derived from frost-ring evidence, falls well within the probable range for the true date of the eruption based on radiocarbon.

Conclusions

The potential importance of frost rings as proxy indicators of past eruptions warrants careful weighing of the physical evidence and of the strength of the postulated linkage between aerosol veils, the response of the circulation, and their relation to the climate and weather of the study region. The tree-ring data base is adequate for the past several hundred years, but frost events may be greatly under-represented before about AD 600, because of the decreasing size and geographical extent of the sample. Our method of stratifying 'notable' frost events seems valid, but the summing of magnitudes of closely spaced eruptions (Table 1) may not be justifiable physically in all cases[20]. The comparison of dates of eruptions and frost rings shows that there is not a one-to-one correspondence. Frost events that are unrelated to aerosol veils have occurred, for example, in 1941. Conversely, there were notable eruptions with no associated frost ring in the western USA. That is, some eruptions may result in a frost record at a certain locality while others may not, and frost damage can be due to atmospheric variability from other causes. We also emphasize that comparisons of the kind that we have attempted become increasingly unreliable in the earlier part of the record. This is due partly to uneven reportage of eruptions and partly to the uncertainties in dating of geological, archaeological, glaciological and other sources of evidence.

Further testing of our hypothesis might include the analysis of an improved frost-event chronology for the western USA using an expanded tree-ring data base. Because frost rings are found in trees in the sub-Arctic and in the high mountains of lower latitudes in many parts of the world[36–40], other frost-event records from suitable regions could be compared independently with volcanic chronologies. Our preliminary findings also suggest the need for empirical studies of regional climatic variability and analysis of modelling results to see how important volcanogenic aerosol veils may be as a causal factor in frost-ring formation.

We thank the NSF for financial support of much of the original work on which this research is based, J. M. Mitchell and H. Flohn for stimulating discussions on aerosol veil climatology and P. I. Kuniholm for an introduction to the fascinating but often vexing problems of the late Bronze Age in the Aegean and V. A. S. McCord and A. Allen for help in preparation of the manuscript. We thank W. J. Robinson for permission to reproduce Fig. 2, which originally appeared in the *Tree-Ring Bulletin.*

Received 20 June; accepted 6 October 1983.

1. Lamb, H. H. *Phil. Trans. R. Soc.* A**226,** 425–533 (1970).
2. Stothers, R. B. & Rampino, M. R. *J. geophys. Res.* **88,** 6357–6371 (1983).
3. Kerr, R. A. *Science* **219,** 157 (1983).
4. Newell, R. E. & Deepak, A. (eds) *Mount St Helens Eruptions of 1980—Atmospheric Effects and Potential Climatic Impact* (NASA SP-458, US Government Printing Office, 1982).
5. Hammer, C. U., Clausen, H. B. & Dansgaard, W. *Nature* **288,** 230–235 (1980).
6. Herron, M. M. *J. geophys. Res.* **87,** 3052–3060 (1982).
7. Mosley-Thompson, E. & Thompson, L. G. *Quat. Res.* **17,** 1–13 (1982).
8. Glerum, C. & Farrar, J. L. *Can. J. Bot.* **44,** 879–886 (1966).
9. Glock, W. S. & Reed, E. L. *Science* **91,** 98–99 (1940).
10. LaMarche, V. C. Jr *Science* **183,** 1043–1048 (1974).
11. LaMarche, V. C. Jr *Nature* **276,** 334–338 (1978).
12. LaMarche, V. C. Jr & Stockton, C. W. *Tree-Ring Bull.* **34,** 21–45 (1974).
13. LaMarche, V. C. Jr *Quat. Res.* **3,** 632–660 (1973).
14. LaMarche, V. C. Jr. & Mooney, H. A. *Arct. Alp. Res.* **4,** 61–72 (1972).
15. LaMarche, V. C. Jr. in *Tree-Ring Analysis with Special Reference to Northwest America* (eds Smith, J. H. G. & Worral, J.) (University of British Columbia Faculty of Forestry Bull. 7, 1971).
16. LaMarche, V. C. Jr. & Harlan, T. P. *J. geophys. Res.* **78,** 8849–8858 (1973).
17. US War Department, Signal Office *Mon. Weath. Rev.* 12 (1884).
18. US Department of Commerce *Climatological Data: Utah.* (1966).
19. Fritts, H. C. *Papers Laboratory of Tree-Ring Research,* 4, (University of Arizona Press, Tucson, 1969).
20. Self, S., Rampino, M. R., & Barbera, J. J. *J. Volcanol. Geotherm. Res.* **11,** 41–60 (1981).
21. Lamb, H. H. *Climate: Present, Past and Future* Vol. 1 (Methuen, London, 1972).
22. Flohn, H. in *Climatic Change and Variability* (eds Pittock, A. B., Frakes, L. A., Jenssen, D., Peterson, J. A. & Zillman, J. W.) (Cambridge University Press, 1978).
23. Rampino, M. R. & Self, S. *Quat. Res.* **18,** 127–143 (1982).
24. Wexler, H. *Tellus* **8,** 480–494 (1956).
25. Flohn, H. in *The Physical Basis of Climate and Climate Modelling,* (Global Atmos. Res. Prog. Pub. Ser. 16, 1975).
26. Hunt, B. G. in *Climatic Change and Variability* (eds Pittock, A. B., Frakes, L. A., Jenssen, D., Peterson, J. A. & Zillman, J. W.) (Cambridge University Press, 1978).
27. Mitchell, J. M. *Weatherwise* **35,** 252–259 (1982).
28. Simkin, T., Siebert, L., McClelland, L., Bridge, D., Newhall, C. & Latter, J. H. *Volcanoes of the World* (Hutchinson Ross, Stroudsburg, 1981).
29. Newhall, C. G. & Self, S. *J. geophys. Res.* **87,** 1231–1238 (1982).
30. Mosimann, J. E. *Elementary Probability for the Biological Sciences* (Appleton-Century-Crofts, New York, 1968).
31. Blalock, H. M. Jr *Social Statistics* (McGraw-Hill, New York, 1972).
32. Lamb, H. H. *Climate: Present, Past and Future* Vol. 2 (Methuen, London, 1977).
33. Betancourt, P. P. & Weinstein, G. A. *Am. J. Archaeol.* **80,** 329–348 (1976).
34. Michael, H. N. in *Proc. Temple University Aegean Symp.* (ed Betancourt, P. P.) (Temple University Press, 1976).
35. Michael, H. N in *Thera and the Aegean World* Vol. 1 (ed. Doumas, C.) 791–795 (Aris & Phillips, London, 1978).
36. Bailey, I. W. *Bot. Gaz.* **80,** 93–101 (1925).
37. LaMarche, V. C. Jr & Fritts, H. C. *Z. Gletscher. Glazialgeol.* **7,** 125–131 (1971).
38. Dunwiddie, P. W. & LaMarche, V. C. Jr *Aust. For.* **43,** 124–135 (1980).
39. Morrow, P. A. & LaMarche, V. C. Jr *Science* **201,** 1244–1246 (1978).
40. Dunwiddie, P. W. & LaMarche, V. C. Jr *Nature* **286,** 796–797 (1980).

DNA sequence of the maize transposable element *Dissociation*

H. P. Döring, E. Tillmann & P. Starlinger

Institut für Genetik, Universität Köln, 5000 Köln 41, FRG

The DNA sequence of the terminal 4.2 kilobases (kb) of the 30-kb insertion in the endosperm sucrose synthase gene of maize mutant sh-m5933 shows that it comprises two identical 2,040-base pair (bp) segments, one inserted in the reverse direction into the other. We suggest that the 2,040-bp sequence is an example of the transposable element Dissociation *described by Barbara McClintock.*

TRANSPOSABLE elements were first detected and characterized in considerable detail by McClintock[1,2]. Nearly 20 years later, similar elements were studied at the DNA level first in bacteria[3–5] and later in a variety of eukaryotic organisms, notably *Drosophila melanogaster* and *Saccharomyces cerevisiae*[6,7]. The structure of these latter elements has been elucidated rapidly, including their complete DNA sequences. It would be desirable to study isolated maize transposable elements because of the variety of genetic phenomena associated with them[1,2,8]. Even when such elements are inserted at the same gene locus, the resulting phenotypes can be quite different. Sometimes, the phenotype resulting from a particular element remaining at the same site is variable in a clonally heritable way ('change in state')[9,10]. It would be interesting to study the structural correlate of these functional changes. DNA probes derived from the transposable elements might be used to isolate genes previously tagged by the insertion of the element. Eventually, the elements might be used as a vector for the introduction of new genes into chromosomes, as has been demonstrated first in bacteria[11,12] and recently in *Drosophila*[13,14].

As no gene products of transposable elements in plants are known, conventional means of isolating these elements cannot be used. It is possible, however, to isolate DNA clones of genes that have previously been mutated by the insertion of a particular transposable element. Isolation of DNA from wild type and mutant genes reveals the presence of DNA foreign to the gene in question, which can then be identified as the DNA of the transposable element[15]. The first plant gene to be used in this way was that coding for endosperm sucrose synthase at the *Shrunken* locus[16]. Mutants were available that were caused by the insertion of the transposable element *Dissociation* (*Ds*)[17,18]. This element cannot transpose by itself, but it can be transposed in the presence of an additional, transposable element called *Activator* (*Ac*) which acts in *trans* on *Ds*[2]. On isolation of genomic clones from *Sh*[19,20], two *Ds*-caused mutations were found (ref. 20 and E. Weck, U. Courage-Tebbe, H.-P.D. and P.S., in preparation).

In one of these mutants, *sh-m5933*, the mutation was shown to be due to a complicated event, leading to the insertion of a 30-kilobase (kb) DNA segment, and a duplication of part of this genetic region was found[21]. The DNA contained in the clone was characterized by restriction analysis, and the DNA adjacent to the cloned segment was studied by Southern blot analysis. From these studies, we hypothesized that only the parts of the insertion located at both of its junctions with *Shrunken* DNA, are transposable elements. The DNA located between the two regions of transposable elements is of unknown origin; part of it is unique, other sequences comprise repeated DNA[20] (K. Theres, unpublished observations). To test this hypothesis, we sequenced 4.2 kb of DNA adjacent to the 3′ end of the *Shrunken* gene. We show here that this sequence corresponds to two identical copies of a 2-kb segment of DNA. One of these segments is inserted in the reverse direction into the other. We discuss evidence that these 2-kb DNA segments correspond to the genetically defined transposable element *Ds* described by McClintock[1,2].

Transposable element insertion

The first 4.2 kb adjacent to the junction between the 3′ end of the *Shrunken* gene and the large insertion mentioned above were sequenced. Figure 1 shows the sequencing scheme and the sequence itself is given in Fig. 2. The 4.2 kb sequence is made up of two identical sequences of 2,040 bp. These sequences are found, however, in an unusual arrangement, one being inserted into the other. The two sequences are inverted with respect to each other (see Fig. 3).

We have reported previously that two pairs of inverted sequences are present near the junction between wild type sequences and the insert[20]. The DNA sequence analysis presented here shows that this really is the case, and also shows that the two pairs of inverted repeats described previously[20] are adjacent to each other.

We can ask whether the two 2-kb sequences are transposable elements, and whether the 4-kb structure has been formed by the insertion of one such element into a copy of itself. Many transposable elements terminate in inverted repeats, and almost all of them are flanked by short direct repeats of the recipient sequence. If these structures are found at the termini of a DNA sequence, this sequence may be a transposable element. Figure 2 shows that by these criteria, the 2-kb sequence described above may be a transposable element; both the inserted and the recipient copy of this sequence terminate in an 11-bp inverted repeat. Thus, this 11-bp sequence is found four times, the two right-hand and the two left-hand ones being oriented in the same direction with respect to each other (Fig. 3).

The internal 11-bp inverted repeats are flanked by 8-bp direct repeats. If these are the result of insertion of one copy of the 2-kb element into the other, the 8-bp sequence should be found only once in the internal copy of the transposable element. This is borne out by the sequence. No 8-bp duplication flanks the recipient 2-kb structure. We expect that the 8 bp of *Sh* DNA bordering the inserted DNA at the 3′ junction will be repeated at the 5′ junction of the 30-kb insert. However, this has not yet been cloned. We conclude, therefore, that the 2-kb sequence is indeed a transposable element and that the more complicated 4-kb structure was created by the insertion of one copy of this element, in inverted orientation, into the other one. We will show below that this transposable element is an example of McClintock's *Ds* and we will refer to the structure described here as a double-*Ds*, or *D*(*s*′*D*′)*s*.

Relationship to *Dissociation* elements?

It is conceivable that the large, ~30-kb insertion in *sh-m5933* is in fact *Ds*. We argue here, however, that the small, ~2-kb

0028-0836/84/020127-04$01.00

© 1984 Macmillan Journals Ltd

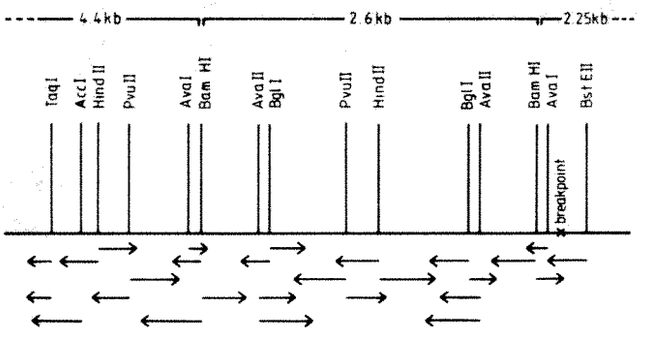


Fig. 1 Sequencing strategy. All sequences were determined (usually twice) by 5′ end labelling and chemical degradation according to Maxam and Gilbert[32]. The arrows indicate the sites of labelling and the extent of sequence read.

structure is *Ds* and that the large insertion in *sh-m5933* consists of several copies of *Ds* and some unique DNA not belonging to this transposable element. Such a conclusion cannot be drawn from the sequence analysis of one element alone; our structure must be compared with other mutations caused by the integration of *Ds*. However, we present the following evidence:

(1) We have isolated a null mutation, *adh1-2F11*, in the gene *Adh1* which encodes alcohol dehydrogenase[22]. Genetically, this mutation is caused by *Ds*, because it is unstable in the presence of *Ac*, while no reversion to the wild type phenotype is observed in the absence of *Ac*. DNA blotting experiments show the presence of a 1.3-kb insertion in the gene. RNA blotting experiments show an increase in the RNA size corresponding to the insert size within the gene. The enlarged RNA hybridizes to a probe derived from the 4-kb sequence described above, while the wild type RNA does not, indicating that the two inserts in the genes *Sh* and *Adh1* have some DNA sequences in common.

(2) Another mutation, *Adh1-Fm335*, was isolated previously by Osterman and Schwartz[23]. By genetic criteria, the mutation is caused by insertion of *Ds*. Peacock and collaborators have sequenced the wild type, mutant and revertant genes[24,25], and found that the mutation is caused by the insertion of a 405-bp DNA segment. The 11 nucleotides at the termini are inverted repeats. The sequence found at the termini of the 2-kb transposable element in *sh-m5933* is identical to the inverted repeat first described by Peacock *et al.*[24] in the *Adh1 Ds* insertion. The insertion in *Adh1* is also flanked by a direct repeat of 8 bp. These findings argue that different copies of *Ds* terminate in the same 11-bp repeat and generate 8-bp duplications of the recipient sequence on insertion. Analysis of revertants at the *Adh1* locus[24] has demonstrated that the 405-bp insert bounded by 11-bp inverted repeats is a *Ds* element, supporting our contention that the 2-kb structure which we have described is also a *Ds* element, that the inverted 11-bp sequence may be sufficient for transposition by an *Ac*-encoded function of a DNA sequence bracketed by these inverted repeats, and that more complicated structures can be built from the simple unit *Ds*.

(3) Another *Ds*-induced mutation at the *Shrunken* locus, *sh-m6233*, has been analysed by the blotting technique[20]. A genomic DNA clone containing one terminus of the insertion and adjacent DNA of the *sh* locus has been isolated and the junction sequenced (E. Weck, U. Courage-Tebbe, H.-P.D. and P.S., in preparation). The first 70 nucleotides adjacent to the junction including the 11 nucleotides at the junction, are identical to the sequence of the 2-kb transposable element found in *sh-m5933*.

These biochemical findings are strongly supported by the genetic observation that excision of the 30-kb structure inserted in *sh-m5933* and reversion to the *Shrunken* wild type occurs only in the presence of *Ac*[21]. Thus, the mobile transposable element-like structure ending in 11-bp termini is probably an example of *Ds*.

Length variation of *Dissociation* elements

From the above, it is clear that *Ds* isolates can differ in length. The *Ds1* of *adh Fm335* sequenced by Peacock *et al.*[24] is only 405 bp long, while our unit *Ds* is 2 kb long. The *Ds* in *adh1-2F11* has an intermediate length of ~1.3 kb. Fedoroff *et al.*[26] found a *Ds* in a clone of the gene *Waxy* isolated from mutant *wx-m6*, which closely resembles our 2-kb *Ds* by restriction mapping. The finding of *Ds* isolates of different length and the observation that *Ds* is deficient in several functions such as excision from a gene locus, transposition and production of chromosome breaks at the site of insertion, but that it can be complemented *in trans* by *Ac* suggests a hypothesis for the origin of different *Ds* copies[8]. It is conceivable that these arise from *Ac* by internal deletions which both reduce the length and abolish the function. Fedoroff *et al.*[26] have isolated *Ac* from another *Waxy* mutant, *wx-m9*, and found that it is ~4.2 kb long. Heteroduplex analysis and restriction mapping has shown that *Ds6* of *wx-m6*, is an internal deletion of *Ac* of *wx-m9*.

Comparison of the *Ds* sequenced by Peacock *et al.*[24,25] and *Ds5933* shows that the situation may be more complex. Apart from the 11-bp repeats at the termini, there is little sequence similarity. Thus, *Ds* can either be formed from *Ac* by a multi-step event, or there exist different families of *Ac* and *Ds*[27].

Evolution of double *Ds*

The creation of independent *Ds* copies by deletions from *Ac* has been discussed above. Another possible means of evolution of different *Ds* structures is evident from the structure of the double *Ds* at the 3′ junction in *sh-m5933*. From Fig. 3 it is apparent that different structures bordered by two inverted repeats of 11 bp could be transposed from the double *Ds* (these are indicated by brackets). The data obtained by DNA blotting analysis of the inserted DNA adjacent to the 5′ junction in *sh-m5933* are compatible with this assumption[21]. The restriction pattern of the mutant and of several of its revertants is most easily explained if the sequence located at the 5′ junction of *Shrunken* and *Ds* DNA is an inverted copy of part of the double *Ds* at the 3′ junction (indicated by the bracket marked II in Fig. 3). If this interpretation is correct, the structure at the 5′ junction of the insertion consists of 1.5 copies of the 2-kb *Ds*. Part of the 2-kb sequence is present only once at the 5′ junction, while the other part is present twice (Fig. 3).

The deletion of part of the structure in one of the revertants, *sh-r5*, and the restriction pattern of the non-deleted part are compatible with the assumption that a unit *Ds* has been excised (Fig. 3). We assume that the remaining half *Ds* element carries the characteristic 11-bp sequence at one terminus only. This will have to be checked by cloning and sequencing. We anticipate that this structure can no longer be excised. However, chromosome breaks at this site are still detected[21]. It is possible that the occurrence of such breaks is not dependent on the presence of the 11-bp sequence at both termini.

Other sequence features of *Ds*

The sequence described here has a few peculiarities, including similarities to other transposable elements, direct or inverted short duplications and insertion sites, repeated oligonucleotides within *Ds* as well as an open reading frame. In none of these cases is a functional significance known.

Termini of *Ds*: Several transposable elements have the dinucleotide TG at the 5′ terminus of their long terminal repeat. In some of the retroviruses, the characteristic TG is preceded by the dinucleotide AA[28]. The hexanucleotide ATGAAA is shared between the termini of the murine leukaemia and sarcoma viruses and *Ds*. In the case of *Ds*, this represents the inner hexanucleotide of the characteristic terminal 11-bp sequence.

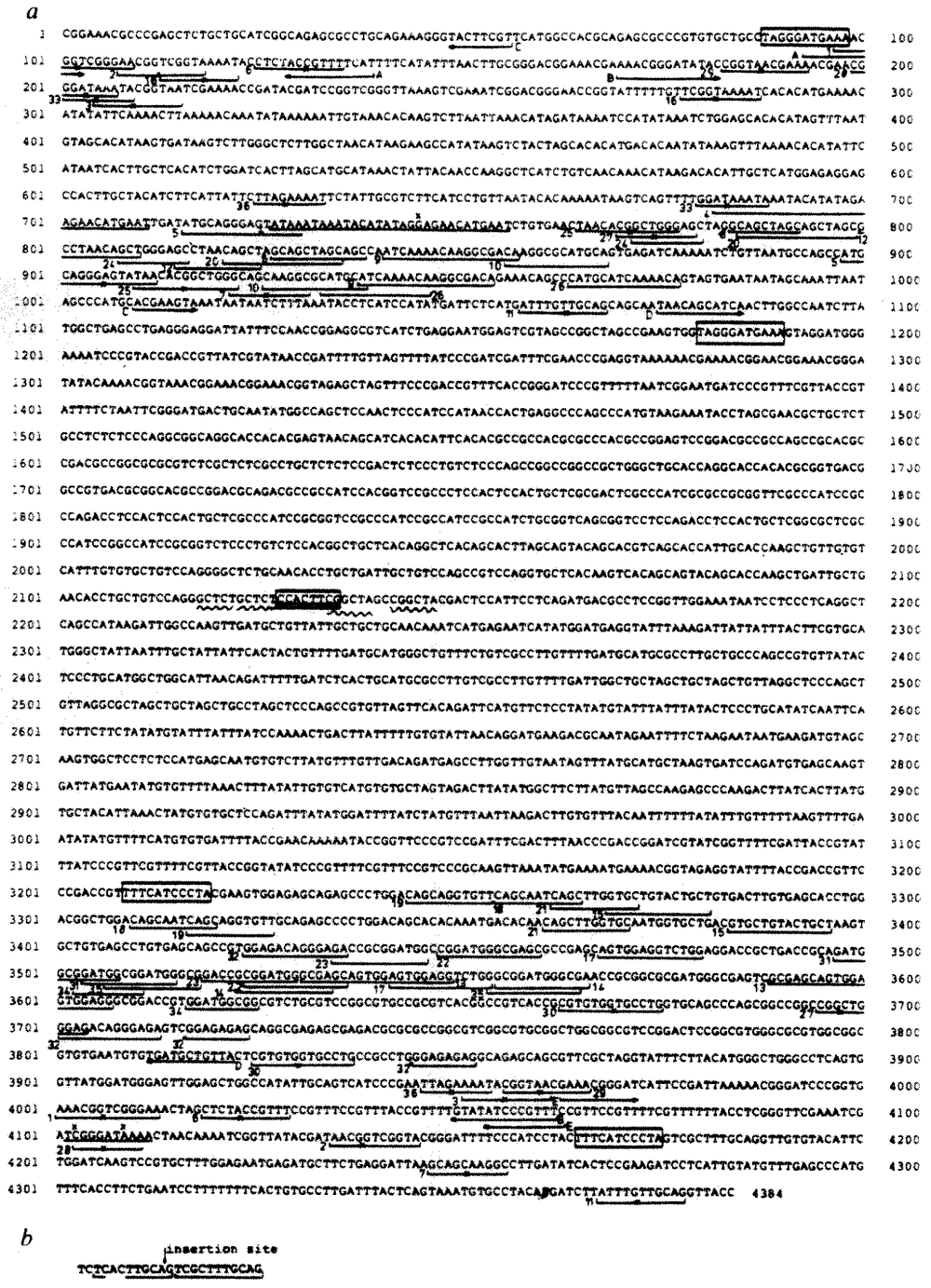


Fig. 2 *a*, Sequence of double *Ds* from *sh-m5933*. The sequence of the transposable element-like structure starts at position 88 and ends at position 4,175. The sequence adjacent to these termini is an 8-bp direct repeat, CGAAGTGG, which is found only once at position 2,128–2,135. The 11-bp inverted repeats and the internal 8-bp insertion site are boxed. The direct repeats (numbered) and the inverted repeats (lettered arrows) are indicated by underlining the outer sequence only. The region between positions 3,960 and 4,080 contains eight copies of CCGTTT, four copies of AAACGG and several near relatives of this sequence. The outer and the inner sequence of *Ds* are identical except for the 8-bp duplication in the outer sequence flanking the insertion of the inner one. At position 955 an ambiguity exists, due to differences in reading the sequencing gels of the opposite strands. Between positions 955 and 956, a C may have to be inserted. The same is true between positions 2,351 and 2,352, where a G might have to be inserted. *b*, Insertion site of *Ds* in *sh-m5933*.

The three adenine residues at the end of the sequence form part of a pentanucleotide AAAGA which is also found at the terminus of the FB transposon in *Drosophila*[29]. Another similarity between *FB* and *Ds* will be described below. Similarities between the termini of the maize transposon *Cin1* and the *Drosophila* transposon *copia* have been described by Saedler *et al.*[30]. In this case, the first pentanucleotide was shared between the two transposons.

Many transposable DNA elements terminate in inverted repeats which can be mismatched. We have screened the sequence inwards from the 11-bp terminal inverted sequences, and have found two penta- and one hexanucleotide on one side which are found in inverted orientation near the other terminus, and which are separated from each other by no more than 10 bp. We have also observed a perfect 11-bp repeat on the left-hand side of *Ds* near one end, at positions 93–103 and 129–139. Near the right-hand end of *Ds* we find a mismatched inverted repeat at positions 4,102–4,112 and 4,165–4,175. These two inverted repeats provide pairing capabilities alternative to that between the two opposite ends of the *Ds* elements.

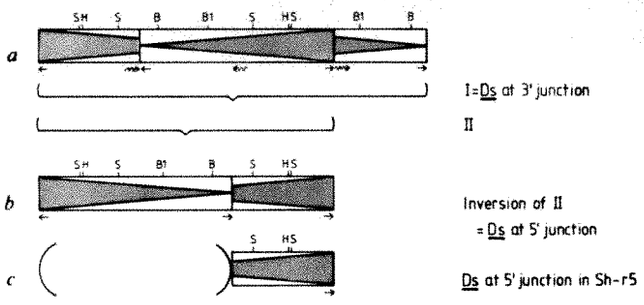


Fig. 3 Schematic representation of double *Ds* and derivatives of it. *a*, Double *Ds* at the 3′ junction of *sh-m5933*, as sequenced by us. The solid arrows below the figure indicate the 11-bp inverted repeats (not to scale). The wavy arrows indicate the 8-bp duplication. The brackets indicate two different structures that can presumably be transposed. *b*, Hypothetical structure of *Ds* at the 5′ junction of *sh-m5933* both in the complete gene and in the duplication, as derived from restriction mapping on the assumption that structures having 11-bp inverted repeats at their termini can be transposed. *c*, Hypothetical structure of *Ds* at the 5′ junction in the duplication of revertant *sh-r5*. This structure is deduced from restriction mapping, which shows a decrease in fragment size for several restriction enzymes cleaving outside *Ds* and a smaller decrease of an *Sph*I fragment which is in accord with the map given here. (B = *Bam*HI, S = *Sph*I, B1 = *Bgl*I, H = *Hin*dII.)

Insertion sites: In *sh-m5933* there are two *Ds* insertion sites, one located near the centre of *Ds*, where one *Ds* copy is inserted into the other one, the other localized in the sucrose synthase gene, where the 30-kb sequence is inserted. Both of the insertion sites are characterized by short sequence duplications. These are present before the insertion and must not be confused with the 8-bp duplications created by the insertion. In the case of the insertion in the sucrose synthase gene, the duplication is 11 bp long, with one mismatch and one base deletion. The insertion site of *Ds* into *Ds* is characterized by the presence of one hexanucleotide and one pentanucleotide as direct repeats. In both cases, the insertion site is located in one of the duplicated oligonucleotides (see Fig. 2*a*, wavy lines, and Fig. 2*b*).

Direct and inverted repeats: The *Ds* sequence is characterized by the presence of many direct and several inverted repeats of variable length. The perfect repeats exceeding 8 bp are indicated in Fig. 2 by underlining. There are additional examples of slightly mismatched repeats which have not yet been analysed in detail.

The position of the direct repeats is non-random. Many are separated by short stretches of DNA, others are adjacent to each other. Some pairs of repeats partially overlap others. We cannot suggest a mechanism for the origin of these repeats apart from the vague notion that in some *Ac–Ds* functions replication and/or recombination events may be involved that are not encountered regularly with other DNA segments.

The repeats are found in most of the *Ds* sequence; only the regions from nucleotides 310–670 and 1,080 to 1,180 are relatively free of them. These regions do not coincide with open reading frames. The 609-bp open reading frame mentioned below is full of repeated sequences. The DNA segments containing the repeated sequences may provide an explanation for the observation that independently isolated *Ds* elements can be of different length and may be derived from *Ac* by internal deletions. Whether a sequence variability generated by recombination events in *Ds* is the basis for the known 'states' of *Ds*[2,9] may be the subject for future experiments.

Repeated hexanucleotide: Near one end of *Ds*, a region of ~230 bp is characterized by the presence of 15 copies of the hexanucleotide CCGTTT and close relatives of it, which differ from the hexanucleotide by one base substitution. Four of these hexanucleotides are oriented opposite to the others. Some of them lie adjacent to one another, while others are separated by one or a few nucleotides. A similar structure has been described for the *FB* transposon of *Drosophila*[29], in which the repeated structure is a decanucleotide. It shares the first five nucleotides, CGTTT, with our hexanucleotide, which may be coincidental. Given the increasing number of similarities between *Drosophila* and maize transposons (see above) the possibility of horizontal transmission of DNA between these two species should be considered.

Open reading frames: The only sizeable open reading frame, starting with an ATG, is located between nucleotides 3,373 and 3,981, possibly encoding a protein of 203 amino acids. The sequence around the ATG at position 3,373, GNNATGG, has been observed to be a translation start site in animals but not yet in plants[31]. We have observed no promoter-like structure preceding it. Whether this open reading frame is transcribed and/or translated is unknown.

Conclusions

The DNA sequence described here resembles other transposons in having inverted DNA repeats at its termini and in creating short direct DNA duplications at the site of insertion. The DNA sequence between the 11-bp inverted repeats does not contain long open reading frames. Whether the abundance of direct and inverted repeats in this structure has a functional significance is unknown. Rearrangements involving these sequences are conceivable and may form the basis for 'changes in state'.

The sequence dissimilarity between *Ds1*[25] and *Ds5933* described here suggests that DNA located between the 11-bp inverted repeats is transposed passively. This fact may be exploited in gene transfer experiments. The observation that sequences hybridizing to *Ds5933* are present throughout the maize genome in multiple copies[20] will make the identification of particular genes mutated by the insertion of *Ds* difficult, but not impossible. Experiments along these lines are in progress in our laboratory.

We thank K. Stüber for invaluable help in the analysis of DNA sequences and N. Fedoroff and J. Peacock for communicating unpublished results. The work was supported by Deutsche Forschungsgemeinschaft through SFB 74.

Received 4 August; accepted 28 September 1983.

1. McClintock, B. *Carnegie Instn. Wash. Yb.* **45,** 176–186 (1946).
2. McClintock, B. *Cold Spring Harb. Symp. quant. Biol.* **16,** 13–47 (1951).
3. Jordan, E., Saedler, H. & Starlinger, P. *Molec. gen. Genet.* **100,** 296–306 (1968).
4. Shapiro, J. A. *J. molec. Biol.* **40,** 93–105 (1969).
5. Starlinger, P. & Saedler, H. *Biochimie* **54,** 177–185 (1972).
6. *Cold Spring Harb. Symp. quant. Biol.* **45,** (1980).
7. *Mobile Genetic Elements* (ed. Shapiro, J. A.) (Academic, New York, 1983).
8. Fincham, J. R. S. & Sastry, G. R. K. *A. Rev. Genet.* **8,** 15–50 (1974).
9. McClintock, B. *Brookhaven Symp. Biol.* **18,** 162–184 (1965).
10. McClintock, B. *Devl. Biol. Suppl.* **1,** 84–112 (1967).
11. Reif, H. J. & Arber, W. *Cold Spring Harb. Symp. quant. Biol.* **45,** 40–45 (1981).
12. Saint-Girons, I., Fritz, H. J., Shaw, C., Tillmann, E. & Starlinger, P. *Molec. gen. Genet.* **183,** 45–50 (1981).
13. Rubin, G. M. & Spradling, A. C. *Science* **218,** 348–353 (1982).
14. Spradling, A. C. & Rubin, G. M. *Science* **218,** 341–348 (1982).
15. Döring, H. P., Ehring, R., Geiser, M., Wöstemeyer, J. & Starlinger, P. in *The Plant Genome* (eds Davis, D. R. & Hopwood, D. A.) 73–78 (John Innes, Norwich, 1980).
16. Chourey, P. S. & Nelson, O. E. *Biochem. Genet.* **14,** 1041–1055 (1976).
17. McClintock, B. *Carnegie Instn. Wash. Yb.* **51,** 212–219 (1952).
18. McClintock, B. *Carnegie Instn. Wash. Yb.* **52,** 227–237 (1953).
19. Burr, B. & Burr, F. A. *Cell* **29,** 977–986 (1982).
20. Geiser, M. *et al. EMBO J.* **1,** 1455–1460 (1982).
21. Courage-Tebbe, U., Döring, H. P., Fedoroff, N. & Starlinger, P. *Cell* **34,** 383–393 (1983).
22. Döring, H. P. *et al. Molec. gen. Genet.* **193,** 199–204 (1984).
23. Osterman, J. C. & Schwartz, D. *Genetics* **99,** 267–273 (1981).
24. Peacock, W. J. *et al. Proc. Miami Winter Symp.* (Academic, New York, in the press).
25. Sutton, W. D. *et al. Science* (submitted).
26. Fedoroff, N., Wessler, S. & Shure, M. *Cell* **35,** 235–242 (1983).
27. Sachs, M. M., Peacock, W. J., Dennis, E. S. & Gerlach, W. L. *Maydica* **XXVIII,** 289–302 (1983).
28. Varmus, H. E. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 411–503 (Academic, New York, 1983).
29. Potter, S. S. *Nature* **297,** 201–204 (1982).
30. Saedler, H. *et al. 5th John Innes Symp.* 107–116 (1982).
31. Messing, J. *et al.* in *Genetic Engineering of Plants* (eds Kusuge, T., Meredith, C. P. & Hollaender, A.) 221–227 (Plenum, New York, 1982).
32. Maxam, A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74,** 560–564 (1977).

Mode of proviral activation of a putative mammary oncogene (*int*-1) on mouse chromosome 15

Roel Nusse*, Albert van Ooyen*, David Cox†, Yuen Kai T. Fung‡ & Harold Varmus‡

* Department of Virology, Netherlands Cancer Institute, Antoni van Leeuwenhoekhuis, Plesmanlaan 121, Amsterdam, The Netherlands
† Department of Pediatrics and ‡ Department of Microbiology, University of California, San Francisco, California 94143, USA

Most mammary carcinomas induced in C3H mice by the mouse mammary tumour virus (MMTV) bear a new proviral insertion within a highly conserved locus on chromosome 15 called int-*1. A transcriptional unit within this locus is inactive in all tested normal tissues but expressed at low levels in mammary tumours with proviral insertions positioned on either the 5′ and 3′ sides of the gene. Transcription of the proviruses proceeds away from* int-*1; thus an indirect mechanism appears to activate expression of this putative oncogene.*

THE proviruses of retroviruses can integrate at many sites in host genomes; thus, like other movable genetic elements, they act as insertion mutagens[1]. Two classes of proviral insertion mutations have been described: recessive mutations that disrupt and prevent expression of genes[2–6] and presumably dominant mutations that activate gene expression, leading to induction of tumours by retroviruses without their own oncogenes[7–13]. For example, during the induction of B-cell lymphoma by the avian leukosis virus (ALV), proviral insertion mutations augment transcription of c-*myc*, the cellular progenitor of a retroviral oncogene[7], either by provision of a retroviral promoter[7–10] or by an indirect effect on a host promoter[10].

The proposal that retroviruses lacking oncogenes induce tumours by insertion mutation of host genes suggests a strategy for the isolation of novel oncogenes. By molecular cloning of a single new mouse mammary tumour virus (MMTV) provirus from a C3H mouse mammary tumour, we have isolated a region of the mouse genome that contains an MMTV proviral insertion in the majority of C3H mammary tumours[13]. Although this region lacks homology with any of the available retroviral oncogenes, we have previously argued that it harbours an oncogene, called *int*-1, instrumental in mammary tumorigenesis because (1) MMTV proviruses can integrate at many sites in the host genome (implying that insertions near *int*-1 confer a selective growth advantage) and (2) because the insertions are accompanied by the appearance of transcriptional products from *int*-1, a silent domain in non-neoplastic mammary tissue.

We demonstrate here that insertion mutations in the *int*-1 locus usually stimulate transcriptional activity by an indirect mechanism: MMTV proviruses are located either upstream from transcribed *int*-1 sequences in the opposite orientation or downstream in the same orientation. We also assign the *int*-1 gene to mouse chromosome 15 and show that *int*-1, like the progenitors of viral oncogenes, has been conserved during evolution.

Locating and orienting MMTV proviruses

We reported previously[13] that 18 of 26 C3H mouse mammary tumours contain MMTV proviral DNA within a common region of approximately 20 kilobases (kb). The insertions are distributed with roughly equal frequency on both sides of a portion of the locus that is represented in tumour-specific, 2.6-kb polyadenylated RNA. To assess the mechanisms involved in the activation of the *int*-1 transcriptional unit, we have now determined the sites of proviral insertion more precisely and ascertained the transcriptional directions of the proviruses and the *int*-1 gene. These studies involved physical mapping of the interrupted *int*-1 loci with restriction endonucleases, using the DNA transfer method and a collection of molecular annealing reagents specific for portions of *int*-1 and for the 5′ and 3′ halves of MMTV proviral DNA. The *int*-1 probes were derived by plasmid subcloning of unique sequence DNA from recombinant λ bacteriophages containing overlapping inserts of the *int*-1 domain from a library of normal BALB/c mouse DNA. Probe C detects *int*-1 RNA in mammary tumours; probe D represents a region located leftward of probe C on the *int*-1 map as it is conventionally drawn (see Fig. 3). The probes used to orientate proviral DNA [MMTV-*env* and MMTV-*gag* (Fig. 1)] represent portions of MMTV DNA that reside near the 5′ and 3′ ends of the provirus but exclude sequences from the long terminal repeats (LTRs). In general, our strategy was to use enzymes that cleaved *int*-1 at widely separated sites and divided MMTV proviral DNA into two portions distinguishable with MMTV-*env* and MMTV-*gag* probes.

Proviruses on the 5′ side of *int*-1

Two enzymes, *Bgl*II and *Kpn*I, proved to be particularly useful for the analysis of MMTV proviruses present to the left of the *int*-1 transcriptional unit. (We will show below that transcription of *int*-1 proceeds from left to right on the physical map; thus, proviruses inserted to the left of the region represented by probe C can be considered to lie 5′ to at least a portion of the transcriptional template.) *Bgl*II cleaves the normal C3H mouse DNA at a leftward site (position −21 on the map in Fig. 3) to generate a 14-kb fragment that anneals with probe C. In addition, *Bgl*II cleaves the MMTV provirus at two sites, one of them near the middle of the element (Fig. 1). Proviral DNA in eight tumours was found to disrupt the normal 14-kb *Bgl*II fragment from one of the two *int*-1 alleles. As illustrated for six of these tumours in Fig. 1*a* and *b*, the novel fragments also annealed with the MMTV-*gag* probe, indicating that the proviruses are positioned either to the right of the probe C homology in the same transcriptional orientation as *int*-1 or to the left of the probe C region in the opposite orientation. In all eight tumours, additional mapping, illustrated here for a few tumours with *Kpn*I and probe D, showed that the latter possibility was correct (Fig. 1*c*, *d*). *Kpn*I cleaves the *int*-1 domain at the most leftward site we have mapped (position −23) and at position −11, to generate a fragment of 12 kb that hybridizes with probe D. The MMTV provirus is cut once by *Kpn*I. Novel *Kpn*I fragments annealing with probe D (Fig. 1*c*) were also detected by the MMTV-*env* probe (Fig. 1*d*), demonstrating that the proviruses are inserted upstream from the probe C homology region in orientations opposite to the transcriptional direction of *int*-1.

© 1984 Macmillan Journals Ltd

Table 1 Segregation of mouse *int*-1 and mouse chromosome in Chinese hamster–mouse somatic cell hybrids

Chromosome	Marker enzyme	*Int*-1/chromosome (no. of clones) +/+	+/−	−/+	−/−	% Discordance
1	PEP-3	5	4	1	2	42
2	AK-1	15	2	8	4	34
3	—	2	7	1	2	67
4	PGD	13	5	4	8	30
5	—	2	7	1	2	67
6	TPI-1	3	6	7	5	62
7	GPI-1	4	5	2	1	58
8	GR-1	4	5	1	2	50
9	MOD-1	1	8	1	2	75
10	PEP-2	2	7	2	1	75
11	—	0	9	0	3	75
12	ACP-1	5	4	1	2	42
13	—	4	5	0	3	42
14	NP-1	10	6	5	7	39
15	AS-2	17	0	1	10	4
16	SOD-1	3	6	2	0	73
17	GLO-1	2	7	1	2	67
18	PEP-1	7	2	5	8	32
19	GOT-1	12	6	1	11	23
X	HPRT	15	3	12	0	50
Y	—	0	9	0	3	75

Thirty clones of hybrids formed by polyethylene glycol fusion of mouse spleen cells or macrophages with an established Chinese hamster cell line (380-6), deficient for hypoxanthine phosphoribosyl transferase (HPRT), were analysed for mouse *int*-1 and enzyme markers, although not all clones were analysed for every enzyme marker. Twelve of the hybrid clones were also analysed by karyotype analysis as decribed previously[22]. The symbols for the marker enzymes, their chromosome assignments and the electrophoretic procedures used to separate the Chinese hamster and mouse enzymes have been previously described[22]. In general, the presence or absence of a given mouse chromosome, as determined by karyotypic analysis, agreed with the presence or absence, respectively, of the enzyme marker for that chromosome. However, in several instances, the enzyme marker for a given chromosome was present in a clone that karyotypically lacked that mouse chromosome, indicating chromosome breakage and/or rearrangement. The presence of mouse chromosomes 3, 5, 11, 13 and Y was based on karyotype analysis alone.

Proviruses on the 3′ side of *int*-1

Similar analytical strategies were applied to DNA from 10 tumours previously shown to have insertions between positions 0 and −8 on the *int*-1 map; an 11th tumour (number 24), previously thought to lack an *int*-1 insertion, was also found to have a provirus within this region during the extended tests. Comparative annealings with *int*-1 and MMTV probes (ref. 13 and data not shown) revealed that all 11 proviruses were oriented in the same direction, with transcription proceeding from left to right on the *int*-1 map.

A particularly interesting part of this analysis, pertinent to the mechanism of expression of *int*-1, is illustrated in Fig. 2. When DNA from tumour 53 was cleaved with *Bam*HI and annealed with probe C, two novel fragments were detected in digests of each sample, indicating that the insert is positioned within the region detected by the probe. Similar results were obtained with DNA from tumour 11 (not shown). Proviruses in three other tumours (numbers 55, 12 and 15) were found to be positioned at very similar sites, about 0.6–0.8 kb to the right of the insertion site in tumour 53 (Fig. 2 and unpublished data). The inserts in these tumours lie within the transcriptional unit, and affect the size of resulting transcripts (see below).

We conclude that 19 of 26 C3H mammary tumours carry MMTV proviruses in the *int*-1 locus (Fig. 3). Eight proviruses are distributed between positions −11 and −18; each of these is oriented so that transcription of the provirus proceeds from right to left. Eleven proviruses are located within the region extending from position −8 to 0, and all are oriented so that transcription proceeds from left to right. Mapping with several endonucleases provides no evidence for deletions within either proviral DNA or *int*-1; the several atypical restriction maps of proviral DNA we encountered are best explained by restriction-site polymorphisms (data not shown).

int-1 transcripts

We have previously shown that tumours bearing proviruses in the *int*-1 region contain polyadenylated transcripts that anneal with probe C, whereas no RNA homologous to probe C is detectable in normal mammary glands[13]. The abundance of the *int*-1 transcripts is not great; less than 10 copies per cell have been estimated by three independent methods (unpublished results).

To judge the mechanism by which the insertion mutations activate expression of *int*-1, it was necessary to determine the direction of transcription of the cellular gene. The *Bam*HI–*Eco*RI fragment that constitutes probe C was recloned in both orientations in the bacteriophage vector M13. Restriction mapping of the replicative forms established the orientation of each insert and hence the polarity of strands present in single-stranded virion DNA (data not shown). Radioactive probes were synthesized by random priming on templates provided by two phages, each bearing one of the two strands. Only one of these probes was found to anneal to RNA from tumour 44, previously shown to contain an *int*-1 transcript 2.6 kb in length (Fig. 4*a*); the composition of the probe established the direction of transcription to be from left to right on the *int*-1 map as presented in Fig. 3.

The *int*-1 transcripts characterized in our earlier report were, like those in tumour 44, approximately 2.6 kb long[13]. In a wider survey of all those tumours from which undegraded polyadenylated RNA could be obtained, we discovered atypical *int*-1 transcripts of 3.2 kb in tumour 11 and of 3.8 kb in tumours 12 and 15 (Fig. 4*b*). In these three tumours, no 2.6-kb *int*-1 RNA was found. The conventional 2.6 kb *int*-1 RNA species was present in all the other tested tumours with *int*-1 insertions (for example, in tumours 24 and 13 in Fig. 4*b*) and in one tumour (number 14) in which an *int*-1 mutation has not been found (unpublished data).

The probable composition of the larger, atypical RNA species was suggested by an intriguing correlation with the site of insertion: in tumours 11, 12 and 15, integration of proviral DNA occurred within or near the right-hand boundary of the region represented by probe C (see Fig. 2). (Suitable RNA samples were not available from the other tumours, 53 and 55, with insertions in this region.) If the proviruses were located within the transcribed domain, upstream from the natural polyadenylation site for *int*-1 RNA, the 5′ LTR would be appropriately positioned to provide a surrogate polyadenylation site. The enlarged size of the *int*-1 transcript could then be ascribed to the length of the U3 region of the MMTV LTR (~1.2 kb)[14–16]. A similar situation has been encountered in an avian leukosis virus (ALV) insertion mutant affecting the chicken c-*myc* locus[10].

It was not possible to test this explanation by attempting to anneal a labelled probe for the MMTV LTR directly to gel fractionated RNA, because virus-specific RNA transcribed in a normal fashion from proviral DNA was vastly more abundant than transcripts from *int*-1. We attempted to circumvent this problem by using the 'sandwich hybridization' procedure[10,16] to test for RNA molecules containing MMTV U3 sequences covalently linked to *int*-1 sequences. Restriction fragments of phage DNA containing a portion of the *int*-1 domain were fractionated in an agarose gel, transferred to nitrocellulose filters, and annealed with unlabelled RNA from tumour 12 (containing a 3.2 kb transcript) or from tumour 13 (with a conventional 2.6 kb transcript). After removal of unannealed RNA, the filter was incubated with a ^{32}P-labelled *Pst*I fragment containing virtually all of the MMTV LTR. One autoradio-

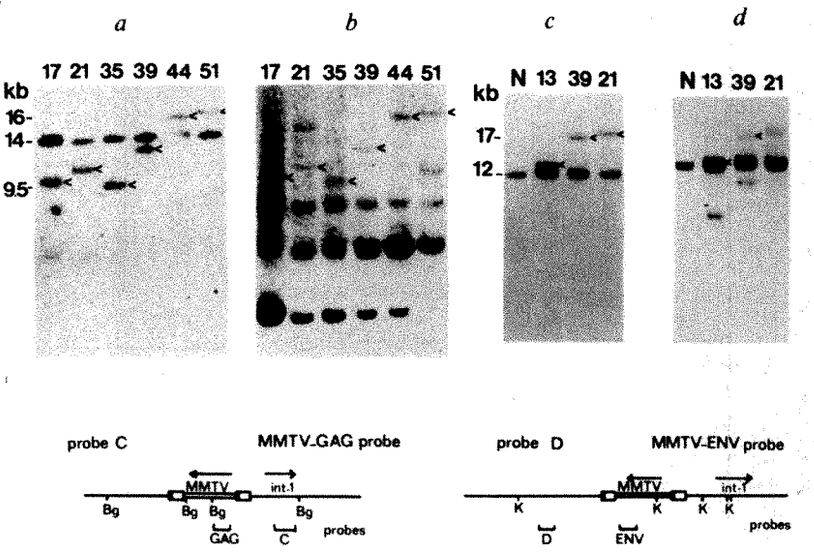


Fig. 1 MMTV proviruses on the 5′ side of the *int*-1 transcriptional unit are in the transcriptional orientation opposite to that of *int*-1. DNA samples (10 μg) from mammary tumours (numbers indicated on top) were digested with *Bgl*II (Bg, panels *a* and *b*) or *Kpn*I (K, panels *c* and *d*), electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose[13]. The filters were first incubated with probe C (*a*) or probe D (*c*), washed and exposed to X-ray film. Subsequently, the probes were removed from the filters and the filters were incubated with MMTV-*gag* probe (*b*) or with MMTV-*env* probe (*d*). The origin of the probes is indicated in the diagrams at the bottom of the figure and in Fig. 3; MMTV-*gag* (3.2 kb) is a *Pvu*II–*Bgl*II fragment obtained from a clone of proviral DNA endogenous to GR mice (ref. 56); MMTV-*env* (1.8 kb) is a *Pst*I fragment derived from MMTV (C3H) DNA and cloned into pBR322 (ref. 57). Arrows indicate tumour-specific bands that show rearrangements of the *int*-1 locus detected with probes C and D and bands in the same position detected with the MMTV probes.

graphic signal, at the position of the *int*-1 fragment homologous to probe C, was observed in the experiment conducted with RNA from tumour 12; no reaction was detected with the sample from tumour 13 (Fig. 4*c*). From this experiment we conclude that *int*-1 and MMTV sequences are covalently joined in transcripts from tumour 12; we presume, but have not proved, that the MMTV sequences are located at the 3′ end of the 3.2-kb transcript in this tumour.

Conservation of the *int*-1 locus

We have proposed that the *int*-1 domain harbours an oncogene that contributes to mammary tumorigenesis following transcriptional activation by proviral integration. However, the locus lacks homology to all tested retroviral oncogenes including *sis*, *ski*, *fos*, *fms* and *yes* (data not shown), as well as the 11 examined previously[13], and it does not anneal with the NIH/3T3 cell transforming gene detected in mammary tumour DNA by Lane *et al.* (ref. 17 and our unpublished results with M. A. Lane and G. M. Cooper). Moreover, we have been unable to measure *int*-1 RNA in a variety of tissues from mature mice (liver, spleen or brain), in cultured mouse cells, or in normal mammary glands from pregnant or lactating females (data not shown). Since no protein product of *int*-1 has been identified, we sought support for the idea that the *int*-1 region contains a coding domain by testing for conserved *int*-1 sequences, homologous to probe C, in the DNA of other vertebrates. As illustrated in Fig. 5, *Bam*HI fragments that anneal effectively with probe C in conditions of only moderately reduced stringency are present in digests of DNA from fish, birds and several mammals, including man. These results are similar to those observed with conserved cellular genes, including the known oncogenes[18]. In addition, as found for the progenitors of some retroviral oncogenes[19], sequences homologous to *int*-1 can be detected in the genome of *Drosophila melanogaster* (lane 1).

Mouse chromosome 15

Specific karyotypic anomalies, some involving putative oncogenes, have recently been found to be associated with a number of different neoplasms[20]. Trisomy of mouse chromosome 13, for example, is frequently observed in MMTV-induced mammary tumours[21]. It was therefore of interest to determine the chromosomal location of *int*-1. To assign *int*-1 to a specific mouse chromosome, we used probe C to detect restriction fragments containing *int*-1 in digests of genomic DNA from 30 Chinese hamster–mouse somatic cell hybrid clones segregating

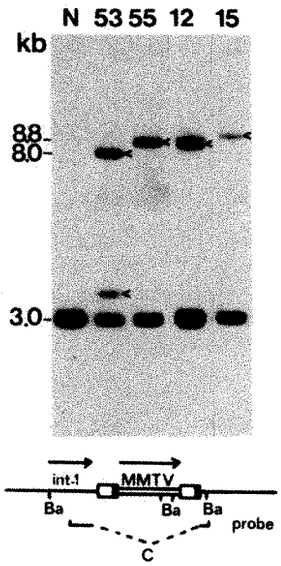


Fig. 2 Proviruses on the 3′ side of *int*-1 are in the same transcriptional orientation as *int*-1. Tumour DNA (number indicated on top, N: normal DNA) was digested with *Bam*HI (Ba), transferred to nitrocellulose filters and annealed with probe C. The sizes of the arrowed, novel restriction fragments and additional mapping data (not shown) show that the proviruses were integrated as drawn at the bottom of the figure. This illustration shows the provirus from tumour 53, providing an explanation for the two novel *int*-1 fragments detected with probe C. Proviruses in the other tumours are located further rightward (see Fig. 3).

mouse chromosomes. These hybrids together contain the full complement of murine chromosomes except for chromosomes 11 and Y (ref. 22). The mouse chromosome complement of each hybrid clone was determined by isozyme and/or karyotype analyses as previously described[22]. The single *int*-1-specific *Eco*RI fragment of 8.6 kb present in digests of genomic mouse DNA could be easily distinguished from the *int*-1-specific *Eco*RI fragment of 2.8 kb derived from Chinese hamster DNA (not shown). An analysis of the segregation of murine *int*-1 and enzyme markers for each of 16 mouse chromosomes (all but 3, 5, 11, 13 and Y) revealed 96% concordant segregation of *int*-1 and arylsulphatase A (AS-1), an enzyme marker for mouse chromosome 15, but discordant segregation of *int*-1 and the

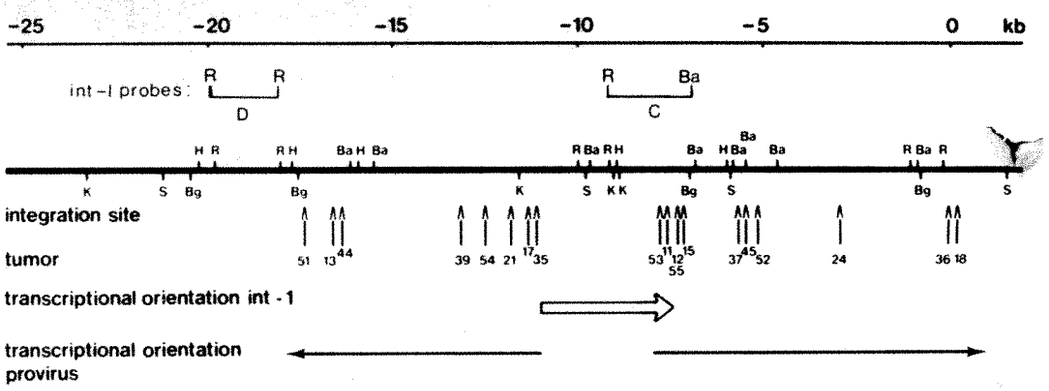


Fig. 3 A map of the proviral integration sites in the *int*-1 locus in C3H mice. Restriction enzyme sites were mapped using C3H and BALB/c chromosomal DNA and DNA from several clones of recombinant bacteriophage containing overlapping inserts from normal BALB/c DNA. Position 0 is defined as the insertion site of the MMTV provirus in tumour 18; this provirus was originally cloned with its flanking DNA to obtain DNA from the *int*-1 locus. Other molecular clones, encompassing positions −22 to +20 on the *int*-1 map, were obtained by sequential screening of a Charon 4A bacteriophage library of normal BALB/c mouse DNA with probes from the *int*-1 region, as described previously[13]. R, *Eco*RI; Ba, *Bam*HI; Bg, *Bgl*II; K, *Kpn*I; S, *Sac*I; H, *Hin*dIII. Probes C and D are fragments of single copy DNA positioned as indicated on the map. Vertical arrows above tumour numbers designate sites of integration. The wide horizontal arrow denotes transcriptional orientation of *int*-1 and the approximate extent of the transcribed domain. The narrow horizontal arrows show the direction of transcription of proviruses integrated in the regions demarcated by the arrows.

other enzyme markers (Table 1). When probe C was removed from the DNA blots and the samples reannealed with a probe for the c-*myc* oncogene (data not shown), we observed perfect concordance between *int*-1 and the mouse *Eco*RI DNA fragment derived from the c-*myc* locus previously assigned to mouse chromosome 15 (refs 23, 24). This finding, consistent with the isoenzyme results, provided further evidence for the assignment of *int*-1 to mouse chromosome 15. Karyotype analyses of 12 selected hybrid clones ruled out an association between *int*-1 and chromosomes 3, 5, 11 13 or Y and were also consistent with the assignment of *int*-1 to chromosome 15 (Table 1). The single discordant clone, which was weakly positive for AS-2 but negative for *int*-1, revealed no evidence of a recognizable mouse chromosome 15. Presumably, this hybrid contains a small, non-recognizable fragment of chromosome 15 which includes AS-2 but not *int*-1. Taken together, these data indicate that *int*-1 is located on mouse chromosome 15.

Implications

The results described here support our previous contention that tumour induction by MMTV involves insertion mutations that activate a previously unknown gene called *int*-1. At the same time they promote confidence in the general proposition that oncogenic viruses lacking viral oncogenes may initiate tumour growth by insertional mechanisms. This hypothesis fosters an experimental strategy for isolation of oncogenes that is formally analogous to what has been termed 'transposon tagging' in other systems[25]: identification of the mutagenic provirus in tumour DNA should lead directly to the isolation of a linked, mutated cellular oncogene. These ideas now receive support from studies of avian B-cell lymphomas induced by at least two different retroviruses[7,11], erythroleukaemia induced by an avian virus that also causes B-cell lymphoma[26], T-cell lymphoma induced by murine leukaemia virus[27] and mammary tumours induced by MMTV[13,28]. At present only circumstantial evidence implicates the affected cellular genes in the oncogenic process: (1) mutation of the same gene in several tumours of the same type; (2) augmented expression and (3) in some cases homology to progenitors of retroviral oncogenes.

Application of the transposon tagging method has led to the discovery of at least two potential mammary oncogenes: the *int*-1 gene commonly implicated in tumours arising in C3H mice and the *int*-2 domain recently found by Peters *et al.*[28] to be interrupted by proviral insertions in about half of the mammary tumours arising in the BR6 mouse strain. These two targets lack evidence of homology with known retroviral oncogenes or with each other, and they are located on different chromosomes (C. Dickson and R. N., unpublished results). In addition, since substantial numbers of tumours lack insertions in either domain, there may be yet other targets for MMTV insertion mutations.

The case for oncogenic roles for *int*-1 and *int*-2 rests primarily on the argument that MMTV proviral DNA appears capable of integration into many different regions in the mouse genome; hence the repeated finding of proviruses at these two loci implies that the tumours are clonal outgrowths of cells[29] with a growth advantage conferred at least in part by the insertion mutations. The case for *int*-1 also rests on the evidence that the locus is transcriptionally active in tumours bearing *int*-1 mutations but not in normal tissues. Although a protein product of *int*-1 has not been identified, a coding function is implied by the conservation of at least part of the transcribed domain during vertebrate evolution (Fig. 5); these findings conform to those obtained with the cellular genes accepted as potential oncogenes by virtue of their homology with retroviral oncogenes.

Our assignment of *int*-1 to mouse chromosome 15 does not offer additional insight into its role as an oncogene: karyotypic abnormalities involving this chromosome have not been reported in mouse mammary tumours, and no endogenous MMTV proviruses have been assigned to chromosome 15 (ref. 30). However, an unrelated oncogene, c-*myc*, also present on mouse chromosome 15, recombines with an immunoglobulin locus on chromosome 12 to produce the translocations found in several plasmacytoma lines[24,31]. Although the distance between *int*-1 and c-*myc* on mouse chromosome 15 is not known, mammary tumours with proviral insertions at *int*-1 do not display abnormal expression of c-*myc* (unpublished data of authors).

The core of the present report concerns the mechanisms by which MMTV proviruses activate expression of *int*-1. In ALV-induced bursal lymphomas, augmented expression of c-*myc* is most commonly achieved by a mechanism referred to as promoter insertion: ALV proviral DNA on the 5′ side of c-*myc* exons in the same transcriptional orientation provides an efficient transcriptional promoter in the form of an LTR[7–10]. However, in the 19 MMTV-induced mammary tumours studied here we have found no instance in which a promoter insertion mechanism activates expression of *int*-1. Instead, MMTV proviruses are positioned either on the 5′ side of the transcriptional template for *int*-1 in the opposite orientation or on the 3′ side in the same orientation. (This rule, however, is not inviolable: in one tumour recently obtained from a BALB/cfC3H mouse, a provirus is present on the 5′ side of the *int*-1 transcriptional domain in the same orientation (R.N., unpublished.)

Previous examples of the two arrangements observed here, in which an LTR cannot serve as promoter for an activated gene, have been described in ALV-induced bursal lymphomas[10]. In these few instances, the effect on expression of c-*myc* was ascribed to a retroviral 'enhancer' element, akin to similar elements first identified in papovavirus genomes[32–38]. Using

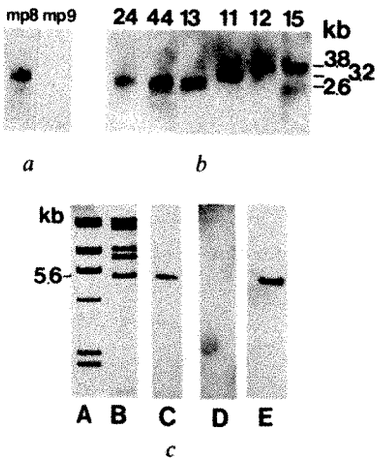


Fig. 4 Analysis of *int*-1 transcription in mammary tumours. *a*, The direction of transcription. 5 μg of polyadenylated RNA from mammary tumour 44 was subjected to electrophoresis in a 1% agarose gel with 2.2 M formaldehyde, transferred to nitrocellulose filters and hybridized with a strand-specific radioactive probe derived by subcloning probe C (a *Bam*HI–*Eco*RI fragment) in the single-stranded bacteriophage derivatives of M13, mp8 and mp9, in both orientations. The autoradiogram shows the hybridization to 2.6-kb RNA with probes made by transcribing the recombinant M13mp8 DNA with reverse transcriptase in the presence of ^{32}P-dCTP with oligomers of calf thymus DNA as random primers. A probe made similarly from the recombinant M13mp9 DNA did not detect a transcript. The direction of transcription of the *Eco*RI–*Bam*HI fragment must proceed from the *Eco*RI site to the *Bam*HI site or from left to right on the *int*-1 map as shown in Fig. 1. *b*, The length of *int*-1 polyadenylated RNA in mammary tumours varies from 2.6 to 3.8 kb. Polyadenylated RNA (5 mg) from mammary tumours with MMTV proviral integrations at the *int*-1 locus was subjected to electrophoresis in 1% agarose gels containing 2.2 M formaldehyde[13] and hybridized with probe C. Tumour numbers are indicated on top (see Fig. 3 for the positions of the proviruses within *int*-1). Tumours 24, 44 and 13 (and also tumours 17, 18, 21, 35, 36, 37, 39, 45) contain a 2.6-kb RNA hybridizing with probe C. Tumour 11 has a 3.2-kb RNA and tumours 12 and 15 have a 3.8-kb RNA species. *c*, Sandwich hybridization demonstrates covalent linkage of *int*-1 sequences to MMTV LTR sequences in tumour 12. DNA from a bacteriophage clone covering the normal *int*-1 region from positions −20 to −3.4 was digested with *Eco*RI and subjected to electrophoresis (lane *B*), next to a marker lane (A) containing wild-type λ DNA digested with *Hin*dIII. DNA was transferred to nitrocellulose filters (several identical filters were prepared from one gel). Direct annealing to radioactive probe C identified a 5.6-kb *Eco*RI fragment (arrow) that contained the *int*-1 transcriptional domain (lane *C*). (This fragment maps from the *Eco*RI site at position −9 in *int*-1 to an *Eco*RI linker at position −3.4.) Other filters were first incubated with unlabelled RNA from tumour 13 (lane *D*) or tumour 12 (lane *E*). These filters were washed and then hybridized to a radioactive probe from the MMTV LTR (a 1.4-kb *Pst*I fragment containing all but 10 bp of the LTR). Only the filter that was annealed first with the RNA from tumour 12 shows hybridization with the *Eco*RI fragment that also hybridized with probe C. Lanes *A* and *B* are photographs of the ethidium-bromide stained gel, the other lanes are autoradiograms.

Methods: 20 μg of poly(A)$^+$ RNA was resuspended in 2 ml of an annealing mix consisting of 50% formamide, 0.5 M NaCl, 20 mM PIPES *p*H 6.8, 5 mM Na_2EDTA, 0.4% SDS, 250 μg ml^{-1} poly(A), 2×Denhardt's solution, and 200 μg ml^{-1} carrier yeast RNA. This solution was applied to the filter-bound DNA and incubated for 24 h at 41 °C. The filter was washed twice at 53 °C for 15 min with 0.1×SSC, 0.1% SDS. The filter was blotted dry, annealed to MMTV–LTR probe (2×10^6 c.p.m.), washed twice for 1 h at 37 °C with 0.1×SSC, 0.1% SDS, dried and autoradiographed.

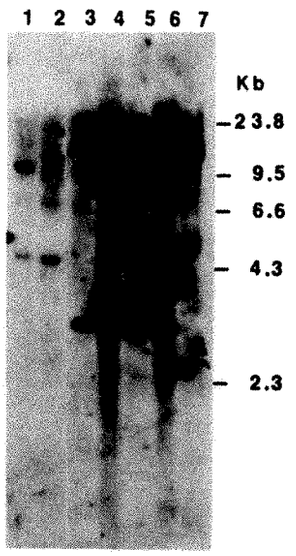


Fig. 5 Sequences homologous to *int*-1 are present in all vertebrates and *Drosophila*. Cellular DNA (10–15 μg) from the indicated sources was digested with *Bam*HI, subjected to electrophoresis in an 0.8% agarose gel, transferred to nitrocellulose filters, and annealed with probe C in 37.5% formamide, 3×SSC, at 42 °C. After washing in 2×SSC at 50 °C, the filter was exposed to X-ray film for 6 days. Lane 1, adult *Drosophila melanogaster* (Oregon-S strain); lane 2, fish (*X. helleri*); lane 3, chicken erythrocytes (strain 15×7); lane 4, mouse liver (BALB/c strain); lane 5, rat hepatoma cells (H4IIE line, ATCC); lane 6, domestic cat liver; lane 7, human mammary epithelial cells (HBL100 line; ATCC). Numbers at the right (in kb) refer to the positions of *Hin*dIII fragments of λ phage DNA present in the same gel.

DNA transformation techniques, retroviral[39–42] and cellular[43] sequences with enhancer activity have been shown to act in *cis*, over variable distances and independent of polarity, to improve the efficiency of heterologous promoters.

Our evidence that MMTV DNA can activate the expression of a previously silent gene in tumours has several of the characteristics of enhancement: the mechanism of activation operates in a manner independent of orientation, over distances of at least 10 kb, and in *cis* rather than *trans*. The basis for claiming *cis* activation is our observation that insertions between positions −8 and −6 on the *int*-1 map are associated only with transcripts of atypically large size (Fig. 4*b*), despite the persistence of the chromosome bearing a normal *int*-1 allele; we conclude that only the physically rearranged locus has been activated. Other aspects of the situation may also be germane to a consideration of mechanism. (1) The observed effect is conversion of an ostensibly silent gene to one expressed at low levels (less than 10 RNA molecules per cell), rather than to amplify expression of a gene normally transcribed. (2) Enhancer functions are likely to be cell specific[44–49] and perhaps target gene specific, so it is plausible that the MMTV enhancer works preferentially in mammary cells and upon *int*-1. (3) Initiation of MMTV transcription is greatly stimulated by glucocorticoid hormones[50], and the hormonal response displays some of the properties of enhancement[51–53]; hence it is possible that activation of *int*-1 is also affected by physiological doses of hormone. This final point should be amenable to direct test by growing mouse mammary tumour cells with *int*-1 insertions in controlled conditions in culture.

The use of an MMTV enhancer function to activate expression of *int*-1 could explain the uniform orientations of proviruses on each side of the proposed transcriptional unit (see Fig. 3). Enhancers are thought to act only or preferentially upon proximal promoters[54,55]; if the MMTV enhancer is located on the 5′ side of the initiation site for viral RNA, within the 1.2-kb U3 sequence of the LTR, then proviruses would require the orientations observed to avoid interposing an MMTV promoter between the enhancer and its *int*-1 target.

We thank P. Borst, C. Dickson, J. Majors and W. Hayward for discussions and comments on the manuscript, M. Simon, M. Schwab and C. Hammond for materials, M. Paape for technical assistance, N. van Nuland and J. Marinos for preparing the manuscript, and E. Stavnezer, C. Sherr, I. Verma, R. Gallo and M. Yoshida for provision of cloned viral oncogenes. A.v.O. was supported by the Netherlands Cancer Society Koningin Wilhelmina Fonds. This work was supported by grants from the NIH and the American Cancer Society (to H.E.V. and D.C.) and Koningin Wilhelmina Fonds (to R.N.).

Received 22 July; accepted 30 September 1983.

1. Varmus, H. E. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 411–503 (Academic, New York, 1983).
2. Varmus, H. E., Quintrell, N. & Ortiz, S. *Cell* **25,** 23–36 (1981).
3. Jenkins, N. A., Copeland, M. G., Taylor, B. A. & Lee, B. K. *Nature* **293,** 370–374 (1981).
4. Copeland, N. G., Jenkins, N. A. & Lee, B. K. *Proc. natn. Acad. Sci. U.S.A.* **80,** 247–249 (1983).
5. Jaenisch, R. *et al. Cell* **32,** 209–216 (1983).
6. Kuff, E. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 1992–1996 (1983).
7. Hayward, W. S., Neel, B. G. & Astrin, S. M. *Nature* **290,** 475–480 (1981).
8. Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. & Astrin, S. M. *Cell* **23,** 323–324 (1981).
9. Payne, G. S. *et al. Cell* **23,** 311–322 (1981).
10. Payne, G. S., Bishop, J. M. & Varmus, H. E. *Nature* **295,** 209–213 (1982).
11. Noori-Daloii, M. R., Swift, R. A., Kung, H. J., Crittenden, L. B. & Witter, R. L. *Nature* **294,** 574–575 (1981).
12. Fung, Y. K. T., Fadly, A. M., Crittenden, L. B. & Kung, K. J. *Proc. natn. Acad. Sci. U.S.A.* **78,** 3418–3422 (1981).
13. Nusse, R. & Varmus, H. E. *Cell* **31,** 99–109 (1982).
14. Shank, P. R., Cohen, J. C., Varmus, H. E., Yamamoto, K. R. & Ringold, G. M. *Proc. natn. Acad. Sci. U.S.A.* **75,** 2112–2116 (1978).
15. Donehower, L. A., Huang, A. L. & Hager, G. L. *J. Virol.* **37,** 226–238 (1981).
16. Dunn, A. R. & Hassel, J. A. *Cell* **12,** 23–36 (1977).
17. Lane, M. A., Sainten, A. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78,** 5185–5189 (1981).
18. Bishop, J. M. & Varmus, H. E. in *The Molecular Biology of Tumor Viruses. Pt III* (eds Weiss, R. A., Teich, N., Varmus, H. E. & Coffin, J. M.) 999–1109 (Cold Spring Harbor Laboratory, New York, 1982).
19. Shilo, B. Z. & Weinberg, R. A. *Proc. natn. Acad. Sci. U.S.A.* **78,** 6789–6797 (1981).
20. Rowley, J. D. *Nature* **301,** 290–291 (1982).
21. Dofuku, R., Utakoji, T. & Matsuzawa, A. *J. natn. Cancer Inst.* **63,** 651–656 (1979).
22. Cox, D. R., Sawicki, J. A., Yee, D., Appella, E. & Epstein, C. J. *Proc. natn. Acad. U.S.A.* **79,** 1930–1934 (1982).
23. Crews, S., Barth, R., Hood, L., L., Prehn, J. & Calame, K. *Science* **218,** 1319–1321 (1982).
24. Sakaguchi, A. Y., Lalley, P. A. & Naylor, S. L. *Somat. Cell Genet.* **9,** 391–406 (1983).
25. Bingham, P. M., Levis, R. & Rubin, G. M. *Cell* **25,** 693–704 (1981).
26. Fung, Y. K., Lewis, W. G., Crittenden, L. B. & Kung, H.-J. *Cell* **33,** 357–368 (1983).
27. Tsichlis, P. N., Gunter-Strauss, P. & Hu, L. F. *Nature* **302,** 445–449 (1983).
28. Peters, G., Brooke, S., Smith, R. & Dickson, C. *Cell* **33,** 369–377 (1983).
29. Cohen, J. C., Shank, P., Morris, V. L., Cardiff, R. & Varmus, H. E. *Cell* **16,** 333–345 (1978).
30. Coffin, J. M. in *The Molecular Biology of Tumor Viruses. Pt III* (eds Weiss, R. A., Teich N., Varmus, H. E. & Coffin, J. M.) 1109–1203 (Cold Spring Harbor Laboratory, Ne York, 1982).
31. Sheng Ong, G. L., Keath, B. J., Piccoli, S. P. & Cole, M. D. *Cell* **31,** 443–452 (1982).
32. Banerji, J., Rusconi, S. & Schnaffer, W. *Cell* **27,** 299–308 (1981).
33. Moreau, P., Hen, R., Everett, R. & Gaub, M. P. *Nucleic Acids Res.* **9,** 6047–6069 (1981).
34. DeVilliers, J. & Schaffner, W. *Nucleic Acids Res.* **9,** 6251–6264 (1981).
35. Capecchi, M. R. *Cell* **22,** 479–488 (1980).
36. Weiher, H., Konig, M. & Gruss, P. *Science* **219,** 626–631 (1983).
37. Lusky, M., Berg, L., Weiher, H. & Botchan, M. *Molec. Cell. Biol.* **3,** 1108–1122 (1983)
38. Fromm, M. & Berg, P. *Molec. cell. Biol.* **3,** 991–999 (1983).
39. Levinson, B., Khoury, G., Vande Woude, G. & Gruss, P. *Nature* **295,** 568–572 (1982).
40. Kriegler, M. & Botchan, M. *Molec. cell. Biol.* **3,** 325–339 (1983).
41. Jolly, P. J., Esty, A. C., Sbramani, S., Friedmann, T. & Verma, I. M. *Nucleic Acids. Re.* **11,** 1855–1872 (1983).
42. Luciw, P., Bishop, J. M., Varmus, H. E. & Capecchi, M. *Cell* **33,** 705–716 (1983).
43. Conrad, S. E. & Botchan, M. *Molec. cell. Biol.* **2,** 949–965 (1982).
44. Fujimura, F. K., Deininger, P. L., Friedman, T. & Linney, E. *Cell* **23,** 809–814 (1981).
45. Katinka, M., Vasseur, M., Montreau, N., Yaniv, M. & Blangy, D. *Nature* **290,** 720–72 (1981).
46. Sekikawa, K. & Levine, A. J. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1100–1104 (1981).
47. Laimins, L. A., Khoury, G., Gorman, C., Howard, B. & Gruss, P. *Proc. natn. Acad. Sc U.S.A.* **79,** 6453–6456 (1982).
48. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33,** 717–728 (1983).
49. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33,** 729–740 (1983).
50. Varmus, H. E., Ringold, G. & Yamamoto, K. R. in *Glucocorticoid Hormone Action* (ed Baxter, J. D. & Rousseau, G. G.) 253–289 (Springer, New York, 1979).
51. Chandler, V. L., Maler, B. A. & Yamamoto, K. R. *Cell* **33,** 489–499 (1983).
52. Hynes, N., Van Ooyen, A. J. J., Kennedy, N., Herrlich, P., Ponta, H. & Groner, B. *Pro natn. Acad. Sci. U.S.A.* **80,** 3637–3641 (1983).
53. Majors, J. E. & Varmus, H. E. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5866–5870 (1983).
54. Wasylyk, B., Wasylyk, C., Augereau, P. & Chambon, P. *Cell* **32,** 503–514 (1983).
55. DeVilliers, J., Olson, L., Banerji, J. & Schaffner, W. *Cold Spring Harbor Symp. Quan Biol.* **47,** 911–920 (1982).
56. Hynes, N. E., Kennedy, N., Rahmsdorf, V. & Groner, B. *Proc. natn. Acad. Sci. U.S.A* **78,** 2038–2041 (1981).
57. Majors, J. E. & Varmus, H. E. *Nature* **289,** 253–258 (1981).

Correlation between segmental flexibility and effector function of antibodies

V. T. Oi, T. M. Vuong, R. Hardy, J. Reidler, J. Dangl, L. A. Herzenberg & L. Stryer

Department of Structural Biology, Sherman Fairchild Center and the Department of Genetics, Stanford University School of Medicine, Stanford California 94305, USA

Mouse monoclonal anti-dansyl antibodies with the same antigen-binding sites but different heavy chain constant region were generated. The extent of segmental flexibility in times of nanoseconds and the capacity to fix complement wer greatest for IgG2b, intermediate for IgG2a, and least for IgG1 and IgE. Hence, the effector functions of immunoglobuli isotypes may be controlled in part by the freedom of movement of their Fab arms.

IMMUNOGLOBULIN G is a flexible Y-shaped molecule. The two antigen-binding Fab units of IgG are joined to an Fc unit at a hinge that allows the angle between the Fab parts to vary over a broad angular range, as shown by hydrodynamic[1], electron microscopic[2,3], and X-ray crystallographic studies[4–6]. Nanosecond fluorescence polarization measurements have demonstrated that immunoglobulin molecules exhibit segmental flexibility in the nanosecond time range[7–9]. Segmental flexibility is likely to be important in enabling immunoglobulins to bind optimally to multivalent antigens and to carry out certain effector functions. Studies of polyclonal antibody populations have suggested that immunoglobulin classes differ in their degree of segmental flexibility[10–11]. We have explored the relationship between hinge motion and effector function in specially constructed families of homogeneous immunoglobulins. Mouse monoclonal anti-dansyl antibodies with the same antigen-combining sites but different heavy chain constant regions (Fig. 1) were generated by selecting somatic variants in hybridoma cell lines. The extent of segmental flexibility in times of nanoseconds (measured by fluorescence spectroscopy) and the capacity to fix complement were greatest for IgG2b, intermediate for IgG2a, and least for IgG1 and IgE. Hence, the effector functions of immunoglobulin isotypes may be controlled in part by the freedom of movement of their Fab arms.

In most antibody-producing hybridoma cell lines, variant cell arise which produce antibody containing a different heav chain[12]. For example, cells producing IgG2b sometimes aris from a cell line producing IgG1. The frequency of these heavy chain switch variants is ordinarily low (10^{-5}–10^{-6} per gener ation). These rare cells can be separated by fluorescence-acti vated cell sorting on the basis of the appearance of a differen heavy chain on their cell surface[13,14]. In these switch variants the light chain remains the same, whereas V_H becomes joine to a different C_H. Consequently, the antigen-combining site i the same as in the parent, despite the change in the heavy-chai constant region. Many of these switch variants are stable an can be cloned.

We chose to generate a family of homogeneous mouse anti dansyl (DNS) antibodies for two reasons. First, the fluorescenc emission spectrum of the bound dansyl chromophore is ver responsive to the polarity of its environment[15], making it convenient and sensitive indicator of whether the antigen combining site of the switch variant antibody is in fact the sam as that of the parent line. Second, the bound dansyl chromophor has an excited state lifetime suitable for determining the seg mental flexibility of immunoglobulin isotypes by nanosecon fluorescence polarization spectroscopy[16]. Variant hybridom cell lines producing different immunoglobulin isotypes wer

0028–0836/84/020136—05$01.00

© 1984 Macmillan

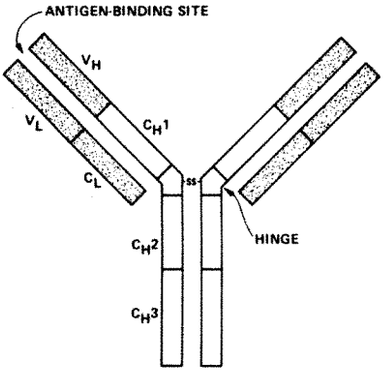


Fig. 1 Schematic diagram of immunoglobulin G. In each family of isotypes, the V_L, C_L and V_H regions are the same. The hinge is located between the C_H1 and C_H2 domains.

Table 1 Mean rotational correlation times of immunoglobulin isotypes and their Fab fragments

	$\langle\phi\rangle$ (ns)	
	No DTT	With DTT
DNS1 family		
Intact immunoglobulin		
IgE	124	88
IgG1	81	58
IgG2a	60	51
IgG2b	47	42
Fab fragment		
IgG1	28	28
IgG2a	28	28
IgG2b	29	29
DNS3 family		
Intact immunoglobulin		
IgG1	93	55
IgG2b	46	45

selected by using affinity-purified goat and rabbit anti-mouse heavy-chain sera and a panel of hybridoma anti-mouse immunoglobulin allotype antibodies[14]. IgG2b, IgG2a and IgE anti-DNS antibody-producing cell lines were isolated starting with the mouse IgG1 anti-DNS hybridoma cell line DNS1 (Igh^a allotype, also designated as 27-44). Our previous nanosecond fluorescence study[16] showed that IgG1 from this mouse cell line is more rigid than is polyclonal rabbit anti-dansyl antibody. The cell lineage of the DNS1 family is: IgG1 (27-44) → IgG2b (27-35) → IgGa (27-13) → IgE (27-74). A cell line containing a different IgG1 anti-dansyl combining site, DNS3 (Igh^b allotype, also designated as 44-10), served as the source of another anti-dansyl IgG2b (44-32).

Two-dimensional gel electrophoresis of antibodies[17,18] produced by the DNS1 family shows that the heavy chain synthesized by each member has the electrophoretic mobility expected for γ1, γ2b, γ2a and ε chains (Fig. 2). Also, these gels show that the light chains produced by this family are identical. Corresponding results were obtained for the DNS3 family. Heavy chains synthesized by these variant cells in the presence of tunicamycin, a known inhibitor of asparagine-linked glycosylation[19-21], have a greater electrophoretic mobility than do those synthesized in its absence. This finding suggests that each variant isotype is normally glycosylated in the absence of tunicamycin. The fluorescence emission spectra of ε-dansyl-L-lysine bound to switch variants of the DNS1 cell line are identical to that of the parent cell antibody (Fig. 3), strongly suggesting that the DNS1 family has the same antigen-combining site. Likewise, both DNS3 isotypes have identical dansyl fluorescence emission spectra. Also, the dissociation constant of the antibody-hapten complex is the same within the DNS family ($K_d = 17 \pm 1$ nM) and the DNS3 family ($K_d = 3 \pm 1$ nM).

Segmental flexibility

The segmental flexibility of these anti-dansyl immunoglobulins was determined by measuring their fluorescence polarization kinetics in the nanosecond time range[7,16,22]. A solution of immunoglobulin containing bound ε-dansyl-L-lysine is excited by a picosecond pulse of vertically-polarized light. Photoselection produces an ensemble of excited choromophores preferentially aligned in the vertical direction. The orientations of these excited molecules then become randomized by rotational brownian motion. The rate of randomization depends on the size and shape of the immunoglobulin, and also on its modes of flexibility. These motions can be monitored by measuring the intensities of the vertically-polarized [$y(t)$] and horizontally-polarized [$x(t)$] components of the fluorescence emission as a function of time following the excitation pulse. The most informative parameter is the emission anisotropy $A(t)$, which is defined as

$$A(t) = \frac{y(t) - x(t)}{y(t) + 2x(t)}$$

The emission anisotropy kinetics of a chromophore rotating in common with a rigid sphere is given by $A(t) = A_0 \exp(-t/\phi)$, where A_0 is the initial value of the emission anisotropy and ϕ is the rotational correlation time. For a nonspherical particle, $A(t)$ is more complex, consisting of up to five exponential terms. For a rigid ellipsoid, $A(t)$ is a sum of three exponential decays that depend on the volume and axial ratio of the ellipsoid and on the directions of the transition moment of the emitting chromophore. A rigorous theory for the emission anisotropy kinetics of rigid and segmentally flexible Y-shaped molecules has not yet been developed. Emission anisotropy data for immunoglobulins have instead been interpreted phenomenologically by comparing observed $A(t)$ curves with those

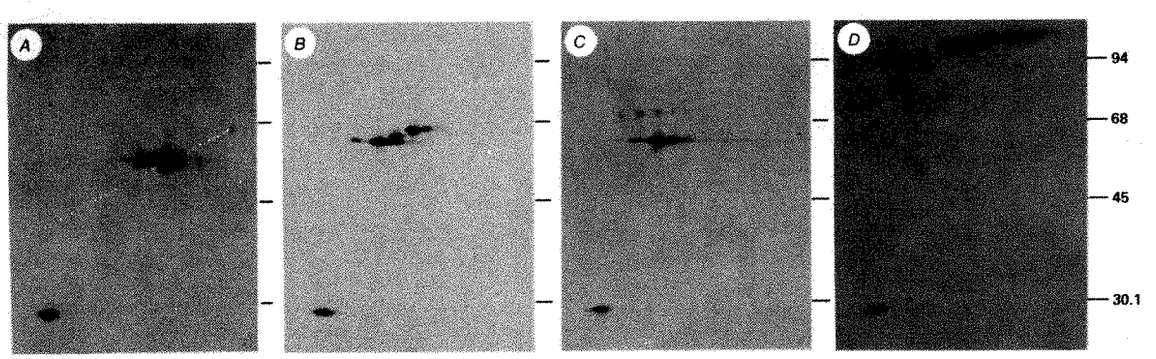


Fig. 2 Two-dimensional gel electrophoresis autoradiogram patterns of antibodies produced by the DNS1 family. *A*, IgG1; *B*, IgG2b; *C*, IgG2a; and *D*, IgE. The horizontal direction corresponds to a non-equilibrium *p*H gradient (low *p*I at the left) and the vertical direction corresponds to sodium dodecyl sulphate polyacrylamide gel electrophoresis[17,18]. Antibodies were labelled biosynthetically with ^{35}S-methionine. Secreted anti-dansyl antibodies in the culture supernatants were precipitated with monoclonal antibodies specific for γ1 (*A*), γ2b (*B*), γ2a (*C*) and a rabbit anti-mouse IgE (*D*). The spot in the lower left-hand corner appears in the same position in each autoradiogram, providing strong confirmation that the κ light chain is the same in each. The multiple spots in the upper half of each gel are typical for myeloma and hybridoma heavy chains. The heterogeneity probably arises from differences in deamidation and glycosylation.

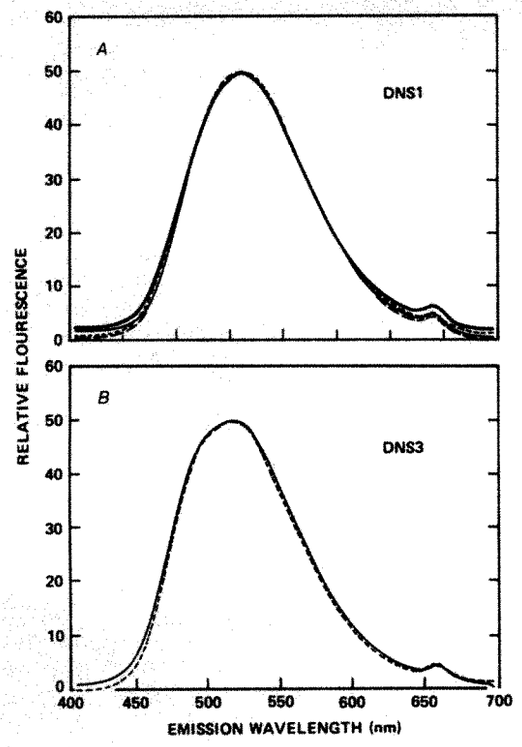


Fig. 3 Fluorescence emission spectra of ε-dansyl-L-lysine bound to isotypes of the DNS1 (*A*) and DNS3 (*B*) families. The spectra of isotypes within a family are virtually superposable. The excitation wavelength was 340 nm.

calculated for rigid spheres and ellipsoids. In this regard, the mean rotational correlation time $\langle\phi\rangle$ can be informative. $A(t$ is fitted to a sum of two exponential decays, $a_1 \exp(-t/\phi_1) + a_2 \exp(-t/\phi_2)$, to give $\langle\phi\rangle$, which is equal to $(a_1\phi_1 + a_2\phi_2)/(a_1 + a_2)$. Rigid nonspherical particles have a $\langle\phi\rangle$ greater than ϕ for a rigid sphere of the same volume. Consequently, a particle irrespective of shape, must be flexible if its $\langle\phi\rangle$ is less than ϕ_{sphere}

Emission anisotropy kinetics were measured using a mode locked argon-ion laser and synchronously pumped rhodamin 6G dye laser as the excitation source and a single-photon counting detection system, as described previously[16]. Picosecond pulses at 325 nm were produced by doubling the frequency of the 650 nm output of the dye laser. All fluorescence studies were carried out at 20 °C. $A(t)$ curves for the DNS1 family are shown in Fig. 4*A*. The striking finding is that the IgG1, IgG2a and IgG2b isotypes of this family display markedly different $A(t$ curves. Their $\langle\phi\rangle$ values are 81, 60 and 47 ns, respectively. These immunoglobulins have virtually identical molecular weights and sedimentation coefficients (6.8, 6.67, and 6.70, respectively; V Schumaker and M. Phillips, personal communication), which means that their molecular volumes and time-averaged shapes are nearly the same. Furthermore, the Fab fragments obtained from these isotypes have nearly the same $\langle\phi\rangle$ values (Table 1) The orientation of the bound dansyl chromophore with respect to the long axis of the Fab unit is identical or nearly so in these isotypes because they possess identical antigen-combining sites Thus, the large difference in the $A(t)$ curves (Fig. 4*A*) are almost certainly due to different extents of segmental flexibility in the intact immunoglobulins. IgG1 is the most rigid, IgG2a is intermediate, and IgG2b is the most flexible of the three isotypes

IgE is like IgG1 in being more rigid than IgG2a and IgG2b (Fig. 4*A*). The $\langle\phi\rangle$ value for IgE can be compared with those of the three IgG isotypes by scaling its $\langle\phi\rangle$ to take into account its 190,000 molecular weight[23] compared with 150,000 for IgG

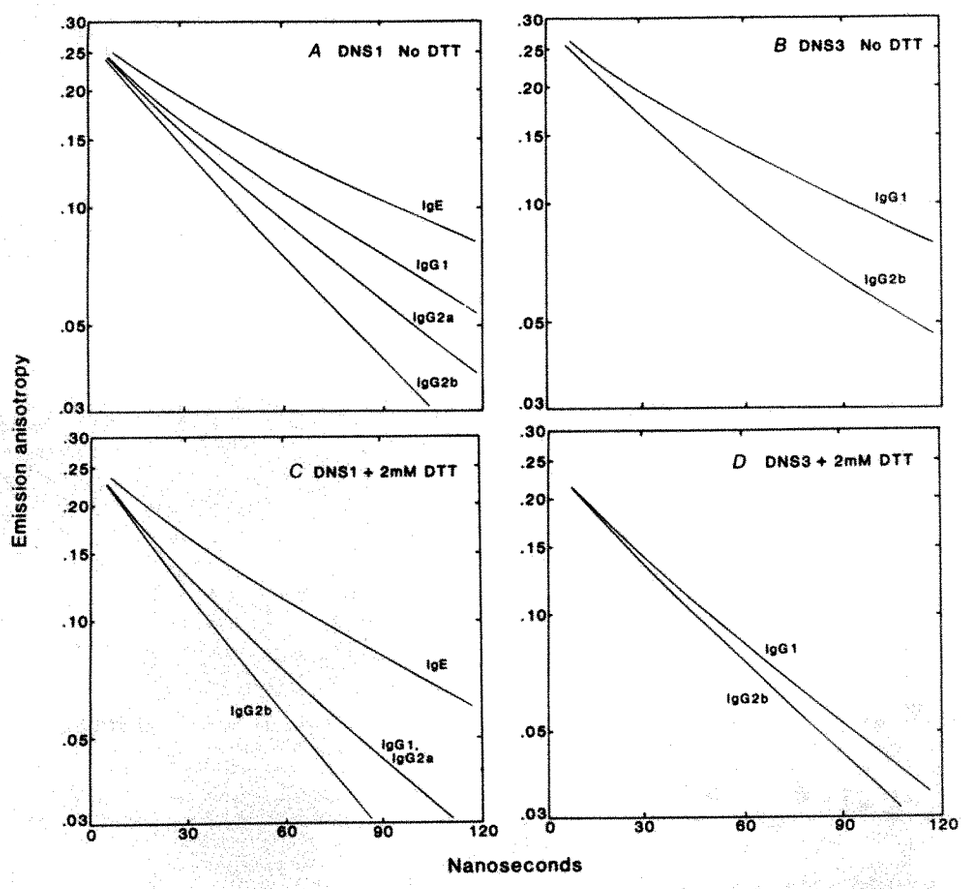


Fig. 4 Nanosecond emission anisotropy kinetics of ε-dansyl-L-lysine bound to immunoglobulins of the DNS1 family in the absence (*A*) and presence (*B*) of 2 mM DTT; and the DNS3 family in the absence (*C*) and presence (*D*) of 2 mM DTT. The temperature was 20 °C.

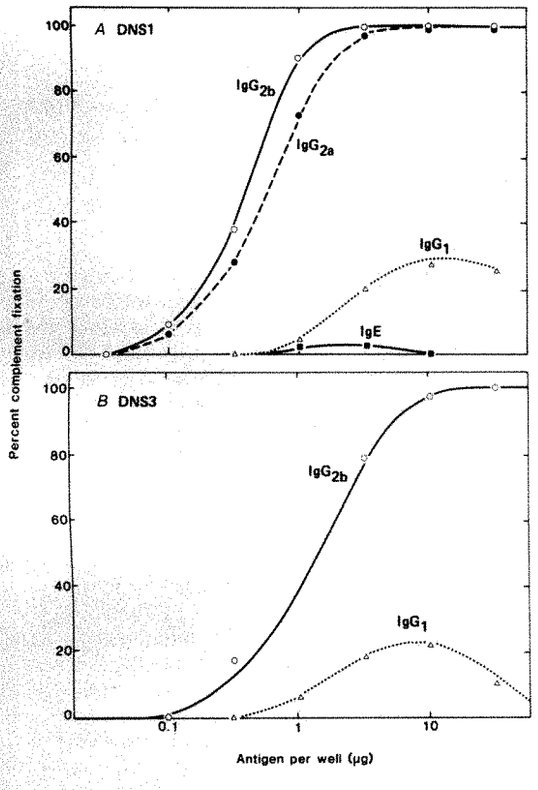


Fig. 5 Complement-fixation by the DNS1 (*A*) and DNS3 (*B*) family. A microcomplement consumption assay using ^{51}Cr release from haemolysin-treated sheep erythrocytes similar to that described previously[25] was used to measure complement fixation by the DNS1 and DNS3 family. Immunoglobulin (10 μg per 25 μl), DNS-bovine serum albumin dilutions (in 25 μl) and complement (2 CH-50 units in 25 μl) were combined in wells of a 96 well microtitre plate and incubated for 45 min at 37 °C. Next, 50 μl of a 2% solution of activated, ^{51}Cr-loaded erythrocytes was added to each well and after a further 45 min incubation at 37 °C the non-lysed cells were pelleted by centrifugation of the plates. 50-μl aliquots of supernatant were collected from each well and counted on a gamma counter set for ^{51}Cr.

The scaled $\langle\phi\rangle$ value for IgE is $124\times(150/190)=98$ ns, which is even larger than the 81 ns value for IgGl. The sedimentation coefficient of 8.35 S for this IgE (V. Schumaker and M. Phillips, personal communication) corresponds to 6.59 S when scaled for the molecular weight difference between IgE and IgG. This is consistent with the time-averaged shape (as expressed by the translational diffusion coefficient) of IgE being very similar to that of the three IgG isotypes.

Reduction of the disulphide bonds between the heavy chains in the vicinity of the hinge between the Fab and Fc units[16,24] provided additional information concerning the degree of segmental flexibility of the isotypes. The addition of 2 mM dithiothreitol (DTT) leads to a large decrease in $\langle\phi\rangle$ for IgE and IgG1 (Fig. 4*B*, Table 1). Reduction of the interchain disulphides lowers $\langle\phi\rangle$ of IgE from 124 to 88 ns, and that of IgG1 from 81 to 58 ns. DTT has no effect on $\langle\phi\rangle$ for the Fab fragment of IgG1. Thus, DTT lowers $\langle\phi\rangle$ of intact IgG1 by opening up a restricted hinge region. In contrast, DTT has almost no effect on $\langle\phi\rangle$ of IgG2a and IgG2b because the Fab units of these antibody molecules have a high degree of rotational freedom even before reduction of the interchain disulphides.

A large difference in segmental flexibility was also observed within the DNS3 family (Fig. 4*C* and Table 1). IgG2b is again much more flexible than IgG1, as evidenced by their $A(t)$ curves and $\langle\phi\rangle$ values. Also, DTT markedly decreases $\langle\phi\rangle$ of IgG1 and has little effect on $\langle\phi\rangle$ of the more flexible IgG2b (Fig. 4*D* and Table 1).

Complement-fixation effectiveness

The ability of these antibody molecules to fix complement was measured using a micro-complement consumption assay[25]. Dansylated bovine serum albumin served as the antigen. In the DNS1 family, IgG2b is most effective in fixing complement, and IgG2a is also highly effective (Fig. 5*A*). In constrast, IgG1 fixes less complement and required ten-fold more antigen to trigger complement fixation. IgE exhibits negligible complement-fixation activity. Likewise, in the DNS3 family, IgG2b fixes more complement than does IgG1 and does so at a lower concentration of antigen (Fig. 5*B*). The isotypes that fix complement most effectively, IgG2a and IgG2b, also exhibit the highest degree of segmental flexibility.

Two families of immunoglobulin isotypes with identical antigen-binding sites were constructed using somatic cell genetics techniques. Nanosecond fluorescence polarization studies of these monoclonal anti-dansyl isotypes have revealed striking differences in their extents of segmental flexibility. IgG2b is the most flexible, IgG2a is less flexible, and IgG1 and IgE are relatively rigid. The degree of molecular motion at the hinge in times of nanoseconds is correlated with the ability of these molecules to fix complement. This correlation may be more than fortuitous. Segmental flexibility may promote complement fixation by enhancing multivalent binding, facilitating the transmission of conformational changes from the Fab units to the effector domain on the Fc unit, or enabling the Fc unit to assume a conformation favourable for complement fixation. It will be very interesting to further explore the relationship between flexibility and effector function by engineering a series of immunoglobulins in which the hinge is deleted, elongated, or modified to alter the rotational mobility of the Fab arms. For example, it would be instructive to study a chimaeric immunoglobulin containing an IgG1 hinge and IgG2b C_H2 and C_H3 domains. We predict that such an immunoglobulin would fix complement less effectively than a normal IgG2b molecule. Segmental flexibility is likely to be one factor correlated with complement fixation. The construction of immunoglobulin genes with novel combinations of domains or with site-specific mutations and their subsequent insertion into lymphoid cell lines should lead to deeper insights into how immunoglobulins mediate their distinctive functions. Methods for expressing designed immunoglobulins have recently been developed[26,27].

Monoclonal antibodies directed against tumour-specific antigens are now being used to treat certain malignancies[28]. It will be important to learn whether the therapeutic effectiveness of a tumour-specific antibody depends on its isotype as well as on its antigen-binding properties. The capacity of such antibodies to interact optimally with tumour-specific antigens on cell surfaces may be influenced by their segmental flexibility.

We thank Tammy Guion for technical assistance and Drs Verne Schumaker and Juan Yguerabide for discussions. This research was supported by research grants from NIH (GM-24032 to L.S. and CA-04681, AI08917, and GM-17367 to L.A.H.). Fellowship support was provided by NIH (GM-07276 to T.M.V. and AI-06144 to V.T.O.), the Cystic Fibrosis Foundation (to J.R.) and the California Division of the American Cancer Society (J-22-82 to R.R.H.).

Received 22 August; accepted 5 October 1983.

1. Noelken, M. E., Nelson, C. A., Buckley, C. E. & Tanford, C. *J. biol. Chem.* **240,** 218–224 (1965).
2. Valentine, R. C. & Green, N. M. *J. molec. Biol.* **27,** 615–617 (1967).
3. Feinstein, A. & Rowe, A. J. *Nature* **205,** 147–149 (1965).
4. Marquart, M., Deisenhofer, J., Huber, R. & Palm, W. *J. molec. Biol.* **141,** 369–391 (1980).
5. Amzel, L. M. & Poljak, R. J. *A. Rev. Biochem.* **48,** 961–967 (1979).
6. Silverton, E. W., Navia, M. A. & Davies, D. M. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5140–5144 (1977).
7. Yguerabide, J., Epstein, H. E. & Stryer, L. *J. molec. Biol.* **51,** 573–590 (1970).
8. Cathou, R. E. *Compreh. Immun.* **5,** 37–83 (1978).
9. Hanson, D. C., Yguerabide, J. & Schumaker, V. N. *Biochemistry* **20,** 6842–6852 (1981).
10. Cebra, J. J. *et al. Proc. Robert A. Welch Foundation XVIII Immunochem,* Houston, Texas (ed. Milligan, W. O.) 67–96 (Welch Foundation, 1975).
11. Dudich, E., Nezlin, R. S. & Franek, F. *FEBS Lett.* **89,** 89–92 (1978).
12. Coffino, P., Laskov, R. & Scharff, M. D. *Science* **167,** 186–188 (1970).
13. Liesegang, B., Radbruch, A. & Rajewsky, K. *Proc. natn. Acad. Sci. U.S.A.* **75,** 3901–3905 (1978).

14. Dangl, J. L., Parks, D. R., Oi, V. T. & Herzenberg, L. A. *Cytometry* **2**, 395–401 (1982).
15. Stryer, L. *Science* **162**, 526–533 (1968).
16. Reidler, J. *et al. J. molec. Biol.* **158**, 739–746 (1982).
17. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. *Cell* **12**, 1133–1142 (1977).
18. Oi, V. T., Bryan, V. M., Herzenberg L. A. & Herzenberg, L. A. *J. exp. Med.* **151**, 1260–1274 (1980).
19. Hickman, S. & Kornfeld, S. *J. Immun.* **121**, 990–996 (1978).
20. Edelman, G. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **63**, 78–85 (1969).
21. Kornfeld, R. & Kornfeld, S. *A. Rev. Biochem.* **45**, 217–237 (1976).
22. Yguerabide, J. *Meth. Enzym.* **26C**, 498–577 (1972).
23. Ishida, N., Ueda, S., Hayashida, H., Mitata, T. & Honjo, T. *EMBO J.* **1**, 1117–1123 (1982).
24. Chan, L. M. & Cathou, R. E. *J. molec. Biol.* **112**, 653–656 (1977).
25. Bengali, Z. H., Das, S. R. & Levine, P. H. *J. immun. Meth.* **33**, 63–00 (1980).
26. Oi, V. T., Morrison, S. L., Herzenberg, L. A. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **80**, 825–829 (1983).
27. Rice, D. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7862–7865 (1982).
28. Miller, R. A. & Levy, R. *Lancet* **i**, 226–229 (1981).

LETTERS TO NATURE

Large anisotropy in the Universe does not prevent inflation

Marek Demianski*

Center for Relativity, Department of Physics,
The University of Texas at Austin, Austin, Texas 78712, USA

The inflationary model in which the Universe passes through an exponentially expanding phase can explain a few cosmological conundrums of the standard big-bang model. It is therefore interesting to study the stability of this model. It has been demonstrated that the inflationary scenario is stable with respect to density, velocity and gravitational wave perturbations[1–3]. Here I show that the new inflationary model is compatible with large initial anisotropy. The initial anisotropic energy density could be many orders of magnitude larger than the initial radiation energy density.

The standard hot big-bang model of the Universe is remarkably simple and provides an amazingly accurate description of its evolution. There are, however, several observational facts which remain unexplained in this model, for example, the present high degree of isotropy of the microwave background radiation, the large scale homogeneity, and near critical energy density of the Universe.

The inflationary model of the early evolution of the Universe[4–6] explains these facts without altering the basic predictions of the big bang model. Guth[4] noticed that the first-order phase transition predicted by grand unified theories (GUTs), which occurs when temperature T is of the order of 10^{14} GeV, could have profound cosmological consequences. The inflationary scenario requires Higgs field effective potential $V(\phi)$ to have a global minimum at zero temperature. This global minimum is called the true vacuum. The zero temperature effective potential should also have a second metastable extremum at $\phi=0$ (the false vacuum). Furthermore, one assumes that there is a critical temperature $T_c \approx 10^{14}$ GeV above which the finite temperature effective potential has a lower value near the false vacuum than it has near the true vacuum. For any small temperature $T>0$ the false vacuum is stabilized by a barrier in the finite temperature effective potential. The height of this barrier is of the order of T^4 and its width is of the order of T.

If at the very early stages of evolution the Universe contained a hot region ($T>10^{14}$ GeV), which was isotropically expanding fast enough to cool to T_c before gravitational effects could cause it to collapse, then this region will cool to T_c and supercool. The constant false vacuum energy density determines then the rate of expansion and this region expands exponentially. This expansion can be described by the de Sitter metric with the false vacuum energy density having the role of a cosmological constant.

The Higgs field fluctuates around the false vacuum. Some fluctuations begin to grow and at some point become large enough to be described by a classical evolution equation. At the moment when evolution of fluctuations could be described by the classical equation the fluctuations should be small. If the fluctuations grow very slowly, allowing many e folding times to pass, this single fluctuation region could grow to become larger than the present Universe. Finally, having reached the steep part of the potential, the field rapidly rolls down and oscillates about the true vacuum position. These oscillations are quickly damped out through coupling to other fields and the released energy is thermalized. The Universe reheats to temperature $T \approx T_c \approx 10^{14}$ GeV. The subsequent evolution follows the standard model.

The inflationary scenario can explain several cosmological conundrums, namely the isotropy of the microwave background radiation (the horizon problem), the observed large-scale homogeneity, the near critical energy density, and small number density of magnetic monopoles[7].

It has been demonstrated that the inflationary scenario is stable with respect to inhomogeneous density, velocity, and gravitational wave perturbations[1–3]. Hawking and Moss[8] suggested that there is a cosmological 'no hair' theorem and the de Sitter space is dynamically stable. Barrow and Turner[9] pointed out that in the inflationary model proposed by Guth[4] large anisotropy prevents the de Sitter phase from occurring. I now show that the new inflationary scenario can tolerate very large homogeneous initial anisotropy.

Let us consider an anisotropic homogeneous Bianchi type 1 universe filled with radiation and constant false vacuum energy density ρ_v. The evolution of this model is described by

$$\dot{R}^2 = \frac{8\pi G}{3}(\rho_r+\rho_v)R^2 + \frac{8\pi G}{3}\frac{a^2}{R^4} \tag{1}$$

and

$$\rho_r R^4 = \text{constant} \tag{2}$$

where R is related to the volume expansion parameter by $\dot{V}/V = 3\dot{R}/R$, ρ_r is the radiation energy density, and a is a constant related to the initial anisotropy. Equation (1) can be rewritten in the form

$$\frac{dz}{d\tau} = 2\left(z^2+1+\frac{\beta}{z}\right)^{1/2} \tag{3}$$

where

$$\tau = \chi t = \left(\frac{8\pi G}{3}\rho_v\right)^{1/2} t$$

$$z = \left(\frac{R}{R_0}\right)^2 \left(\frac{\rho_v}{\rho_r(0)}\right)^{1/2}$$

and

$$\beta = \frac{a^2}{R_0{}^6\rho_r(0)}\left(\frac{\rho_v}{\rho_r(0)}\right)^{1/2} = \frac{\rho_{an}(0)}{\rho_r(0)}\left(\frac{\rho_v}{\rho_r(0)}\right)^{1/2}$$

where R_0 is a constant, and $\rho_r(0)$ and $\rho_{an}(0)$ are the initial energy densities of radiation and anisotropy. In this equation z^2 is related to the false vacuum energy density, 1 to the radiation energy density, and β/z to the anisotropic energy density.

At the very early stage of expansion, when z is very small, the anisotropic energy density determines the expansion rate of the Universe, so that $z=\beta^{1/3}(3\tau)^{2/3}$. We are interested in a situation when the anisotropic energy density determines the expansion rate of the Universe at an epoch when the Universe

* Present address: Department of Physics and Astronomy, Williams College, Williamstown, Massachusetts 01267, USA. Permanent address: Institute of Theoretical Physics, University of Warsaw, Warsaw, Poland.

0028–0836/84/020140—02$01.00

cools below T_c. When $T \approx T_c$ the radiation energy density and the vacuum energy density are equal, and $z = 1$. The anisotropic energy density will dominate the dynamics of expansion at that epoch if $\beta \gg 1$. Let us assume that the temperature of radiation at the Planck time $t_p = (G\hbar/c^5)^{1/2} = 0.5 \times 10^{-43}$ s is equal to Planck temperature $T_p = 1.2 \times 10^{19}$ GeV, then $\beta \gg 1$ if the initial anisotropic energy density $\rho_{an}(0) \gg 10^{10}\ \rho_r(0)$. The vacuum energy density starts to have a dynamically dominant role when $z = \beta^{1/3}$, $(\tau = \frac{1}{3})$. The de Sitter phase prevails if the small fluctuations of the Higgs field generated at the moment when or after the Universe cools to T_c ($\rho_v = \rho_r$, $z = 1$, $\tau = \frac{1}{3}(\beta)^{1/2}$) do not have enough time to grow significantly by the time when the vacuum energy density starts to determine the expansion rate of the Universe.

Let us assume that the initial fluctuations of the Higgs field are large enough to be described by the classical evolution equation

$$\ddot{\phi} + 3H\dot{\phi} = \lambda\phi^3 \tag{4}$$

where $\lambda \approx \frac{1}{2}$, and H is the Hubble constant, in our case $H = \frac{1}{3}t$. Assuming that initially, when $T = T_c$, $\phi(\tau = \frac{1}{3}(\beta)^{1/2}) = \phi_i$ we obtain an approximate solution of equation (4)

$$\phi = \frac{\phi_i}{\left(1 - \frac{\lambda}{2}\left(\frac{\phi_i}{\chi}\right)^2\left(\tau^2 - \frac{1}{9\beta}\right)\right)^{1/2}} \tag{5}$$

Following Guth[7] we assume that the initial fluctuations are of the order of thermal fluctuations and $\phi_i \approx 0.23\chi$. By the time that the vacuum energy density starts to determine the expansion rate of the Universe the fluctuations grow only insignificantly, $\phi(\tau = \frac{1}{3})/\phi_i - 1 \approx 7 \times 10^{-4}$. Therefore, the vacuum energy survives up to the moment when it starts to have a dominant dynamical role. The Universe enters the de Sitter phase and expands exponentially. The evolution of small fluctuations of the Higgs field in the de Sitter universe has been studied by Guth[7]. He showed that the fluctuations grow slowly and the Universe stays in the false vacuum state for many e folding times. The new inflationary model is, therefore, compatible with very large initial anisotropy. The initial anisotropic energy density can be many orders of magnitude larger than the intial radiation energy density.

One might ask what happens when due to coupling to other fields the anisotropic energy density is dissipated and released in the form of heat. This will only delay the moment of the phase transition but it does not change our main conclusion.

Recently, Futamase[10] noticed that anisotropy will change the form of the effective potential $V(\phi)$. The additional terms are proportional to the anisotropic energy density and they distort the potential in a similar way as the high temperature corrections. Due to this additional bump in the potential the transition from the false to the true vacuum state would be slower, making the inflationary scenario even more plausible.

I thank the members of the Department of Physics of the University of Texas at Austin, Center for Relativity, and Center for Theoretical Physics for hospitality and support.

Received 8 July; accepted 7 October 1983.

1. Frieman, J. A. & Will, C. M. *Astrophys. J.* **259**, 437–441 (1982).
2. Barrow, J. D. in *The Very Early Universe* (eds Gibbons, G., Hawking, S. & Siklos, S.) (Cambridge University Press, 1983).
3. Boucher, P. & Gibbons, G. in *The Very Early Universe* (eds Gibbons, G., Hawking, S. & Siklos, S.) (Cambridge University Press, 1983).
4. Guth, A. H. *Phys. Rev.* **D23**, 347–356 (1981).
5. Linde, A. D. *Phys. Lett.* **108B**, 389–395 (1982).
6. Albrecht, A. & Steinhardt, P. J. *Phys. Rev. Lett.* **48**, 1220–1223 (1982).
7. Guth, A. H. in *The Very Early Universe* (eds Gibbons, G., Hawking, S. & Siklos, S.) (Cambridge University Press, 1983).
8. Hawking, S. & Moss, I. G. *Phys. Lett.* **110B**, 35–38 (1982).
9. Barrow, J. D. & Turner, M. S. *Nature* **292**, 35–38 (1982).
10. Futamase, T. *Coleman-Weinberg Breaking in an Anisotropic Space-time* (Preprint, University College Cardiff, 1983).

Correlation of bristlecone pine ring widths with atmospheric ^{14}C variations: a climate–Sun relation

C. P. Sonett

Department of Planetary Sciences and Lunar and Planetary Laboratory, University of Arizona Tucson, Arizona 85721, USA

H. E. Suess

Department of Chemistry, University of California at San Diego, La Jolla, California 93093, USA

Recent measurements by Stuiver and Quay[1] on wood growth during the past millennium confirm in an impressive way the contention that, in addition to being affected by the geomagnetic dipole moment, the production rate of ^{14}C is mediated by interplanetary modulation arising from solar activity, of which the sunspot index is a measure. But Stuiver[2], like others before him, finds no statistically significant correlation with climate indicators, including ring-width data and $\Delta^{14}C$. However, the data presented here show a case of an unusually convincing correlation between variations of cosmic ray-produced ^{14}C activity of the CO_2 in the terrestrial atmosphere during the past five millennia[3,4] and annual growth ring widths for bristlecone pine wood from one particular area, that of Campito Mountain in eastern California.

The annual values of the Campito Mountain series were supplied by V. C. LaMarche of the University of Arizona Laboratory of Tree Ring Research. Twenty-year averages have been published by Lamb[5]. The bristlecone pine growth ring sequence was determined specifically to investigate the influence of climatic conditions on the growth rates of bristlecone pine trees. This growth ring sequence extends from 3405 BC to AD 1979. The sample trees are from the upper limit of the altitude band in which the bristlecone pine is found on Campito Mountain. For the high altitude (3,384 m) bristlecone pine at the upper growth region limit (from which the bristlecone data discussed here are drawn) the primary stress-induced growth effect is thought to be temperature[6,7].

The correlation of $\Delta^{14}C$ and tree-ring sequences is based on power spectral densities (PSDs) and cross-covariance; the PSDs are discussed first. The power spectrum of $\Delta^{14}C$ deduced from the La Jolla measurements of $\Delta^{14}C$ in samples of bristlecone pine wood from the White Mountains of California, has been published previously[8]. Essentially the same data are used here for a more detailed calculation of the $\Delta^{14}C$ spectrum. Additional comparisons (not shown here) have been made using the $\Delta^{14}C$ series reported by a workshop held at the University of Arizona[9]. A lesser correlation with a limber pine sequence from Mount San Gorgonio in Southern California has also been observed. Wood used for the ^{14}C determinations was preferentially selected from trees with abnormally wide rings.

The $\Delta^{14}C$ La Jolla measurements of bristlecone pine samples, dated dendrochronologically by Ferguson[4], were used. They are unequally spaced in time, and therefore must be detrended and interpolated prior to computation. We first remove the quasi-sinusoid[3] (thought to arise from secular drift in the intensity of the geomagnetic field), followed by application of a cubic spline[10], quadratic detrending and finally interpolation at 10-yr intervals. To avoid extreme tensioning of the spline used for interpolation and yet cover all data points the highest frequency contributions are removed by a ±20 yr moving linear least-squares estimator which replaces each interpolated point by a value which dampens the highest frequency components. The LaMarche bristlecone pine ring-width sequence, consisting of yearly data points, is filtered and decimated by 10; the initial computations were made using the sequences truncated to the

© 1984 Macmillan Journals Ltd

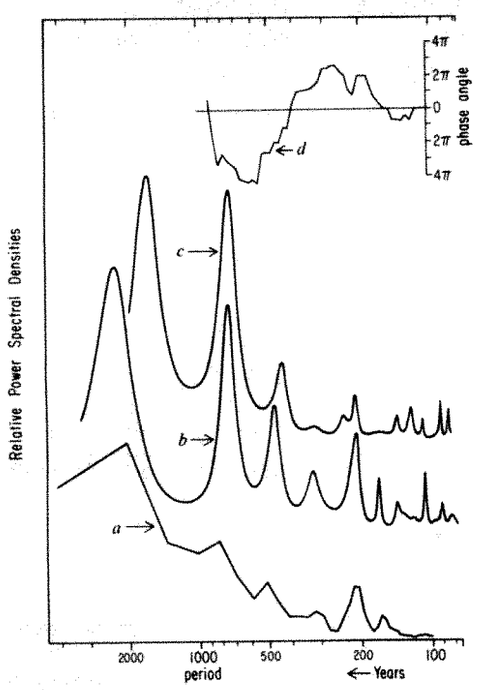


Fig. 1 Power spectral densities of the La Jolla $\Delta^{14}C$ and bristlecone pine tree-ring growth record: *a*, the Neftel *et al.*[8] FFT of the full 8,400-yr record; *b*, the maximum entropy (MEM) spectrum at autoregressive (AR) order 120 of the truncated 5,290-yr (3405 BC–AD 1885) La Jolla record; *c*, the Campito Mountain bristlecone pine (3405 BC–AD 1885) growth ring spectrum at AR order 120; *d*, variation in relative phase between $\Delta^{14}C$ and Campito Mountain bristlecone pine ring growth with frequency and period. The phase is that corresponding to the peak coherence between narrow-banded versions of the original sequences obtained from Fourier spectra filtered to isolate narrow spectral intervals which provide the basis for narrow band $\Delta^{14}C$ and bristlecone pine sequences used for correlation.

maximum common time period (3405 BC–AD 1885; T = 5,290 yr).

As the primary ^{14}C spectrum is 'red' with a slope, using a power law fit, with spectral index approximately -1, and the region of the spectrum of interest here encompasses periods longer than about 80 yr, aliasing is not significant. Filtering and corruption by splining have been studied using Monte Carlo tests and synthetic sequences whose spectra satisfy the 'red' slope characterizing the $\Delta^{14}C$ spectrum. The test sequence realizations are generated using sinusoidal sequences of decreasing mean amplitude, normally distributed, and with random phase[11], upon which may be superimposed signals of specific intensity. These tests show insignificant corruption of the data for periods in excess of several times 10 yr.

The PSDs are computed using the maximum entropy method (MEM)[12,13], although we have confirmed major spectral features using the less constrained Yule–Walker (YW) algorithm[14,15] and also the Singleton fast Fourier transform (FFT)[16]. Both the MEM and YW algorithms involve all-pole filter models which are considered most suitable for data where the major features are spectral lines. Figure 1 shows the PSDs for both the La Jolla $\Delta^{14}C$ and the Campito Mountain bristlecone pine tree-ring sequences. Both have major features in common. As noted the periods of the lines should be regarded as approximate, because the pole positions in the autoregressive (AR) filter are somewhat dependent upon detailed properties of the sequences. (This is thought to be due to the line splitting phenomenon which results in peak shifting depending upon signal to noise ratio[17]. Its correction is under study and is expected to refine the periods reported here.) The La Jolla $\Delta^{14}C$ series has been previously investigated by Kruse[3] and in greater detail by Neftel *et al.*[8] using the standard FFT. The prime purpose of these investigations was to demonstrate that the secular $\Delta^{14}C$ variations are not random and hence cannot b caused by experimental errors. Moreover these investigatior show that the $\Delta^{14}C$ time series contains cyclic features, in pa ticular a prominent spectral line of about 200-yr period. Ou analysis confirms these earlier results in all their details. Becau of its ubiquity, we have centred attention on the 200-yr peric as a key marker of a solar interrelation between the sequenc reported here. Thus the appearance of the 200-yr period in th ^{14}C record, repeated in the tree-ring record, strongly suppor the contention that this solar-based signal is reflected in th growth rate of wood in the bristlecone pines.

The PSDs have normalized amplitudes with the highest pea in each spectrum set to unity for convenience. Table 1 gives th periods of selected lines for each of the two spectra. Althoug MEM estimates are thought to have better intrinsic resolutio than $\Delta f = 1/T$, FFT estimates are conservatively rated at Δf $6/T$. The actual MEM resolution is possibly less than that give above, since MEM carries intrinsic filtering depending inverse upon order; very approximately the MEM frequency resolutic is optimistic by perhaps a factor of 3–4. This asymptotic lim is also given by Ulrych and Bishop[13] based on an analysis Kromer[18]. Thus $\Delta f_r = 2.3\times10^{-4}\,yr^{-1}$ for the bristlecor sequence and $\Delta f_c = 1.9\times10^{-4}\,yr^{-1}$ for La Jolla, assuming r filtering so that $(0)T/20$ independent degrees of freedom a available. Combining the two gives an approximate value $\Delta f_s = 4.2\times10^{-4}\,yr^{-1}$, but more probably greater than $10^{-4}\,yr^{-}$ Inspection of Table 1 discloses that of the lines tabulated, thr pairs differ in frequency by $10^{-4}\,yr^{-1}$, one pair by $3\times10^{-4}\,yr$ and the two high frequency pairs by 7–$8\times10^{-4}\,yr^{-1}$. Thus majority of the line pairs tabulated lie approximately within better than the estimated resolution, and the two exceptio are within two resolution elements.

The calculations reported here have been repeated for th $\Delta^{14}C$ sequence from the Arizona workshop, but for brevity v report only that the agreement is qualitatively reasonable. A a final test we have also compared the La Jolla $\Delta^{14}C$ sequen with a tree-ring growth sequence of limber pine from Mou San Gorgonio in Southern California. The 100- and 200-yr lin match closely; those at longer periods (including the very stro line at 500 yr in the limber pine spectrum) probably correspo to the two long periods in the two ^{14}C spectra.

Note also that these time sequences are statistically no stationary. For a certain period within the full La Jolla reco the 200-yr line disappears as has been pointed out previou by Neftel *et al.*[8], although the 200-yr line is strong before a after this period. Thus the record used for the work report here should be regarded as an integrated composite of spect response over the entire time period examined.

An independent verification of the existence of a correlati between the La Jolla $\Delta^{14}C$ sequence and the BCP ring grow

Table 1 ^{14}C data for La Jolla (3405 BC–AD 1885) (*a*) and Camp Mountain bristlecone (3405 BC–AD 1885) (*b*)

	Frequency (yr^{-1})	Period T (yr)	$T-\Delta T$	$T+\Delta T$
a	0.0004	2,500	1,700	4,740
	0.0014	714	629	826
	0.0047	213	205	222
	0.0087	115	113	118
	0.0096	104	102	106
	0.0114	88	86	89
b	0.0007	1,430	1,125	1,957
	0.0015	667	592	763
	0.0048	208	200	217
	0.0088	114	111	116
	0.0103	97	95	99
	0.0122	82	81	83

Frequency and period of some selected major lines in the ^{14}C a bristlecone ring growth spectra. The two right-hand columns are t period resolution, estimated from the sequence length.

sequence is obtained by a narrow band moving window cross-correlator operating jointly on the two sequences. The two time domain sequences are first Fourier transformed, yielding the complex Fourier coefficients. The fundamental resolution in frequency is defined as follows: as the length of the sequences, $T = 5{,}290$ yr, the frequency resolution, $\Delta f = 1/5{,}290\ \mathrm{yr}^{-1}$. The Nyquist frequency, f_N, is half the final adjusted sampling frequency of $10\ \mathrm{yr}^{-1}$, so the number of spectral estimates $n = f_N/T$. We apply a simple narrow-band filter consisting of a linearly tapered window of full width $7/20\ \mathrm{yr}^{-1}$. This triangular window provides imperfect but reasonable suppression of side lobes. Since there are five non-zero weights (1/3, 2/3, 1, 2/3, 1/3) in this filter, it weights the ends of the sequence asymmetrically. For this reason application of the filter is restricted to those spectral estimates lying in the frequency interval $1/1{,}770 < f < 1/90\ \mathrm{yr}^{-1}$. This eliminates the two lowest and highest frequency estimates.

This procedure yields a set of spectra encompassing only a very narrow band of frequencies in accordance with the discussion just given. Inversion of these spectra into the time domain thus provides two subsets of sequences, one for $\Delta^{14}C$ and the other for bristlecone pine growth rings. We now correlate these in pairs corresponding to each frequency estimates. Such a narrow banded correlation exhibits time lags which may be interpreted as phase lags over narrow frequency intervals. Further, these lags are relatively independent of the coherence. We now choose those lags corresponding to the peak value of coherence for each of the narrow-band sequence pairs. The correspondence between lag and phase with varying frequency yields the phase-frequency relation shown in fig. 1. This figure indicates a continuously varying phase with increasing frequency. The variation in phase exceeds that which could be assigned to reservoir effects, those being limited to a lag of $\pi/2$.

Stuiver[2], for the AD centuries investigated by him, reports spectral features in his ^{14}C production rate curves for the periods $T = 126$–141 and 185–218 yr at the 2σ confidence level or better. Assuming his smoothed FFTs have $\Delta f = 1/15\ \mathrm{yr}^{-1}$, then the 200-yr line is detected; the 120-yr line is less certain, lying slightly outside his lower period window. However, the correlation (of very low confidence) between White Mountain trees and the $\Delta^{14}C$ record exhibits zero lag. Since coherence depends on bandwidth, the independent test for existence of a meaningful relative phase which we use is preferred. The credibility of a detectable phase is checked independently of MEM though supported by calculations using a two channel cross-spectral MEM estimator. We believe that further work is required to interpret the phase relation, but it can be said that its detection is qualitatively consistent with the comparison of the $\Delta^{14}C$ and BPC ring growth auto-spectra.

The relation between $\Delta^{14}C$ and solar activity is well established, though many details are poorly understood. The detection of spectral lines in the bristlecone pine growth ring spectra at periods commensurate with those in the $\Delta^{14}C$ spectra implies that the $\Delta^{14}C$ detection of solar signals is repeated in the ring growth of the Campito Mountain bristlecone pines.

The similarity between the $\Delta^{14}C$ record and the growth record of the Campito Mountain bristlecone pine suggests that a forcing function is present which mediates both the atmospheric ^{14}C level and tree growth. The expectation is that the ^{14}C uptake by trees will lag growth on the premise that growth is a short term response to climate, whereas atmospheric and oceanic reserviors contribute a frequency dependent lag to the net atmospheric ^{14}C budget[19]. Since the two effects discussed here appear correlated, by a process of elimination, the Sun is an obvious candidate for the source of the variations, though expressed through widely differing 'channels', that is, hydromagnetic in the case of ^{14}C and an as yet poorly understood mechanism in the case of tree growth. Further work, especially in regard to the very complex problem of the relative phase, may aid in clarifying the mechanism(s).

As there is a strong stochastic element in the data used for this analysis, the results are fundamentally probabilistic in nature and cannot be assigned a numerically deterministic certainty. This also has the effect that the actual signal strength, for what are generally to be regarded as threshold signals, has a probabilistic distribution and can attain larger or smaller values than measured, with the mean favouring the larger. Corrections for this effect have not been considered here but would have the general effect of increasing the true signal levels above those inferred from measurement[20]. Lastly, because phase is much more sensitive to noise corruption than is amplitude, details of the relative phase should be regarded as tentative.

We thank C. W. Stockton for providing the bristlecone and limber pine tree ring width sequences, T. W. Linick for the La Jolla $\Delta^{14}C$ data and to P. E. Damon for providing the workshop sequence. Programming was carried out by T. Trebisky and by L. D. Smith. CPS was supported by grant ATM-8308378 from the NSF Solar-Terrestrial Program.

Received 25 April; accepted 18 October 1983.

1. Stuiver, M. & Quay, P. D. *Science* **207,** 11 (1980).
2. Stuiver, M. *Nature* **286,** 868 (1980).
3. Suess, H. E. *Radiocarbon* **20,** 1–8 (1978), **22,** 200–209 (1980).
4. Linick, T. W., in preparation.
5. Lamb, H. H., *Climate, Present, Past, and Future* Vol. 2, 59 (Methuen, London, 1977).
6. Fritts, H. C. *Tree Rings and Climate*, (Academic, New York, 1976).
7. LaMarche, V. C. *Science* **183,** 1043 (1974).
8. Neftel, A., Oeschger, M. & Suess, H. E. *Earth planet. Sci. Lett.*, **56,** 127 (1981).
9. Klein, J., Lerman, J. C., Damon, P. E. & Ralph, E. K. *Radiocarbon*, **24,** 103 (1982).
10. Reinsch, C. H. *Num. Math.*, **10,** 177 (1967).
11. Rice, S. O., reprinted from Bell System Tech. Jour. in *Selected Papers on Noise and Stochastic Processes* (ed. Wax, N. Dover, 1954).
12. Burg, J. P. thesis, Stanford Univ. (1975).
13. Ulrych, T. J. and Bishop, T. N. *Rev. Geophys. Space Phys.* **13,** 183 (1975).
14. Yule, U. Y., *Proc. R. Soc.* A**642,** 267 (1927).
15. Box, G. E. P. and Jenkins, G. M. *Times Series Analysis* (Holden-Day 1976).
16. Singleton, R. C. *IEEE Trans. Audio Electroacoust.* **17,** 93 (1969).
17. Jaynes, E. T. *Proc. IEEE* **70,** 939 (1982).
18. Kromer, R. thesis, Stanford Univ. (1970).
19. Houtermans, J. C., Oeschger, M. & Suess, H. E. *J. geophys. Res.* **78,** 1893–1908 (1973).
20. Groth, E. J. *Astrophys. J. Suppl.* Ser. 286, **29,** 285 (1975).

Reconstruction of precipitation history in North American corn belt using tree rings

T. J. Blasing & Daniel Duvick

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA

Tree-ring-based reconstructions of past climate have proved useful for evaluating hydrological statistics calculated from the shorter observational record of climate[1], and particularly for analysing past patterns of drought in the western USA[2]. The independently verified climatic reconstructions obtained here provide a significant contribution to a reliable data base for evaluation of climatic changes on time scales of decades to centuries. Fifteen tree-ring chronologies were developed from specimens of drought-sensitive white oak (*Quercus alba*), many over 300-yr old, in the western part of the North American corn belt. Verification statistics indicate reliable reconstructions of annual precipitation for the two-state area of Iowa and Illinois can be obtained back to 1680 from these chronologies. This extends the network of confirmed drought-sensitive tree-ring chronologies well into the central USA, and provides encouragement for further expansion of the tree-ring chronology network into the North American grain-belt region.

Tree rings have been used to reconstruct past climate in several regions including the western USA[2,3], western Canada and Alaska[4,5], the eastern USA[6,7], and the British Isles[8]. Stockton and Meko[2] reconstructed a 270-yr drought history of the western USA, which contained some evidence of a 22-yr Hale sunspot cycle[9]. Their study region extended into the central USA, but their tree-ring data base was weak there, including only a few sites on the periphery of the North American grain-belt region. Furthermore, the climatic information content of

© 1984 Macmillan Journals Ltd

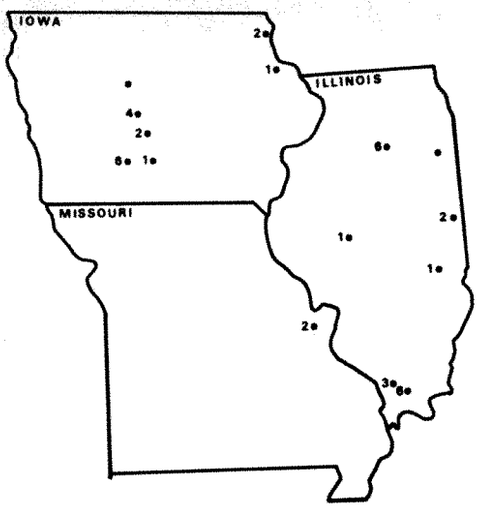


Fig. 1 Site locations of trees and number of trees at each location which date back to at least AD 1680. One site in Iowa and one site in Illinois had no trees that old, but all sites had at least one tree dating back to 1686. Some younger trees from each of the sites were also used in the reconstruction.

the ring-width chronologies from the central United States had not been verified.

Since that study appeared[2], we have published[10] preliminary results, in which the state average annual precipitation for Iowa was reconstructed back to 1680 using three tree-ring-width chronologies from the central part of the state. Our verification results encouraged further investigations leading to the more extensive network of tree sites used in this study (Fig. 1).

Usually two cores, each 4 mm in diameter, were extracted at breast height from opposite sides of each tree sampled. Ring widths from each core were converted to ring-width indices[11] using spline functions to prefilter non-climatic variance due to gradual changes in tree age, competitive status, and so on[12]. Ring-width indices of all cores at all sites in each state, Iowa and Illinois, were averaged for each year (with the Missouri site data included in the Illinois state average), and the two statewide series were averaged to form a regional ring-width-index series. This was used as the predictor variable in linear regression to estimate annual precipitation amounts averaged for the two-state area (Iowa and Illinois). In this study, annual precipitation was computed from August of the preceding year through July of the year corresponding to ring formation. This coincides with the 12-month period for which these tree rings best reflect total precipitation amounts, from the end of the preceding growing season to the end of the season in which the ring is formed[10]. Ring-width data from 1920 to 1980, and the corresponding annual precipitation data as defined above, were used to calculate the regression coefficients. State average-precipitation data available for both states since 1878 [13] were used for independent verification of precipitation estimates up to 1919.

The correlation coefficient between actual and estimated precipitation values was +0.79 for the period 1920–80, and +0.86 for the independent period. For comparison, when annual (August–July) precipitation data at Springfield, Illinois, and Des Moines, Iowa, were averaged, the correlation between that series and the two-state averages for the period 1920–80 was +0.89. All of these are significant well beyond the 99% confidence level, even after first-order autocorrelations (always below +0.25) were accounted for[14]. For the independent data (1878–1919) the reduction of error, RE, was +0.66. The RE is described by Fritts *et al.*[3] as "a most rigorous verification statistic". It is, in some ways, analogous to the so-called explained variance, but its range extends from $-\infty$ to +1, and any positive value indicates some information in the reconstruction[3]. Precipitation is often noticeably underestimated for extremely wet years, presumably because moisture is not limiting to growth[10]. Thus, precipitation estimates for normal and dry years are often more accurate than the overall statistics indicate. In this case, the RE increased to +0.76, although the correlation coefficient remained at +0.86, when one-third of the years (14 yr) having the highest estimated precipitation values were removed from the independent data set. The overall accuracy and reliability of these reconstructions of past precipitation amounts, as measured by the above statistics, are as great if not greater than any reported to date.

The reconstructed annual precipitation since 1680 is shown in Fig. 2*a*. No droughts appreciably worse than the 'dust bowl' of the 1930s are indicated, although there apparently were several droughts of roughly equal severity. The reconstructions show no long-term changes such as might occur if the precipitation there were linked to Northern Hemisphere temperature trends[15]. This is also true of the (unfiltered) actual precipitation series which began in 1878 (Fig. 2*a*). Droughty conditions are apparent beginning in the mid 1880s when the Northern Hemisphere was relatively cool[15], and in 1816 when the 'year without a summer' occurred in the northeastern United States and adjoining Canadian provinces[16]. However, the 'dust bowl' drought of the 1930s coincided with one of the warmest periods in the last century and probably one of the warmest periods in the span of our reconstructions which extend about two centuries back into the Little Ice Age[17,18]. Overall, drought in the western corn belt appears to have been about equally probable during the relatively warm and cool periods of the past three centuries. With acknowledgment to projections of increased droughtiness for a CO_2-induced climatic warming[19], our reconstructions indicate that droughts comparable with those of the 1890s and 1930s afflicted the Iowa–Illinois region at various times over the past three centuries and, therefore, should be expected to continue to do so even if no further warming occurs.

Stockton and Meko[20] analysed tree-ring data from our sites in central Iowa and found evidence of a 22-yr cycle. However, the cycle was not persistent throughout the period of record. It seemed to be strongest since about 1890 and was not evident before 1800. The 10-yr moving averages in Fig. 2*b*, based on

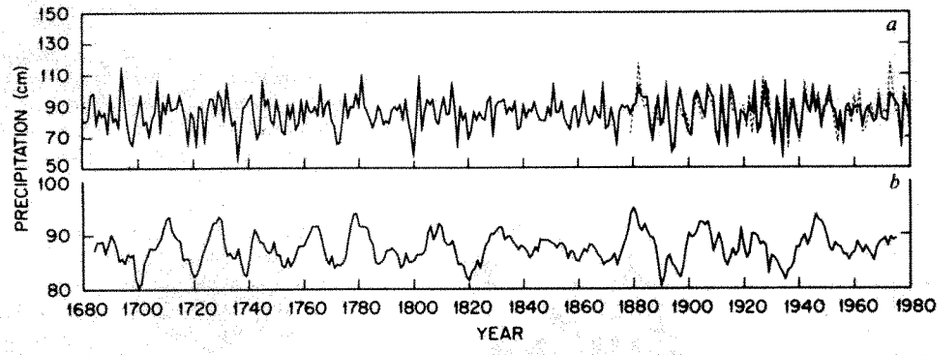


Fig. 2 *a*, Reconstructed annual (August–July) precipitation for Iowa–Illinois as obtained from the regression equation: $y = -2.788 + 90.756\,x$, where y is the precipitation estimate for a particular year and x is the regional ring-width index for the corresponding year. Actual values including independent data are indicated by the dashed line. When this equation, derived using data from young and old trees, was applied to reconstruct precipitation from only the cores dating back to 1680, the RE statistic corresponding to +0.66 in the text changed negligibly to +0.68. *b*, The 10-yr running means of the reconstructions plotted for the fifth year of each 10-yr sequence.

the data now available, suggest a cycle of about 20-yr before 1800. That may be at least partially an artefact of the frequency–response function of the moving average, but the reconstructed drought around 1720, for example, in Fig. 2*b* is much more pronounced than in the 10-yr moving averages presented earlier[10] which were based only on chronologies from central Iowa. It has been suggested that a tendency for drought to occur about every 20 yr may be due to combined effects of the Hale solar cycle of about 22 yr and the regular lunar-tidal cycle of 18.6 yr (refs 21, 22).

Reconstructions of past climate provide an extended data base for formulating or testing hypotheses about possible causes of climatic change (such as solar and lunar cycles), and for evaluating or recalculating probabilities of climatic events (such as drought) calculated from the shorter instrumental records. This work shows that excellent reconstructions of annual precipitation, for at least three centuries, are possible for portions of the North American grain belts.

We thank Dale Huff and John Krummel for helpful comments. The research was sponsored by the NSF, Ecosystem Studies Program, under Interagency Agreement DEB81-15316 with the US Department of Energy under contract W-7405-eng-26 with Union Carbide Corporation. Publication no. 2243, Environmental Sciences Division, ORNL.

Received 6 September; accepted 4 October 1983.

1. Stockton, C. W. *Long-Term Streamflow Records Reconstructed from Tree Rings* (University of Arizona Press, Tucson, 1975).
2. Stockton, C. W. & Meko, D. M. *Weatherwise* **28,** 244–249 (1975).
3. Fritts, H. C., Lofgren, G. R. & Gordon, G. A. *Quat. Res.* **12,** 18–46 (1979).
4. Garfinkel, H. L. & Brubaker, L. B. *Nature* **286,** 872–873 (1980).
5. Jacoby, G. C. & Cook, E. R. *Arctic Alp. Res.* **13,** 409–418 (1981).
6. Cook, E. R. & Jacoby, G. C. *Science* **198,** 399–401 (1977).
7. Cleaveland, M. K. thesis, Clemson Univ., South Carolina (1975).
8. Briffa, K. R., Jones, P. D., Wigley, T. M. L., Pilcher, J. R. & Baillie, M. G. L. *J. Climatol.* (in the press).
9. Mitchell, J. M., Stockton, C. W. & Meko, D. M. in *Solar–Terrestrial Influences on Weather and Climate* (eds McCormac, B. M. & Seliga, T. A.) 125–143 (Reidel, Dordrecht, 1979).
10. Duvick, D. N. & Blasing, T. J. *Wat. Resour. Res.* **17,** 1183–1189 (1981).
11. Fritts, H. C. *Tree Rings and Climate* (Academic, London 1976).
12. Cook, E. R. & Peters, K. *Tree-Ring Bull.* **41,** 45–53 (1981).
13. U.S. Weather Bureau (now, National Weather Service). *Climatological Data for the United States by Sections.* (National Climatic Center, Asheville, various dates).
14. Bartlett, M. S. *J. R. statist. Soc.* **98,** 536–543 (1935).
15. Jones, P. D., Wigley, T. M. L. & Kelly, P. M. *Mon. Weath. Rev.* **110,** 59–70 (1982).
16. Hughes, P. *Weatherwise* **32,** 108–111 (1979).
17. Groveman, B. S. & Landsberg, H. Publ. 79-181 (Meteorology Program, Univ. of Maryland, 1979).
18. Bryson, R. A. & Murray, T. J. *Climates of Hunger* (University of Wisconsin Press, Madison, 1977).
19. Manabe, S., Wetherald, R. T. & Stouffer, R. J. *Climat. Change* **3,** 347–386 (1981).
20. Stockton, C. W. & Meko, D. M. *J. Climatol. appl. Met.* **22,** 17–29 (1983).
21. Vines, R. G. *J. geophys. Res.* **87,** 7303–7311 (1982).
22. Gough, D. *Nature* **288,** 639–640 (1980).

Elemental constituents of atmospheric particulates and particle density

Akiyoshi Sugimae

Environmental Pollution Control Center, Osaka Prefecture, 3-62, 1-chome, Nakamichi, Higashinari-ku, Osaka 537, Japan

Atmospheric particulates are expected to be formed from materials of various densities. Particle motion in the atmosphere is characterized by the product of the density multiplied by the diameter squared. For studies of source characteristics, atmospheric transport processes and removal rates of atmospheric particulates, accurate knowledge of density distribution is essential. Previous studies[1–3] indicated that the value of 1.7 g cm^{-3} for the average density of atmospheric submicrometre particles was quite reasonable. However, there are very few published data on the elemental constituent as a function of density of atmospheric particulates I report here the density distribution of Si, Fe, Mn, Zn and Cu in the atmosphere.

Atmospheric particulates were sampled either by filtration from the suspension in the atmosphere, or by collection of deposited particles as they fall out under gravitational influence, known as dustfall. Samples of suspended particulates were collected on the rooftop (about 10 m above street level) of the Environmental Pollution Control Center in a metropolitan area of Osaka, using both a conventional high-volume sampler (hi-vol) and a low-volume sampler (low-vol). The hi-vol was used to collect samples of total suspended particulates (TSPs) on 8 × 10-inch filters. The sampler is fitted in an aluminium shelter which prevents direct settling of dusts onto the filter. Particles with a terminal settling velocity in air greater than approximately 30 cm s^{-1} are excluded from the shelter[4]. The maximum theoretical particle size has been calculated to be 100 μm (particle density = 1.0 g cm^{-3}). However, collection efficiencies for large particles are quite sensitive to small changes in wind velocity, approaching angle and flow rate[5]. The upper limit of particle sizes actually collected is believed to be 15–20 μm. The Japanese Environment Agency specifies the use of low-vol for the long-term monitoring of inhalable particulate (IP) levels. Coarse particles are preliminarily separated by three cyclones installed in the sampler. The low-vol has a D_{50} (aerodynamic diameter at an effectiveness of 50%) = 8 μm and collects IPs on 48-mm diameter filter at a flow rate of 20 l min^{-1}.

The polystyrene filter (Microsorban type 99/97, Delbag-Luftfilter GMBH, Berlin) was used as the collection medium, because the filter has collection efficiencies, capacities and loading characteristics similar to the more commonly used glass fibre filter, yet has a much lower blank concentrations of trace elements[6].

Particulates which fell as a result of their own settling velocity and which were carried down with precipitation were collected in a deposit gauge. The sample collection continued for a month. After collection, water-insoluble component of dustfall was filtered off with distilled water on a polystyrene filter.

To evaluate the contribution of wind-blown soil to local atmospheric particulates, a soil sample was taken near the sampling station.

I have modified the density gradient separation technique used for the chemical speciation of Pb compounds in street dusts[7–10] and for the multi-element characterization of roadway dusts[11] and applied it to density fractionation of particulate samples collected on polystyrene filters. The use of this type of filter was a good choice because a suspension of particulate samples for the subsequent density separation was prepared by dissolving the filter material in dichloromethane. After centrifugation, the float was transferred and the residue was centrifuged in a series of dichloromethane–diiodomethane mixtures such that the density of each successive mixture was incrementally increased by 0.2 g cm^{-3}. The final centrifugation was in diiodomethane. Particulate samples were thus classified into 12 density fractions from <1.3 to >3.3 g cm^{-3}. Iron, Mn, Zn and Cu were determined in each density fraction by inductively coupled plasma–atomic emission spectrometry (ICP–AES) following acid digestion in hot aqua regia. Silicon was determined by ICP–AES after alkaline fusion. The experimental methods have been described in detail elsewhere[12–15].

The densities of the commonly recognized compounds which exist or are expected to exist in the atmosphere are as follows: SiO_2 (2.65 g cm^{-3}), Fe_2O_3 (5.24 g cm^{-3}), Fe_3O_4 (5.18 g cm^{-3}), $Fe_2(SO_4)_3$ (3.094 g cm^{-3}), MnO (5.026 g cm^{-3}), ZnO (5.61 g cm^{-3}), $ZnSO_4$ (3.54 g cm^{-3}), CuO (6.0 g cm^{-3}) and $CuSO_4$ (3.61 g cm^{-3}).

Commonsense suggests that heavy metal compounds will be present primarily associated with particles of high density (>3.3 g cm^{-3}). However, the particle density is often related to the mode of origin, composition and interaction with the surrounding vapours and with each others[16]. The density of particle formed by comminution, attrition or disintegration will resemble that of parent material. On the other hand, fine particles originating from nucleation of vapours are highly agglomerated. It is generally accepted that the density of agglomerate which includes void spaces is less than that of individual component particles.

© 1984 Macmillan Journals Ltd

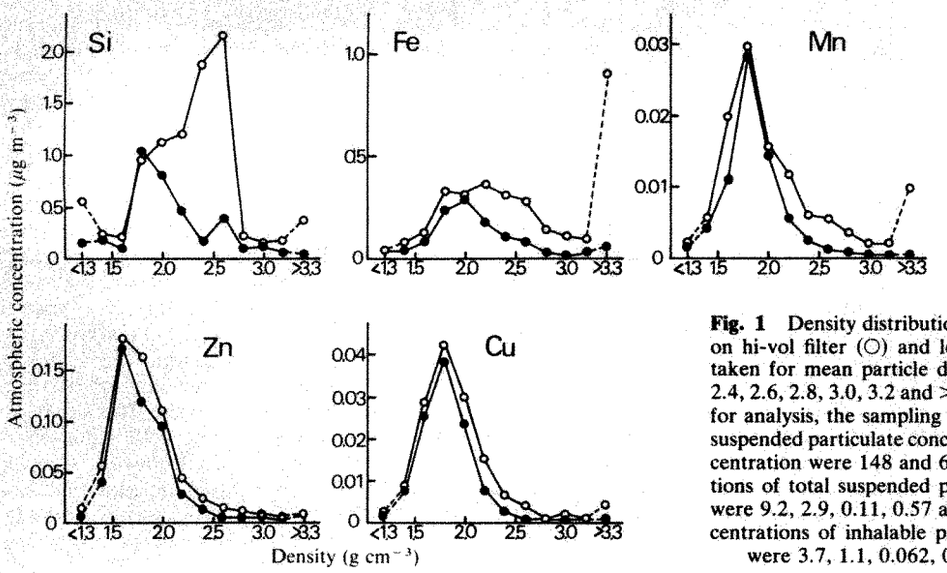


Fig. 1 Density distributions for Si, Fe, Mn, Zn and Cu collected on hi-vol filter (○) and low-vol filter (●). Measurements were taken for mean particle density of <1.3, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2 and >3.3 g cm^{-3}. To collect enough material for analysis, the sampling duration was extended to 100 h. Total suspended particulate concentration and inhalable particulate concentration were 148 and 69 μg m^{-3}, respectively. The concentrations of total suspended particulates for Si, Fe, Mn, Zn and Cu were 9.2, 2.9, 0.11, 0.57 and 0.14 μg m^{-3}, respectively. The concentrations of inhalable particulates for Si, Fe, Mn, Zn and Cu were 3.7, 1.1, 0.062, 0.46 and 0.11 μg m^{-3}, respectively.

The elemental density distributions shown in Fig. 1 represent that the concentration of Si in TSPs is relatively high in the density range of 2.5–2.7 g cm^{-3}, corresponding to the density of SiO_2. Approximately 30% of Fe particles in TSPs collected by means of hi-vol are associated with particles of density higher than 3.3 g cm^{-3}. There is a general tendency for the soil-derived elements in TSPs, typified by Si and Fe, to increase in concentrations in the corresponding density ranges of the parent materials (such as quartz; haematite and magnetite). On the other hand, the concentration of Si in IPs goes through a maximum in the density range of 1.7–1.9 g cm^{-3} and another small maximum in the density range of 2.5–2.7 g cm^{-3}. The density distribution of inhalable Fe particles shows definite peak in the density range of 1.9–2.1 g cm^{-3}. Iron particles with density higher than 3.3 g cm^{-3} can account for only a few per cent of the total mass of Fe particles. It is noticeable that TSPs and IPs display quite different density distributions with respect to Si and Fe particles, which are generally present as coarse particles in suspended particulates[17–24]. The low-vol used for this study is limited to the collection of particles which have aerodynamic diameters <8 μm. The results suggest that the low-vol is relatively less efficient for collecting atmospheric soil particles existing as coarse particles in the atmosphere.

The presence of Si and Fe particles with densities less than those of parent materials is attributable partially to the presence of soil agglomerates with low apparent densities[25,26] and partially to the presence of empty spheres, known as cenospheres and plerospheres[27–29]; both spheres are formed from a particular combustion.

Volatile (chalcophilic) elements such as Zn and Cu are concentrated in low-density fractions which seem to consist of carbonaceous matter judging from their black colour and microscopic observation. In the density distributions of Zn and Cu, only one peak is observed in the density range of 1.5–1.7 and 1.7–1.9 g cm^{-3}, respectively. It is also notable that the density distributions of Zn and Cu do not differ appreciably between hi-vol and low-vol sample. The densities of Zn and Cu compounds are anticipated to be higher than 3.3 g cm^{-3} because the likely compounds of these elements in the atmosphere have the bulk densities, being higher than 3.3 g cm^{-3}. Nevertheless, Zn and Cu particles with the density higher than 3.3 g cm^{-3} can account for only a few per cent of total mass of Zn and Cu, respectively. This is probably because these elements are capable of being volatilized in high-temperature, anthropogenic processes. And fine particles originating from volatilization grow by condensation and coagulation to form agglomerates with low densities.

In the case of Mn particles collected on hi-vol filter, the density distribution shows two maxima, one for particles with density of the 1.7–1.9 g cm^{-3} range and the other for particles with

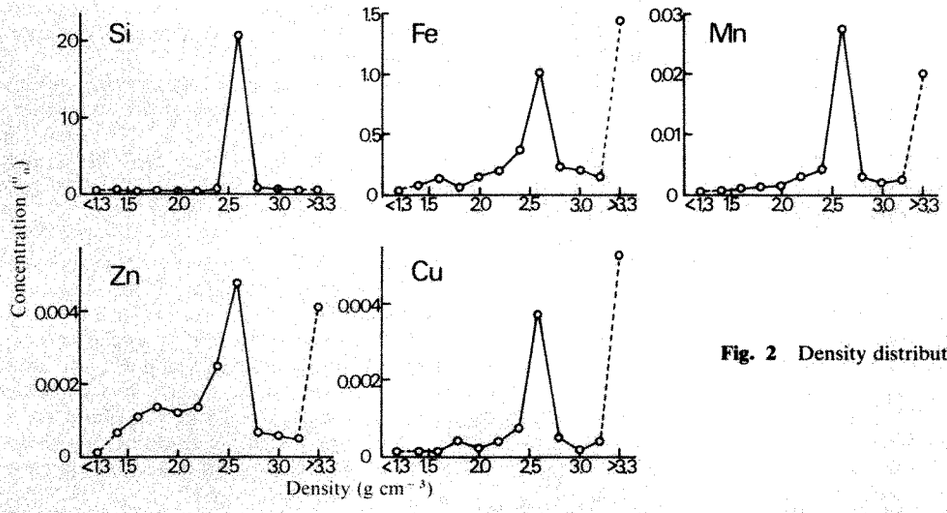


Fig. 2 Density distributions for Si, Fe, Mn, Zn and Cu in soil.

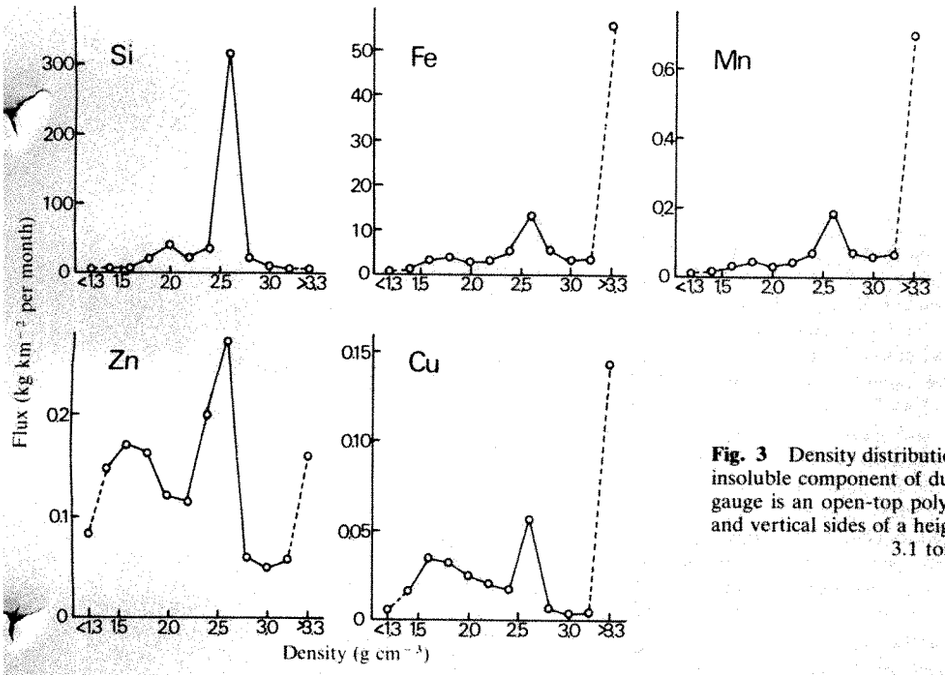


Fig. 3 Density distributions for Si, Fe, Mn, Zn and Cu in water-insoluble component of dustfall collected in a deposit gauge. The gauge is an open-top polyethylene cylinder of diameter 105 mm and vertical sides of a height of 215 mm. The flux of dustfall was 3.1 ton km^{-2} per month.

density higher than 3.3 g cm^{-3}. The density distribution of Mn particles in IPs favours low density. Manganese shows lower volatility than chalcophilic elements and is volatilized in a lesser extent in the high-temperature processes. However, low-density components are probably introduced into the atmosphere after being volatilized. The presence of dense particles (>3.3 g cm^{-3}) in TSPs suggests that abrasional processes such as crustal weathering are also the sources of atmospheric Mn particles.

In urban area, soils, street dusts, construction dusts, and so on should be efficient sources of coarse particles, most of which will rapidly fall out near their sources, but some of which should suspend as atmospheric particulates. The density distributions of Si, Fe, Mn, Zn and Cu in soil samples are shown in Fig. 2. The maximum value was found for Si in the density range of 2.5–2.7 g cm^{-3}, corresponding to the density of SiO_2. However, all elements with the exception of Si show two definite peaks in the density distributions at the density ranges of 2.5–2.7 and >3.3 g cm^{-3}. The presence of particles with density >3.3 g cm^{-3} would be explained by the presence of these elements as oxides. However, we are unaware of any crustal materials, containing these elements, with the density near 2.6 g cm^{-3}. Although the mechanisms responsible for the low density are not yet understood, the presence of these low-density particles would be explained by the presence of coarse SiO_2 particles which are coated with submicrometre platelets containing these elements. The particles associated with the density ranges of 2.5–2.7 and >3.3 g cm^{-3} can be characterized by the natural sources such as wind-blown soil dusts. The soil particles floated into the atmosphere are likely to give additional peaks at the density ranges of 2.5–2.7 and >3.3 g cm^{-3}. The elemental density distributions of suspended particulates are completely different from those of soil. However, the density distributions of Fe and Mn in TSPs show a gradual slope in the density region around 2.6 g cm^{-3}, and imply the presence of soil-derived Fe and Mn particles.

For soil particles, gravitational fallout is an important removal mechanism. The elemental density distributions for the water-insoluble component of dustfall, illustrated in Fig. 3 are essentially similar to those for the soil. The matrix material of dustfall is considered to be composed primarily of soil-derived materials. However, the low-density fractions of dustfall seem to consist of carbonaceous materials judging from their black colour. The density distributions of chalcophilic elements Zn and Cu show a broad peak near the density of 1.6 g cm^{-3}, indicative of anthropogenic sources, although the concentrations are still high in the density ranges of 2.5–2.7 and >3.3 g cm^{-3}.

Even though the most serious criticism of this technique is that the heavy liquids used for the density gradient separation would attack atmospheric particulates dissolving out some of the tarry matter and disagglomerating some clusters of particles, the technique is the only way to fractionate atmospheric particulates within the critical particle density range and provide valuable qualitative information on the physicochemical characteristics of atmospheric particulates. Until more quantitative techniques are developed, most quantitative tests for the formation and transport mechanisms for atmospheric particulates must rely on the concentration versus particle density data as well as on the concentration versus particle size data.

Received 27 June; accepted 7 November 1983.

1. Sverdrup, G. M., Whitby, K. T. & Clark, W. E. *Atmos. Envir.* **9**, 483–494 (1975).
2. Durham, J. L., Wilson, W. E., Ellestad, T. G., Willeke, K. & Whitby, K. T. *Atmos. Envir.* **9**, 717–722 (1975).
3. Watson, J. G., Chow, J. C., Shah, J. J. & Pace, T. G. *J. Air Pollut. Control Ass.* **33**, 114–119 (1983).
4. Jutze, G. A. & Foster, K. I. *J. Air Pollut. Control Ass* **17**, 17–25 (1967).
5. Wedding, J. B., McFarland, A. R. & Cermak, J. E. *Envir. Sci. Technol.* **11**, 387–390 (1977).
6. Dams, R., Rahn, K. A. & Winchester, J. W. *Envir. Sci. Technol.* **6**, 441–448 (1972).
7. Olson, K. W. & Skogerboe, R. K. *Envir. Sci. Technol.* **9**, 227–230 (1975).
8. Biggins, P. D. E. & Harrison, R. M. *Envir. Sci. Technol.* **13**, 558–565 (1979).
9. Biggins, P. D. E. & Harrison, R. M. *Envir. Sci. Technol.* **14**, 336–339 (1980).
10. Linton, R. W., Natusch, D. F. S., Solomon, R. L. & Evans, C. A. Jr *Envir. Sci. Technol.* **14**, 159–164 (1980).
11. Hopke, P. K., Lamb, R. E. & Natusch, D. F. S. *Envir. Sci. Technol.* **14**, 164–172 (1980).
12. Sugimae, A. *Taiki Osen Gakkaishi* **14**, 389–398 (1979).
13. Sugimae, A. *ICP Inform. Newslett.* **6**, 619–644 (1981).
14. Sugimae, A. *Taiki Osen Gakkaishi* **18**, 233–240 (1983).
15. Sugimae, A. *Taiki Osen Gakkaishi* (in the press).
16. Whytlaw-Gray, R. & Paterson, H. S. *Smoke* (Edward Arnold, London, 1932).
17. Gladney, E. S., Zoller, W. H., Jones, A. G. & Gordon, G. E. *Envir. Sci. Technol.* **8**, 551–556 (1974).
18. Dzubay, T. G. & Stevens, R. K. *Envir. Sci. Technol.* **9**, 663–668 (1975).
19. Flocchini, R. G. *et al. Envir. Sci. Technol.* **10**, 76–82 (1976).
20. Hardy, K. A., Akselsson, R., Nelson, J. W. & Winchester, J. W. *Envir. Sci. Technol.* **10**, 176–182 (1976).
21. Paciga, J. J. & Jervis, R. E. *Envir. Sci. Technol.* **10**, 1124–1128 (1976).
22. Kadowaki, S. *Envir. Sci. Technol.* **13**, 1130–1133 (1979).
23. Tanaka, S., Darzi, M. & Winchester, J. W. *Atmos. Envir.* **14**, 1421–1426 (1980).
24. Adams, F., Van Espan, P. & Maenhaut, W. *Atmos. Envir.* **17**, 1521–1536 (1983).
25. Gillette, D. A., Blifford, I. H. & Fenster, C. R. *J. appl. Met.* **11**, 977–987 (1972).
26. Rahn, K. A. *Atmos. Envir.* **10**, 587–601 (1976).
27. Fisher, G. L., Chang, D. P. Y. & Brummer, M. *Science* **192**, 553–555 (1976).
28. Campbel, J. A., Laul, J. C., Nielson, K. K. & Smith, R. D. *Analyt. Chem.* **50**, 1032–1040 (1978).
29. Smith, R. D. *Prog. Energy Combust. Sci.* **6**, 53–119 (1980).

Photochemical production of carbonyl sulphide in marine surface waters

R. J. Ferek & M. O. Andreae

Department of Oceanography, Florida State University, Tallahassee, Florida 32306, USA

Carbonyl sulphide (COS) has been proposed as the major source of the stratospheric sulphate layer in times of low volcanic activity[1] because of its long tropospheric residence time[2], its uniform distribution in both the Northern and Southern Hemispheres[3], and the lack of knowledge of any significant sinks except photodissociation in the stratosphere[4]. Known sources of COS include biomass burning[5], volcanoes[6,7], salt marshes[2,8], fossil fuel combustion[9], and oxidation of atmospheric CS_2 (refs 10, 11). Also, recent measurements[12–14] have shown the surface oceans to be a source of COS. The total flux to the atmosphere from these known sources is substantially greater than the flux to the stratosphere needed to maintain the sulphate layer[10]. Thus questions remain about the sources and sinks of COS, and we have now performed a series of experiments in nearshore ocean and estuarine waters to investigate further the oceanic source of this gas. Dissolved COS in near-surface waters was found to be supersaturated with respect to atmospheric COS, and the dissolved concentrations varied diurnally with light intensity. Similar behaviour has been observed by other investigators for carbon monoxide, which is produced photochemically in surface seawater[15]. Furthermore, COS production appears to be independent of salinity, photosynthetic activity and microbial activity, and is probably the result of photooxidation of organic matter. COS was produced in the laboratory by UV-irradiation of seawater and solutions of several biochemically relevant organosulphur compounds.

Measurements of both atmospheric and dissolved COS were performed using an instrumental system described in detail elsewhere[14]. For air measurements, a small volume (300 cm^3) of ambient air was passed through a tube immersed in liquid nitrogen to trap out gases which were then separated on a chromatographic column and analysed by a flame photometric detector for sulphur. For dissolved COS, a closed volume of air was cycled through a gas bubbler in a glass column containing rapidly exchanging seawater until the air was equilibrated with the dissolved gases in the seawater. The equilibrated air was then analysed as before and compared with the ambient air concentration to obtain directly the COS saturation ratio (SR) in the water (that is $[COS]_{equil\ air}/[COS]_{amb\ air} = SR$). Liquid concentrations can be calculated from the saturation ratio using Henry's law as follows:

$$[COS]_{liq} = \frac{[COS]_{amb\ air}}{h} \times SR$$

where h is the Henry's law constant for COS in seawater[16].

Initial measurements of the diurnal behaviour of dissolved COS were performed at the FSU Marine Laboratory located at Turkey Point on the north coast of the Gulf of Mexico south of Tallahassee, Florida. A continuous supply of seawater was obtained from the seawater circulation system with intake ~650 m offshore in 0.5–1.5 m of water. The residence time of water in the system was ~45 min. Figure 1 shows the results of two sampling periods at the Marine Laboratory. COS supersaturation shows a large diurnal cycle with peaks occurring during mid to late afternoon. No effect of tide (semidiurnal at this site) was observed.

Further measurements of COS saturation in surface waters were made during a cruise of the RV *Cape Hatteras* to Chesapeake Bay (28 March–3 April 1983) and Delaware Bay (4–8 April 1983). Seawater was obtained from the ship's continuously pumping seawater system with intake located 3 m below the surface. Ambient air was drawn through a Teflon

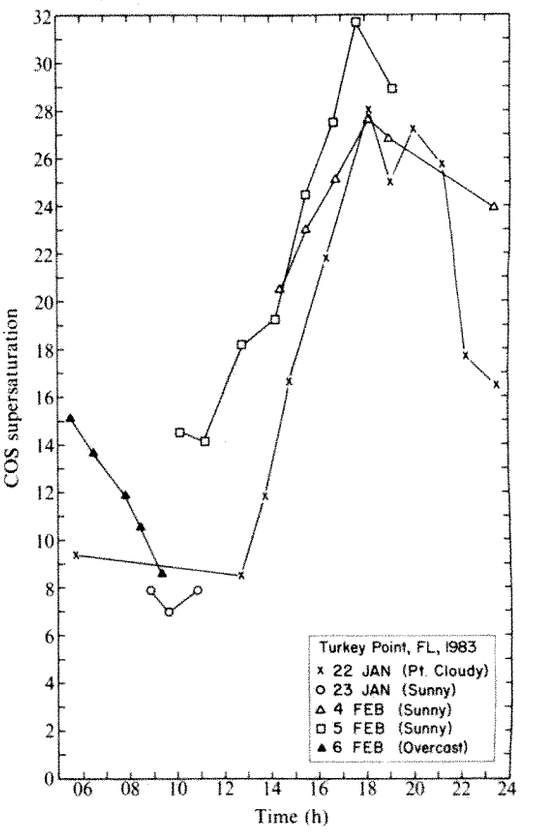


Fig. 1 Diurnal variation of COS in surface seawater measured at FSU Marine Laboratory, Turkey Point, Florida (northeastern Gulf of Mexico). The data are expressed as supersaturation ratio, that is, the ratio between the actual concentration in seawater and the concentration in equilibrium with ambient air.

tube (6.4 mm i.d.) which extended from the top of the mast to the laboratory. Figure 2 shows the diurnal behaviour of COS over a range of locations in both bays together with the hourly totals of light intensity in einsteins per m^2 (T. R. Fisher, personal communication). The highest COS supersaturations occur on days when light intensity was greatest. 28 and 29 March were both north–south transects of Chesapeake Bay over the same route. Although light measurements were not available on 28 March, it was a sunny day compared with partly cloudy conditions on 29 March, hence the higher COS levels on 28 March. Measurements on 30 March, 31 March, 1 April, and 2 April were taken at stations on Chesapeake Bay at surface salinities of 24, 17.4, 11, and 12‰, respectively. The magnitude of the COS saturation is again related to light intensity with no observable effect of salinity. Some of the variation on 1 April between 1100 and 1500 h may be due to vertical mixing as indicated by a lowering of the depth of the pycnocline between those hours. Since the secchi disk transparency was only slightly greater than 1 m, an increase in vertical mixing would be expected to mix in water from below with low COS concentration. This is reflected in the lowering of the COS saturation between 1100 and 1500 h on 1 April as opposed to a continuous rise during the daylight hours as illustrated in the FSU Marine Laboratory data (Fig. 1). The 3 April measurements were made near the mouth of the Susquehanna River (surface salinity = 0.2‰) on a sunny day. The COS saturation remained low relative to the other sunny days, probably due to low light penetration into the very turbid water (secchi disk transparency = 35 cm) at this station. 4 April was a north–south transect of Delaware Bay and 5 April was a station off Cape Henlopen (salinity = 29‰). COS saturations on both days do not appear appreciably different from the earlier Chesapeake stations.

0028-0836/84/020148—03$01.00

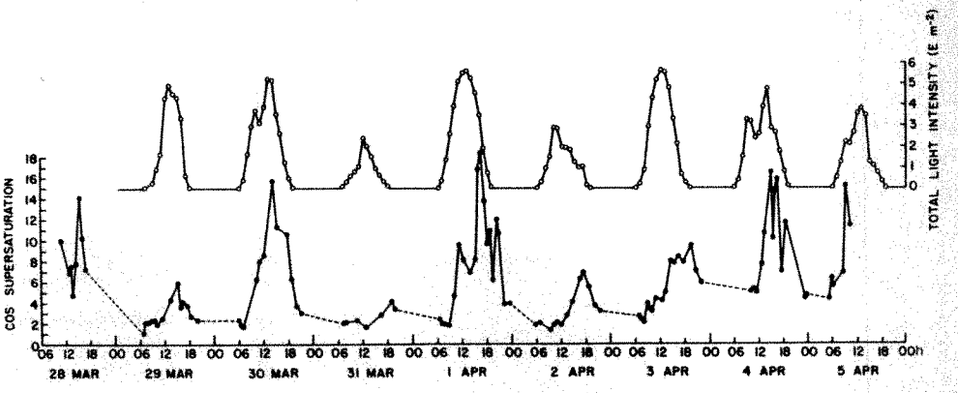


Fig. 2 COS supersaturation and light intensity measured in surface waters during cruise of RV *Cape Hatteras* in Chesapeake and Delaware Bays, spring 1983.

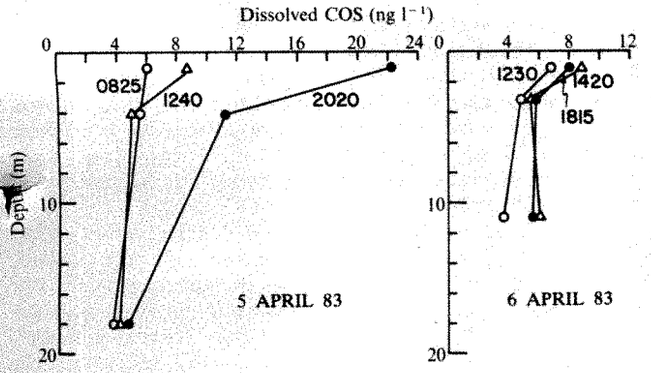


Fig. 3 Variation of the depth profile of dissolved COS concentration for two stations in lower Delaware Bay. 5 April was partly cloudy, 6 April was overcast. The time of sampling is indicated on each profile.

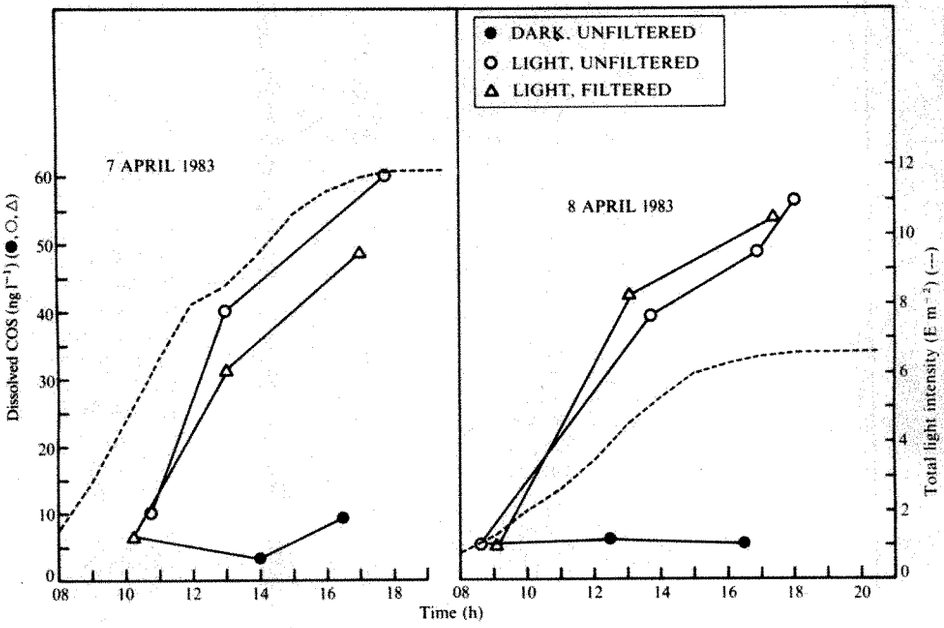


Fig. 4 Increase of COS concentration in surface seawater samples (filtered and unfiltered) incubated in light and dark bottles. The total integrated light intensity is indicated by a dotted line.

While at the Cape Henlopen station (5 April) and the 6 April station in lower Delaware Bay (surface salinity = 19‰), samples were collected at different depths by hydrocasts with 5-l Niskin bottles. 50-ml water samples were filtered through Gelman AE filters and purged with helium for 20 min in a gas stripping column with the effluent passed through a glass bead trap immersed in liquid nitrogen. Successive purges showed 20 min to be sufficient purging time to strip >90% of the dissolved COS from the samples. Figure 3 shows the results of these experiments. 5 April was a partly cloudy day and 6 April was overcast. Secchi disk transparencies at the two stations were 2.6 and 1.4 m, respectively. On both days COS at the deepest level remained fairly constant and 2–5 times lower than the surface concentrations. Diurnal variation was greatest at the surface level, indicating that production of COS was confined to the photic zone.

A final experiment to check the effect of biological activity on the production of COS was performed on 7 and 8 April. Surface samples were collected at two stations in upper Delaware Bay (salinities 7.0 and 3.4‰). One sample was sterilized by filtering through a 0.45-μm Nuclepore filter and, along with an unfiltered sample, analysed immediately by purging with He. Other filtered and unfiltered samples were placed in light and dark bottles and incubated in daylight for different lengths

of time. Figure 4 shows the results of these measurements. Also plotted are the running totals of light intensity throughout the day. The production of COS in the light bottles followed the light intensity, while dark bottles remained essentially constant at the initial COS concentration. There was essentially no difference between the filtered and unfiltered samples within the experimental uncertainty. The production of COS seems to be due to photooxidation of dissolved organic matter and thus only indirectly related to biological activity. As the 0.45-μm filter will remove all phytoplankton cells, but not all bacteria, a microbial production of COS cannot be rigorously excluded on the basis of this experiment. However, repeated irradiation of seawater with UV light from a small mercury discharge lamp ($\sim 400\ \mu W\ cm^{-2}$ of 254 nm light) in the presence of dissolved O_2, followed each time by degassing and determination of the COS produced, released COS in slowly decreasing amounts. Under the high UV intensities used, bacteria would have been killed during the first irradiation–degassing cycle and no further COS production should have taken place. UV irradiation of 100 μM solutions of several biochemically relevant organosulphur compounds (cysteine, methionine, glutathione, dimethylpropiothetin) in deionized, distilled water in the same conditions also produced substantial amounts of COS. This reaction did not occur when the solutions were stripped of dissolved O_2 by purging with He before irradiation.

Further evidence for the sea surface being a source of COS to the atmosphere can be seen in the ambient air concentrations measured at the three different locations. At Tallahassee, the mean ambient concentration of COS on 17 January, 18 January, and 18 February 1983 was $\bar{x} \pm s = 515 \pm 82$ p.p.t.v. (parts per 10^{12} by volume) ($n = 28$). This is very close to the mean tropospheric concentration of 512 ± 60 p.p.t.v. reported by Torres *et al.*[3] for a total of 346 measurements during Project GAMETAG. During the period of sampling at the FSU Marine Laboratory, atmospheric concentrations were 537 ± 59 p.p.t.v. ($n = 65$), not significantly different from Tallahassee. However, during the Chesapeake and Delaware Bays cruise, the ambient air concentration was 627 ± 84 p.p.t.v. ($n = 204$), ~20% higher than the continental and coastal concentrations, and may be due to the closer proximity to the surface seawater source. In addition, air concentrations averaged 641 ± 88 p.p.t.v. ($n = 131$) between the hours of 10 am and 8 pm and 601 ± 70 p.p.t.v. ($n = 73$) between 8 pm and 10 am.

Earlier measurements[12,14] in open ocean areas found COS saturation in surface waters averaging approximately 2.0, which results in a flux to the atmosphere of ~ 0.5–0.9×10^{12} g COS yr^{-1}. The higher saturations observed in coastal waters may make the total flux from the oceans greater than previously estimated. We are attempting to gather data from more diverse areas of the ocean to further refine our estimate of this flux.

We thank the captain and crew of the RV *Cape Hatteras* and chief scientist T. R. Fisher for their cooperation. J. T. Byrd and G. Baptist helped with sample collection. This work was supported by NSF grant ATM-8017574.

Received 14 July; accepted 15 September 1983.

1. Crutzen, P. J. *Geophys. Res. Lett.* **3**, 73–76 (1976).
1. Aneja, V. P., Aneja, A. P. & Adams, D. F. *J. Air. Pollut. Control Ass.* **32**, 803–806 (1982).
3. Torres, A. L., Maroulis, P. J., Goldberg, A. B. & Bandy, A. R. *J. geophys. Res.* **85**, 7357–7360 (1980).
4. Turco, R. P., Whitten, R. C., Toon, O. B., Pollack, J. B. & Hamill, P. *Nature* **283**, 283–286 (1980).
5. Crutzen, P. J., Heidt, L. E., Krasnec, J. P., Pollock, W. H. & Seiler, W. *Nature* **282**, 253–256 (1979).
6. Cadle, R. D. *Rev. Geophys. Space Phys.* **18**, 746–752 (1980).
7. Rasmussen, R. A., Khalil, M. A. K., Dalluge, R. W. & Penkett, S. A. *Science* **215**, 665–667 (1982).
8. Carroll, M. A., Heidt, L. E., Cicerone, R. J. & Prinn, R. G. *EOS* **63**, 893 (1982).
9. Turco, R P., Cicerone, R. J., Inn, E. C. Y. & Capone, L. A. *J. geophys. Res.* **86**, 5373–5377 (1981).
10. Sze, N. D. & Ko, M. K. W. *Nature* **280**, 308–310 (1979).
11. Logan, J. A., McElroy, M. B., Wofsy, S. C. & Prather, M. J. *Nature* **281**, 185–188 (1979).
12. Rasmussen, R. A., Khalil, M. A. K. & Hoyt, S. D. *Atmos. Envir.* **16**, 1591–1594 (1982).
13. Johnson, J. E., Harrison, H. & Heidt, L. E. *EOS* **63**, 894 (1982).
14. Ferek, R. J. & Andreae, M. O. *Geophys. Res. Lett.* **10**, 393–396 (1983).
15. Conrad, R., Seiler, W., Bunse, G. & Giehl, H. *J. geophys. Res.* **87**, 8839–8852 (1982).
16. Rasmussen, R. A., Hoyt, S. D. & Khalil, M. A. K. *Chemosphere* **11**, 869–875 (1982).

0028–0836/84/020150—03$01.00

First observations of the interaction of ocean swell with sea ice using satellite radar altimeter data

C. G. Rapley*

Groupe de Recherche de Géodésie Spatiale,
18 Avenue Edouard Belin, 31055 Toulouse Cedex, France

The action of ocean waves and swell on sea ice in the marginal ice zone (MIZ) controls the size distribution of ice floes and thus affects ice dynamics and the thermodynamics of ice growth and decay. An understanding of ice processes is important because of the influence of the sea-ice cover on high latitude weather and global climate[1]. Also, long-term changes in average sea ice extent could provide a sensitive indication of climatic change if the degree of shorter term variability were better understood[2]. Previous observations of the propagation of swell within the ice pack have been limited in spatial and temporal extent[3–6]. Here we present new results from the US Seasat satellite which suggest that radar altimetry can provide a powerful means of global synoptic monitoring of the interaction between ocean and ice.

The US satellites GEOS-3 and Seasat carried pulse-limited altimeters designed for studies of the geoid and ocean surface[7]. Both instruments obtained data over regions of sea ice. The radar echoes observed were highly variable but were generally stronger and more peaked in time than those from open water[8]. Brown[9] attributes the intense component of first-year ice returns to a coherent signal from smooth ice platelets all lying at the same level amongst the floes. Robin *et al.*[10] show that the peaked component will dominate over the diffuse echo if even as little as 0.01% of the surface contributes a coherent signal. Thus, provided any small areas of new ice or calm water are exposed within the altimeter footprint, we may expect to observe a strong return, and if the sea surface between floes is flat the waveform will be highly peaked.

Figure 1 shows three examples of the evolution of on-board computed values of 'significant wave height' (SWH) and signal strength (automatic gain control, AGC) as the Seasat altimeter passed from open ocean into the ice pack. Obvious features are the step in signal strength at the ice edge (indicated by the arrows) and the greater variability of both parameters over the ice. A particularly interesting feature of the SWH data in Fig. 1*a* and *b* is the gradual overall decline to zero with increasing distance from the ice edge. It is not immediately obvious to what property of the pack ice the SWH value corresponds. An insight into the effect is provided by the altimeter waveform data which typically evolve as shown schematically in Fig. 2 (see also Fig. 1*d*, *e* of Robin *et al.*[10]). Pulse shapes just inside the ice edge (Fig. 2*a*) are often almost identical to those from the nearby ocean, although amplitudes may be as much as 10 times greater. Altimeter pulse risetimes are a measure of the height probability density function of reflecting elements at nadir, and pulse fall times depend on the slope distribution (that is, for specular reflection the maximum duration of the pulse is given by $t_m = (h/c)\theta_{max}^2$ where θ_{max} is the maximum surface slope, and c and h are the velocity of light and satellite altitude respectively). Apparently, neither distribution changes significantly across the ice edge, although the large increase in signal strength implies that the nature of the reflecting elements must alter dramatically. This may be naturally explained if most of the power returned is reflected from thin ice platelets or smooth water between floes as discussed previously. The increasing leading edge slope and more rapid decay of waveforms deeper in the pack (Fig. 2*b*,*c*) then imply a continuous reduction in the interrelated height and slope distributions of the ocean

* Permanent address: Mullard Space Science Laboratory, Department of Physics and Astronomy, University College London, Dorking, Surrey RH5 6NT, UK.

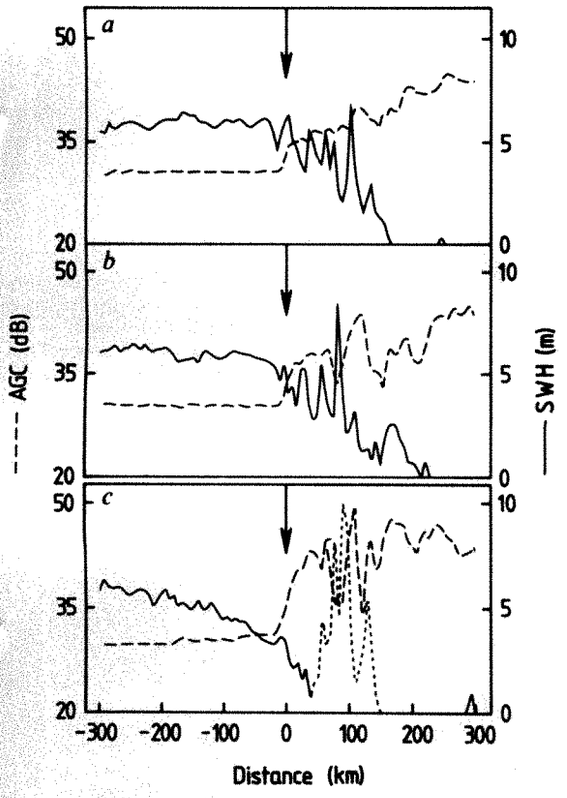


Fig. 1 Three examples of the evolution of on-board computed SWH and AGC values as the Seasat altimeter passed from ocean to sea ice. The ice edge is indicated by the arrow in each case. Note the sudden increase in AGC at the edge and the gradual overall decline of SWH value to zero. Note also the increased SWH noise within the ice pack and the poor data points, particularly in example *c*.

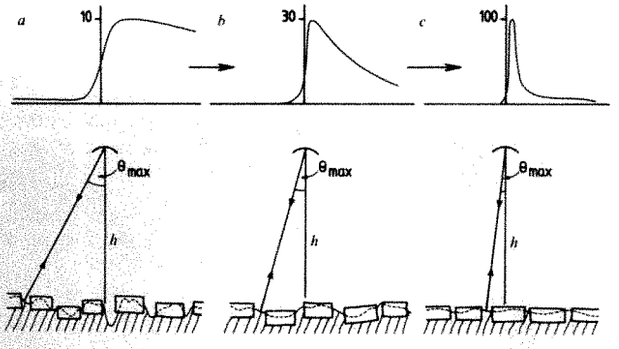


Fig. 2 The evolution of waveform shape and strength as a function of increasing distance into the ice pack. Leading-edge slopes depend on the height distribution of scatterers at nadir just as for ocean returns. Falltimes are determined by the distribution of surface slopes as shown schematically in the lower panels. Pulse strengths are relative to a value unity for a standard ocean return. Pulse shape and strength change as ocean waves and swell are damped out by the action of ice floes within the pack.

(or thin ice) surface as a function of distance from the ice edge. This is consistent with the known behaviour of ocean waves within the ice[5,6].

The relationship between pulse shape and SWH value is not straightforward. The on-board processing methods used by the Seasat altimeter were based on a standard ocean waveform model and the measured instrument performance characteristics[11,12]. An analysis of the response to non-standard pulses is complicated by interactions between all three processing loops[13]. However, an SWH output of zero was special and indicated a value less than the minimum resolvable by the instrument (~40 cm). This could only occur for pulses more peaked than an ocean return, although the degree of 'peakiness' necessary was a function of pulse risetime. Nevertheless, risetime and peakiness are partially interrelated as we have described, and we may therefore use zero SWH as a crude reference level of surface flatness.

Unfortunately, the on-board computed SWH values over sea ice suffered from increased noise and poor data points (note Fig. 1*c* in particular). The latter consist of correlated excursions of the height and SWH signals and resulted from brief attempts by the on-board tracker to follow receding bright features during transitions between areas of high and low surface reflectivity. The effect is similar to that described by Thomas *et al.*[14] for transitions across an ice front. The geographical distribution of extreme events shows a strong concentration in the vicinity of the ice edge and in areas known to be prone to polynya formation, implying an association with transitions from new or first-year ice to open water. Alternatively, 'hunting' by the on-board tracker when processing highly peaked pulses[13] could have introduced similar errors. Occurrences within the ice pack were more frequent towards the coast where AGC values were low, and may have resulted from transitions to areas of continuous ice or snow cover. Much of the contaminated data can be recovered by analysis of the altimeter waveforms but this represents a very major computing task. Thus, an exploratory investigation of swell propagation within the ice was carried out using the SWH data, recognizing its limitations. The use of a spatial resolution of ~70 km (10 point averages) and the averaging effect of adjacent groundtracks reduced the impact of data contamination on the results.

Figure 3 shows data from four successive repeats of the 3-day orbit cycle executed by Seasat during September 1978 at the time of maximum sea ice extent. The ice edge derived using an AGC threshold of 33 dB and the zero SWH contour are shown, the region between being shown as solid areas. Despite the coarse spatial resolution used, inconsistencies in the location of the ice edge occur due to motion within a 3-day cycle. These are represented by small re-entrant features. The SWH contour joins locations at which zero SWH is first encountered on entry into the ice pack. All non-zero SWH values within this contour are ignored as are occasional groundtracks obviously affected by poor data. Also shown are zones of high ocean swell (>5 m) derived using the method of Mognard[15] (see also ref. 16). The zones shown represent major storm systems. An immediate qualitative correspondence can be seen between areas in which the zero SWH contour is furthest from the ice edge and nearby zones of high swell. Furthermore, changes in the width of the band follow the temporal and spatial evolution of the swell storms. For example, the sector between 30° E and 60° W contains little swell storm activity and consistently exhibits a narrow band of swell penetration, whereas the decay of the major swell storm between 30° E and 120° E is matched by a decline in penetration distance.

The altimeter data therefore provide a means of using the ice as a detector of swell or, alternatively, the swell as a probe of the ice. The energy decay of each swell component is known to be exponential with an attenuation coefficient strongly dependent on swell wavelength and ice thickness[5,6]. The width of the band shown here also depends on the amplitude of the penetrating component at the ice edge and may be affected by the inadequacies of the SWH data as described above. Nevertheless, the observed penetration distances of up to 300–400 km are consistent with previous *in situ* measurements[3]. Detailed, quantitative studies will require analysis of the waveform data. Even so, the SWH data provide useful qualitative insights into ice properties. For example, the strong propagation of low swells into the edge features seen between 30° W and 90° W in Fig. 3*a* and at 30° W in Fig. 3*d*, implies that the ice is thin, as does their rapid spatial variability.

In an attempt to relate swell effects to ice edge growth, Fig. 4 shows a detail of ice edge changes over the four periods. A good general correlation is observed between ice edge variability and regions affected by swell, particularly in the case of the

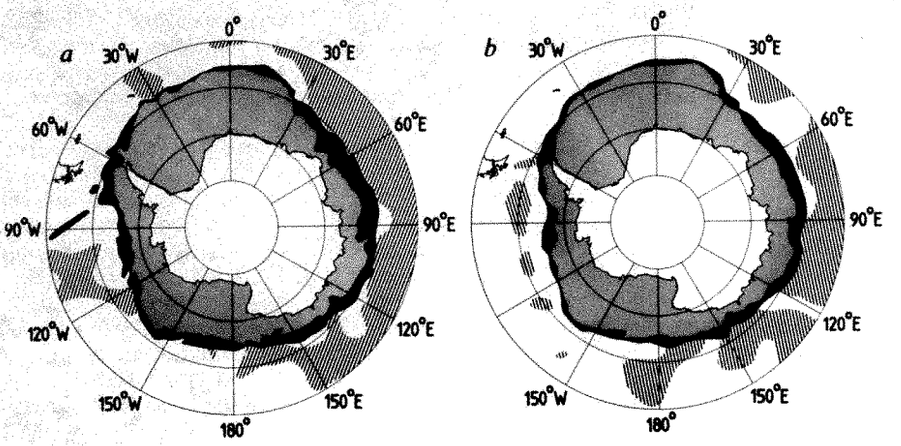


Fig. 3 Four consecutive 3-day maps of Antarctic sea ice (shaded areas) and regions of ocean swell greater than 5 m (cross-hatched areas) during September 1978. Zones of swell penetration into the ice (solid areas) derived from the SWH data contract and expand with the decline and subsequent growth of nearby ocean swell storms. Note the generally limited penetration in the region 30° E to 60° W where swell activity is low during this period, other than the feature suspected to consist of thin ice in *d*.

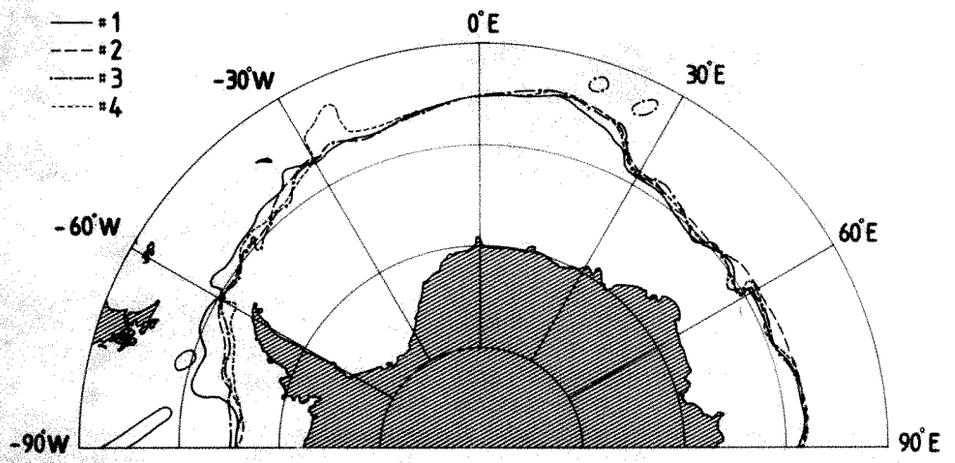


Fig. 4 Detail of the ice-edge variations over the four 3-day cycles. Greater variability occurs in regions affected by swell. Features showing the greatest changes are deeply penetrated by low-amplitude swells (see Fig. 3) implying that the ice is comparatively thin.

(apparently) thin ice features already noted. Keliher[17] has previously reported an association between pack ice disturbances and regions of high sea state.

We may conclude, therefore, that variations in altimeter return pulse shape provide a powerful means of monitoring swell propagation within the ice pack. Difficulties associated with the use of on-board SWH values have important implications on instrument design and data analysis procedures for future satellite altimeter missions.

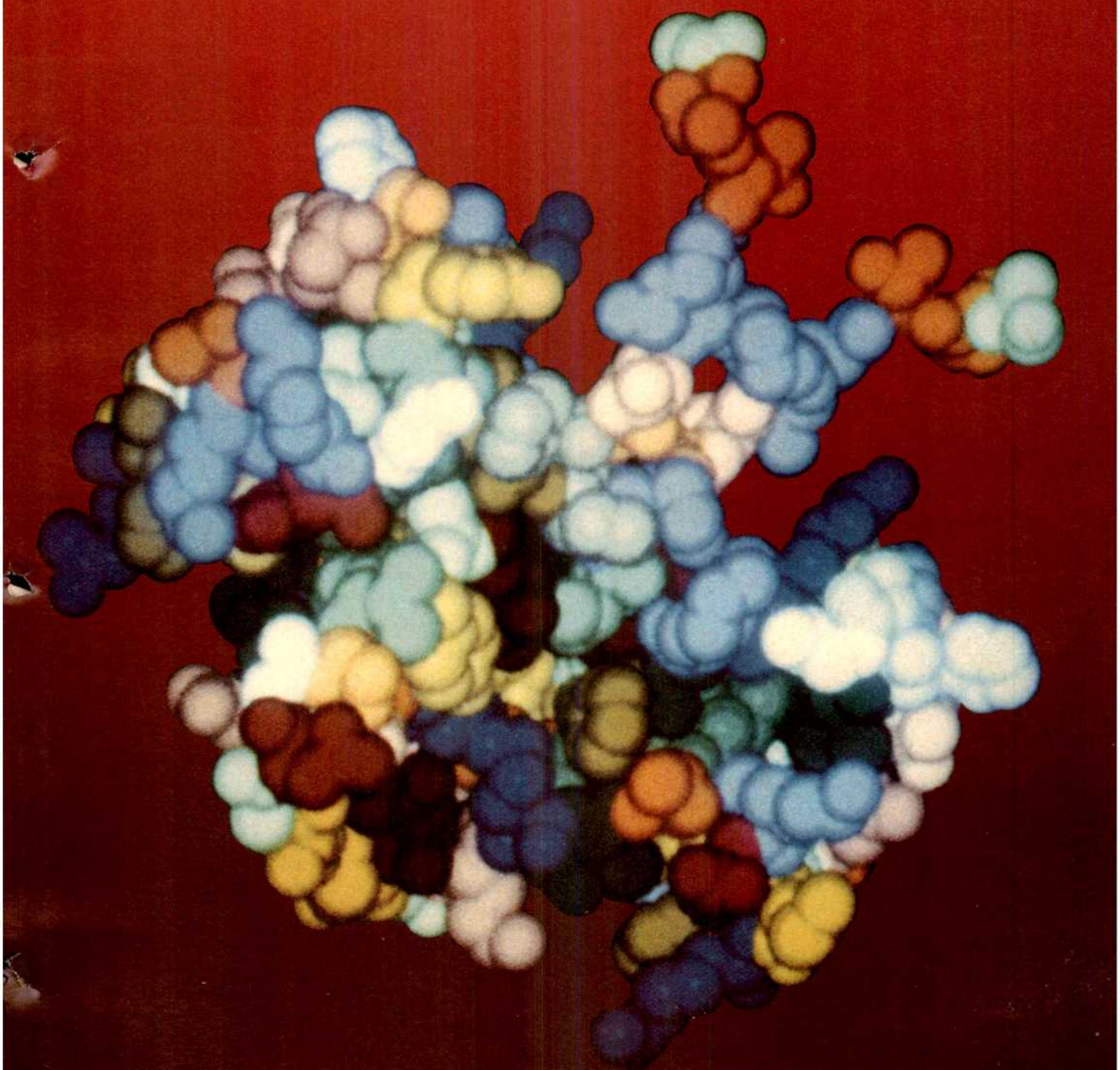
I thank the Centre Nationale d'Etudes Spatiales, France for financial support and the Groupe de Recherche de Géodésie Spatiale for their hospitality and assistance. I am particularly grateful to Mr Claude Brossier for his hard work and enthusiasm in producing the data plots and to Dr Michel Lefebvre for his encouragement. I acknowledge useful discussions with Drs Nelly Mognard and Bill Campbell.

Received 19 September; accepted 28 October 1983.

1. Committee on Glaciology, Polar Research Board *Snow and Ice Research, An Assessment* (National Academy Press, Washington DC, 1983).
2. Zwally, H. J., Parkinson, C. L. & Comiso, J. C. *Science* **220,** 1005–1012 (1983).
3. Robin, G. de Q. *Phil. Trans. R. Soc.* **A255,** 313–339 (1963).
4. Wadhams, P. *J. geophys. Res.* **80,** 4520–4528 (1975).
5. Wadhams, P. *Deep Sea Res.* **25,** 23–40 (1978).
6. Squire, V. A. & Moore, S. C. *Nature* **283,** 365–368 (1980).
7. Townsend, W. F. *IEEE J. Oceanic Engng* **OE-5**(2), 80–92 (1980).
8. Dwyer, R. E. & Godin, R. H. *NASA Contract Report* 156862 (1980).
9. Brown, G. S. *Radio Sci.* **17,** 233–243 (1982).
10. Robin, G. de Q., Drewry, D. J. & Squire, V. A. *Phil. Trans. R. Soc.* **A309,** 447–463 (1983).
11. MacArthur, J. L. *Proc. Oceans-76, MTS-IEEE* 1–8 (1976).
12. MacArthur, J. L. *Applied Physics Laboratory,* SDO-5232 (Johns Hopkins University, 1978).
13. Rapley, C. G. *et al. ESA Contract Report* 5182/82/F/CG(SC) (1983).
14. Thomas, R. H., Martin, T. V. & Zwally, J. H. *Ann. Glaciol.* (in the press).
15. Mognard, N. thesis, Univ. Paul Sabatier de Toulouse (1982).
16. Mognard, N., Campbell, W. J., Cheny, R. E. & Marsh, J. G. *J. geophys. Res.* **88,** 1736–1744 (1983).
17. Keliher, T. E. *AIDJEX Bull.* **34,** 114–135 (1976).

LKB
BROMMA

LKB HPLC systems revolutionize biology, biochemistry and biotechnology.



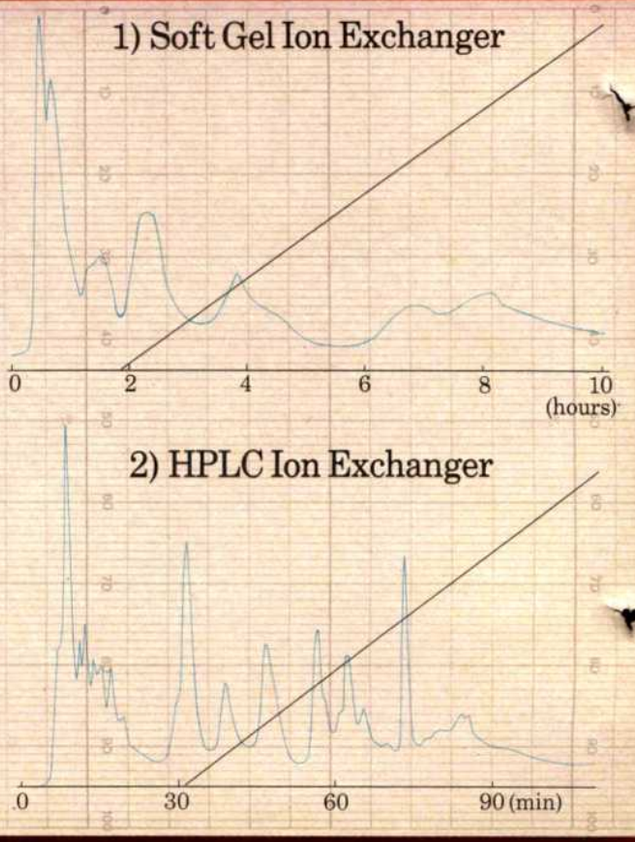
Achieve rapid, high resolution

High resolution

Sample:	Tryptic digest of aldehyde dehydrogenase
Detection:	280 nm, 1) 0.1 AUFS; 2) 0.02 AUFS
Gradient:	0—0.1 M NaCl in 0.02 M ammonium acetate, 8 urea, pH 5.0
HPLC Column:	CM, LKB 2133-200, 7.5×150 mm

Recent developments in high performance liquid chromatography have brought on a veritable revolution in the life sciences—enabling exploration far beyond the resolution limits of conventional liquid chromatography. These extraordinary improvements in resolution can be attributed primarily to the unique character of new HPLC separation media.

LKB HPLC columns are packed with rigid silica particles of approximately 1/10th the diameter of traditional soft gel media. The rigidity of these particles enables better packing than conventional liquid chromatography techniques. The smaller diameter results in more efficient fractionation, sharper peaks, increased resolving power and higher sensitivity.

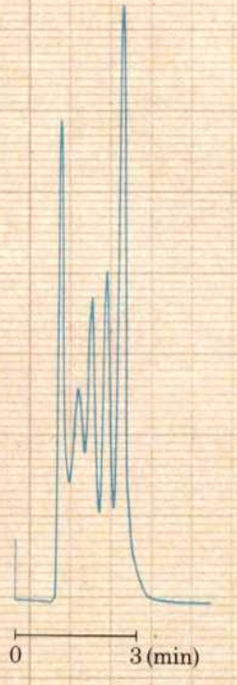


Rapid analysis

Sample:	Mixture of thyroglobulin, gammaglobulin, ovalbumin, myoglobin, vitamin B_{12}
Detection:	280 nm, 0.32 AUFS
Eluent:	0.1 M Na-phosphate buffer, pH 6.8
Flow rate:	1.0 ml/min
Column:	Gel permeation, LKB 2135-075, 7.5×75 mm

HPLC is the most rapid and efficient method available today for the fractionation of biomolecules. The HPLC separation shown here, for example, would have taken several hours by conventional soft gel chromatography! Like improvements in resolution, shorter analysis times are attributable largely to the rigidity and relatively small diameter of the HPLC media—enabling more rapid mass transfer between the stationary and mobile phases.

As a life scientist you are compelled to seek rapid methods for the fractionation of your samples. Not only are you interested in faster sample throughput, but you are also concerned about the danger to labile molecules of prolonged or drastic treatment, or the short half-life of a radioactive isotope. In addition, many conventional methods require harsh and time-consuming separation procedures. In all these cases, LKB HPLC systems are rapid and highly efficient alternatives.

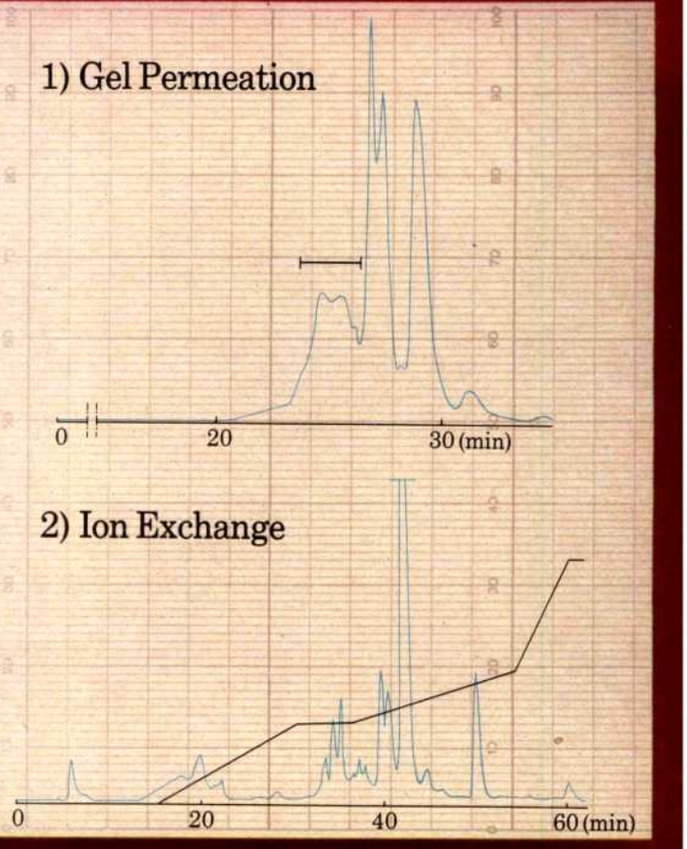


separations of biomolecules

Optimized purification schemes

Detection:	254 nm, 1) 0.5 AUFS; 2) 0.1 AUFS
Gradient:	2) 0.03—0.7 M ammonium acetate, pH 6.2
Columns:	1) Gel permeation, LKB 2135-260, 7.5×600 mm 2) DEAE, LKB 2133-100, 7.5×150 mm

By combining LKB HPLC techniques and thereby increasing the resolving power and speed of critical steps in a separation process, it is now possible to produce significant improvements in the efficiency of purification schemes. In this example, the entire separation was three times faster than conventional techniques and revealed 3—4 times as many components. An ultrafiltrate from the serum of uremic patients was first resolved by high performance gel filtration chromatography. Components in the molecular weight range 350—2000 (those which accumulate in the blood of uremic patients) were then applied to a high performance ion exchange column and resolved into 22 peaks.

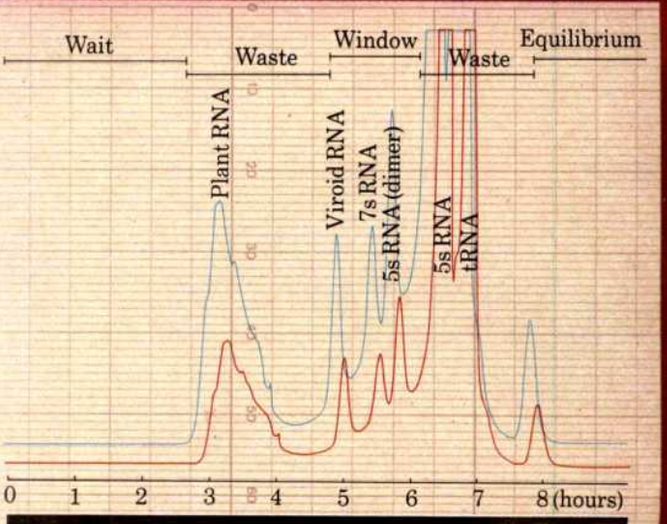


Preparative capabilities

Sample:	RNA from PSTV-infected tomato leaf
Detection:	254 nm, 1.0/2.0 AUFS
Eluent:	0.01 M NaAc, 0.02% NaN_3 0.2 M NaCl, 5 mM $MgCl_2$, pH 5.0
Flow rate:	0.4 ml/min
Column:	Gel permeation, LKB 2135-465, 21.5×600 mm

Courtesy of Sänger et al, Max-Planck-Institut of Biochemistry, Martinsried

Preparative LKB HPLC systems have proven to be a highly attractive alternative to other methods commonly used to separate complex mixtures of proteins and polynucleotides.
In this example, a preparative LKB HPLC system has been used to resolve a mixture of nucleic acids with a resolution comparable to that of analytical electrophoresis. Fractions of interest were collected automatically by the LKB 2211 SuperRac fraction collector. While electrophoretic methods are the traditional choice for analytical separations of polynucleotides, the preparative application of electrophoresis suffers from drawbacks such as limited loading capacity and extraction procedures which are tedious and often result in a loss of purified material. In this example, 400 OD of viroid RNA was eluted in less than 5 hours, with a recovery of better than 90%.





Electropherogram of the same sample.

Select the technique mos

LARGE BIOMOLECULES	FRAGMENTS	SMALLER BIOMOLECULES
i.e. proteins nucleic acids polysaccharides lipids	i.e. peptides oligonucleotides oligosaccharides	i.e. peptides amino acids nucleotides

Gel permeation

Ion exchange

Reversed phase

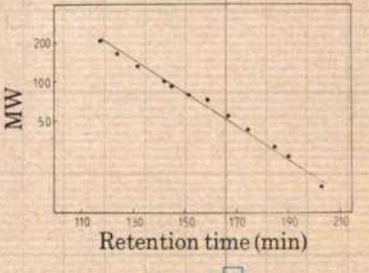
Gel Permeation Chromatography

Sample:	ϕX 174 from RBL incubated with Hinf I
Detection:	257 nm, 0.01/0.02 AUFS
Eluent:	50 mM TEAA, pH 7.0
Flow Rate:	100 µl/min
Column:	LKB 2135-460, 7.5×600 mm

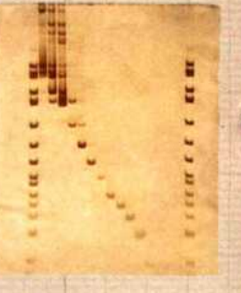
Courtesy of Kruppa et al, Physiol.-Chem. Institut, Hamburg

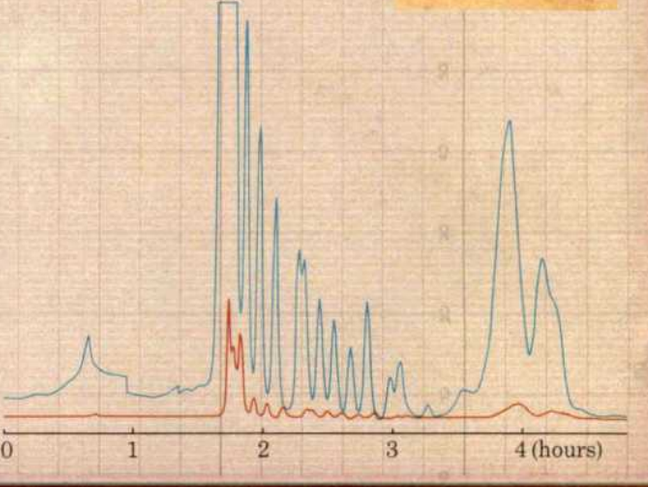
LKB offers a wide range of analytical and preparative Blue Columns for high performance gel permeation chromatography. The column packing material, a macroporous silica particle support, has been chemically modified to provide an inert and non-adsorptive stationary phase appropriate for high resolution separations of a wide variety of biomolecules as well as for use with most commonly used buffer systems and solubilizing agents.
The calibration shown here is just one of several examples of the excellent resolution, linearity and selectivity obtainable with an LKB high performance gel permeation system.

Calibration curve prepared from the chromatogram.



Electropherogram of the same sample.





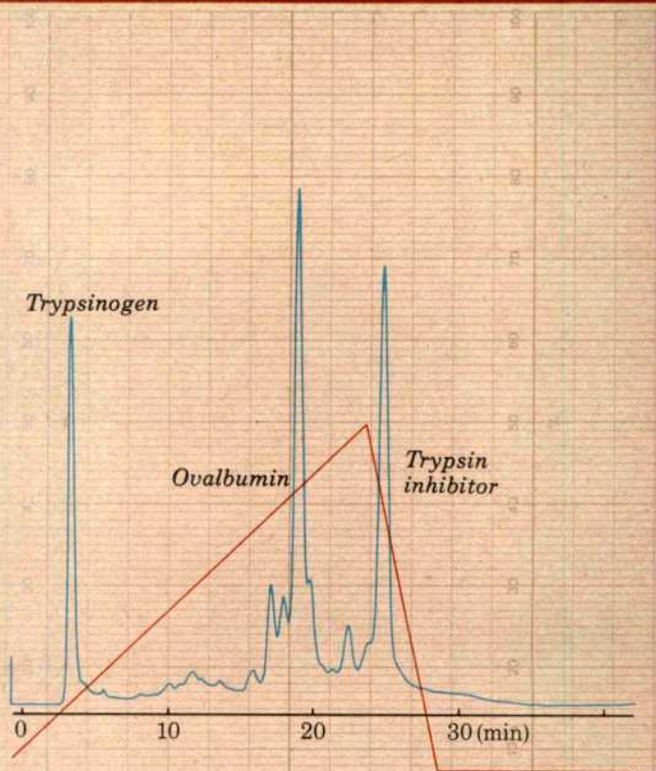
suitable for your sample

Ion Exchange Chromatography

Sample:	Trypsinogen, ovalbumin and trypsin inhibitor
Detection:	280 nm, 0.32 AUFS
Gradient:	0-0.5 M NaCl in 0.05 M Tris-HCl, pH 7.2
Flow rate:	1.0 ml/min
Column:	DEAE, LKB 2133-100, 7.5×150 mm

LKB Blue Column DEAE and CM ion exchangers can be used at high pressures and ionic strengths, and with a wide variety of buffer systems. Due to their speed, resolution and wide range of application, high performance ion exchange techniques are rapidly replacing many methods conventionally used for separation and analysis prior to protein sequencing; they are now also used to purify commercially-available proteins for enzyme assays and structural studies.

LKB ion exchange systems, for instance, permit the use of volatile buffer solutions (ammonium bicarbonate or ammonium acetate) for highly efficient peptide separations. For large peptides, the LKB HPLC ion exchange system gives better resolution than traditional reversed phase chromatography.



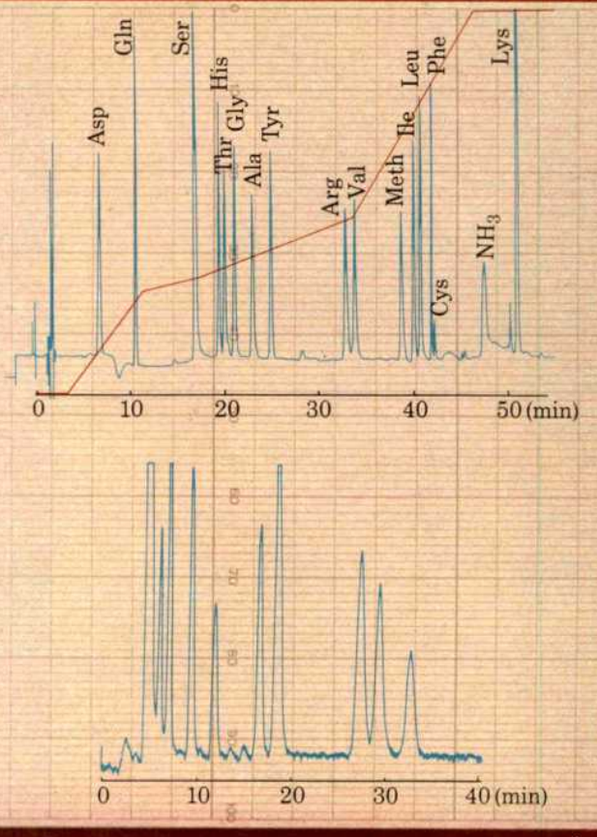
Reversed-phase Chromatography

Sample:	*o*-phthalaldehyde derivatives of amino acids, 1 nMol each
Detection:	330 nm, 0.04 AUFS
Gradient:	10—50 % CH_3CN in 12.5 mM Na-phosphate buffer, pH 7.2
Column:	C18 (5 μm), LKB 2134-215, 4×250 mm

LKB's high performance reversed-phase chromatography systems provide an accurate, reproducible, rapid and inexpensive method for analyzing smaller biomolecules such as peptides, amino acids, PTH-amino acids and nucleic acid components.

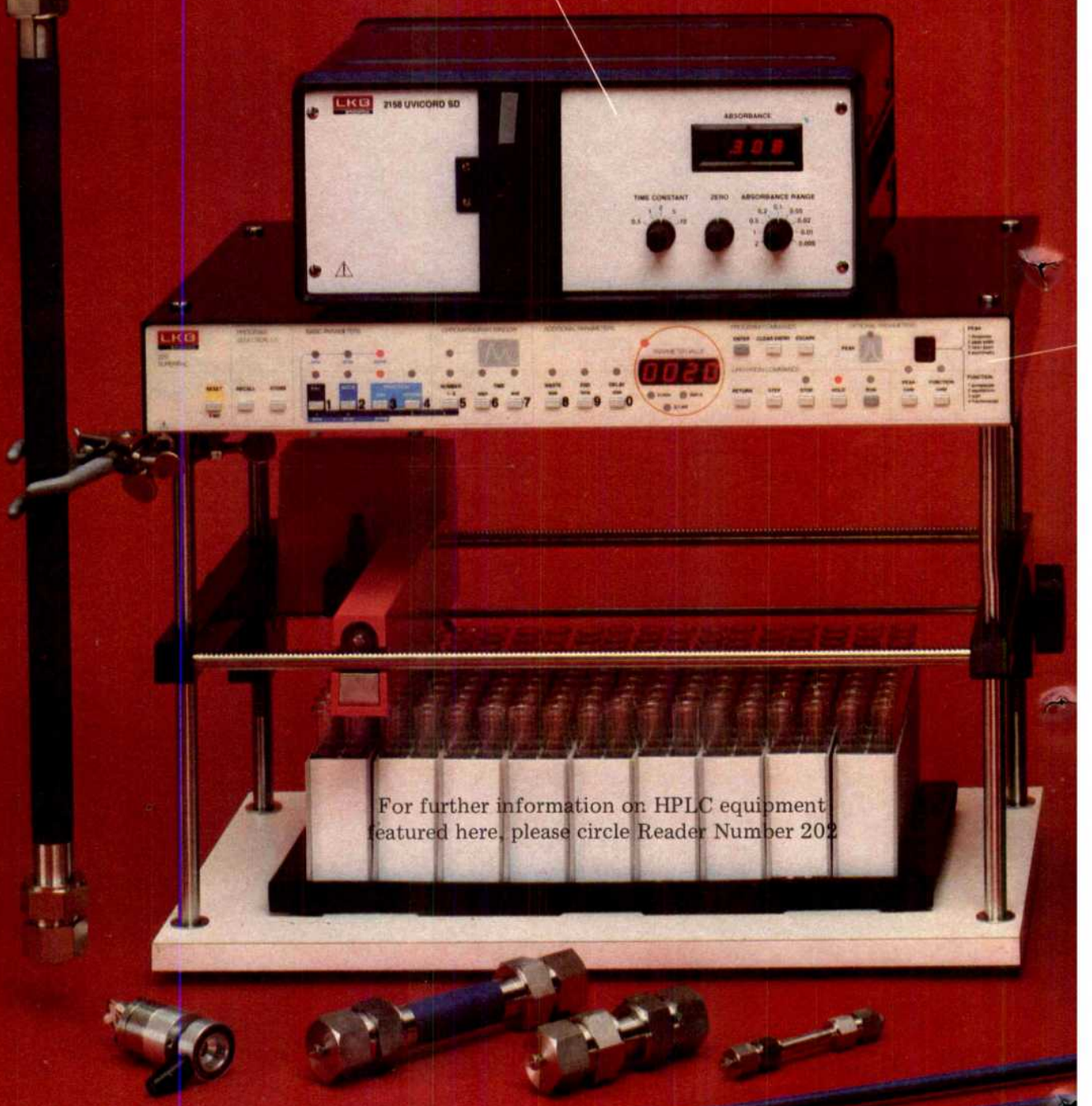
Column dimensions and particle sizes have been selected to give maximum separation efficiency, lower detection limits and a minimum of solvent consumption. An LKB high performance *microbore* system (1 mm ID columns), for example, can achieve up to 14 times lower detection limits and solvent savings of up to 95% compared with conventional HPLC systems.

Sample:	PTH amino acids, 1—5 pMol each
Detection:	254 nm, 0.005 AUFS
Eluent:	CH_3CN/THF in NaAc buffer, pH 5.8
Flow rate:	50 μl/min
Column:	C18 (5 μm), LKB 2134-600, 1×250 mm



Optimize your
LKB's modular

Choice of detectors—The versatile Variable Wavelength Monitor or the highly sensitive Uvicord SD with a choice of 3 flow-optimized cells and 7 interference filters.



For further information on HPLC equipment featured here, please circle Reader Number 202

Extensive range of columns and accessories
Prepacked gel permeation, ion exchange and reversed-phase columns with a choice of particle size and column dimensions (for preparative or analytical applications), injector, capillaries & fittings.

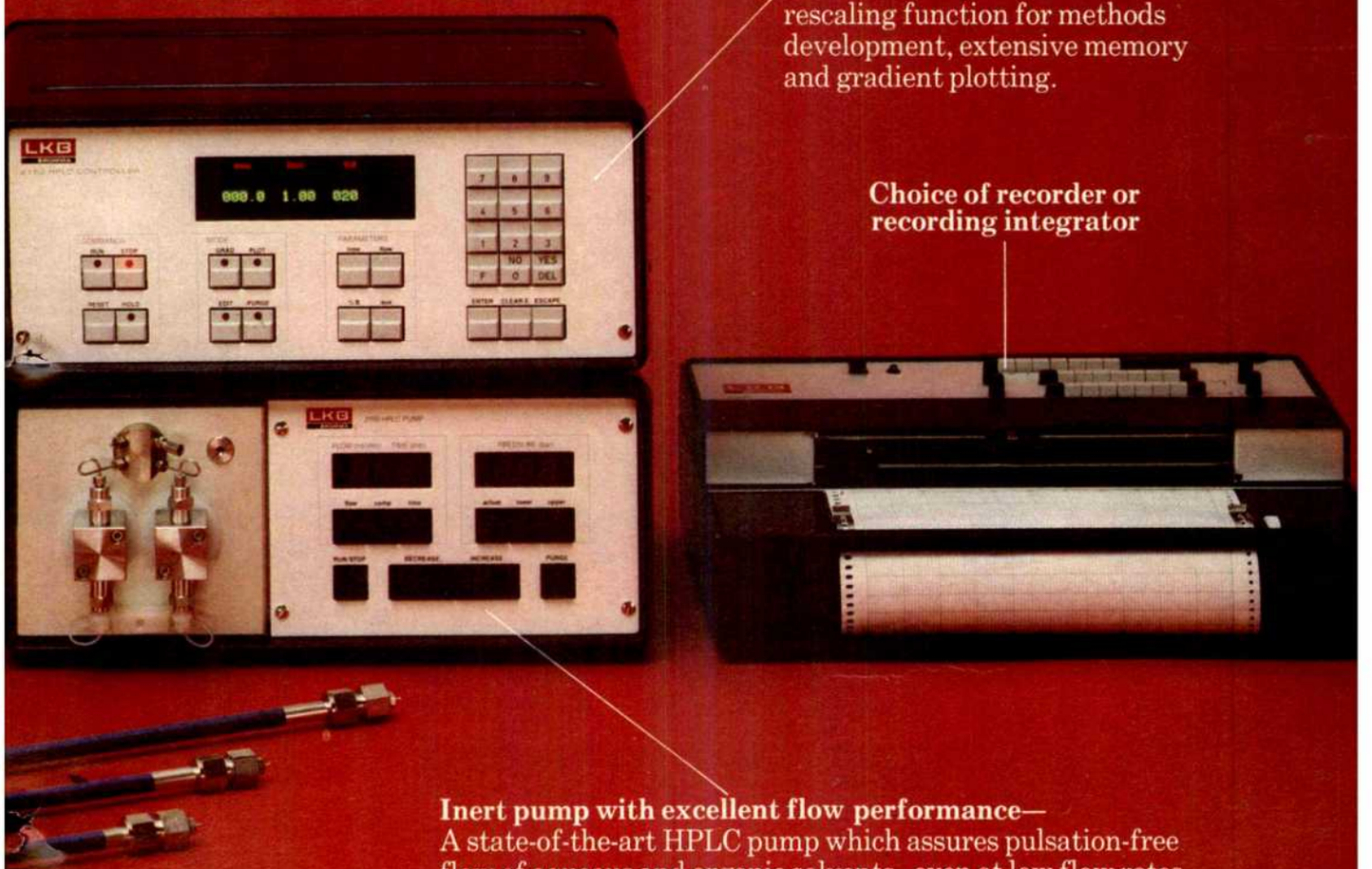
NEW—an expanded range of ion exchange columns.
Ask your LKB representative for more information.

esults with HPLC systems

LKB offers the first modular HPLC system designed to meet both today's general requirements in HPLC as well as the special requirements posed by biomolecular research. Given the relatively low diffusion rates of many large biomolecules, the LKB 2150 HPLC Pump is the only HPLC solvent delivery unit capable of flow-optimized gel permeation and ion exchange chromatography. This unique pump teams up with the 2152 HPLC Controller to provide unprecedented precision, accuracy and complete control of gradient formation—even at the extremes of gradients where very low flow rates are required. Both units are microprocessor-controlled for added precision and reproducibility as well as for ease of use—incorporating convenient pushbuttons and bright and easy-to-read digital displays.

The intelligent fraction collector
SuperRac is the most advanced fraction collector available today, with peak-slope detection, window programming, re-cycling capabilities and an extensive memory.

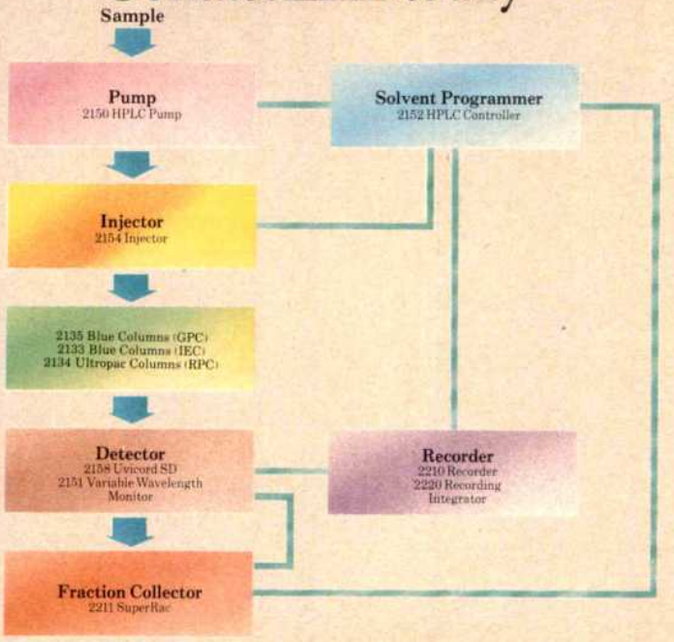
Accurate gradient elution & complete system control—Simple and accurate system control with rescaling function for methods development, extensive memory and gradient plotting.



Choice of recorder or recording integrator

Inert pump with excellent flow performance—
A state-of-the-art HPLC pump which assures pulsation-free flow of aqueous and organic solvents—even at low flow rates (for high resolution separations of large biomolecules and microbore HPLC).

Contact LKB today



The LKB HPLC System configuration

LKB can provide you with all the detailed and up-to-date information you need on HPLC instrumentation, techniques and applications for the separation of biomolecules. Apart from **Descriptive Catalogues** and **Product Brochures** for all HPLC instruments and chemicals, LKB also publishes **Application and Technical Notes** to help you realize the full potential of your HPLC instrumentation. LKB **Workshops and Seminars** give you support on the theory and practice of HPLC for the separation of biomolecules, and provide you with an opportunity to discuss techniques with other scientists and LKB specialists. A 64-page Seminar Note booklet entitled **HPLC for the Biochemist** and an accompanying **Slide Set** are also available from your local LKB representative.



LKB—specialists in HPLC for the Biochemist

LKB
THE INCENTIVE GROUP

LKB-Produkter AB, Box 305, S-161 26 Bromma, Sweden
Stockholm Antwerp Athens (Middle East) Copenhagen Hong Kong London Luzern Munich Paris Rome Turku Vienna Washington Zoetermeer
Representatives in 60 countries

Earthquake seasonality before the 1906 San Francisco earthquake

Patrick H. McClellan

US Department of Interior, Minerals Management Service, 345 Middlefield Road, Menlo Park, California 94025, USA

Since 1855, 40 earthquakes of magnitude ≥5.5 have occurred on the San Andreas Fault (SAF) system in coastal central California, between latitudes 36°30′ N and 39°30′ N (Table 1)[1]. Of the included 27 'independent' earthquakes, which occurred more than 1 yr after or 1 geocentric degree distant from a previous ≥5.5 mag earthquake, 3 occurred in summer, 7 in autumn, 4 in winter and 13 in spring. All of the spring earthquakes occurred during the 25-yr interval that culminated with the great San Francisco earthquake (8.25 mag) in the spring of 1906 (Fig. 1*a*, *b*). I report here that the observed frequency of the spring earthquakes before 1906 significantly exceeds (binomial $P = 0.002$) seasonal frequencies expected from random variation in the earthquake rate, and thus invites serious inquiry into the subject of earthquake seasonality. More importantly, the time-clustering of spring earthquakes before 1906 raises the question of whether accelerated seasonal seismicity may herald the end of the 'seismic cycle' suspected[1] to modulate the long-term pattern of seismicity on the northern segment of the SAF system.

To avoid the *a priori* assumptions inherent in seasonal grouping of the data, the significance of the apparent earthquake seasonality before 1906 was further assessed with the Schuster test[2], which, as used here, measures the tendency of earthquakes to occur near the same time ('phase') of the year. Each earthquake datum within a year was converted to a phase angle ω_i (1 January = 0°) and the n phases were vectorially added. If

$$A = \sum_{i=1}^{n} \cos \omega_i \quad \text{and} \quad B = \sum_{i=1}^{n} \sin \omega_i$$

then the resultant vector has a net phase $\phi = \arctan(B/A)$ and a length $R = \sqrt{A^2 + B^2}$. The probability of observing a vector longer than R (that is, a better correlation) from a set of n random phases is $P = \exp(-R^2/n)$, where $n \geq 10$ (refs 3–5). The results are listed in Table 2. For the complete set of $n = 27$ independent earthquakes since 1855, $\phi = 119°$ (corresponding to a date of 30 April) with a random probability $P = 0.21$ (not significant). However, for the 19 earthquakes between 1855 and the end of 1905, $\phi = 104°$ and $P = 0.029$. In other words, leading into the year 1906, there was a significant tendency for moderate and large earthquakes to occur not only in the spring but around the date of 15 April. The great San Francisco earthquake occurred on 18 April 1906.

Considering only the 'strongest' independent earthquakes (≥6.0 mag, as defined, for example, in ref. 6) between 1855 and 1906 ($n = 7$) leaves too few events ($n < 10$) to be tested reliably for correlation by the Schuster method[5]. If the magnitude threshold is lowered below 6.0 mag, to enlarge the set of strongest events between those years, seasonality becomes increasingly apparent. Although clustering is not evident for the 10 earthquakes of ≥5.9 mag ($P = 0.35$), it is significant at the $P = 0.07$ level for the 13 events of ≥5.7 mag, and, as noted above, at the $P = 0.029$ level for the 19 events of ≥5.5 mag. This indicates that the weaker earthquakes were, in large part, responsible for the observed seasonality. Alternatively, the set of seven strongest (≥6.0 mag) earthquakes before 1906 may be enlarged by adding to it the three strongest known pre-1855 central California earthquakes (Table 3), which reasonably are assumed also to have exceeded 6.0 mag[7]. Those 10 strongest earthquakes collectively are correlated, albeit marginally ($P = 0.07$–0.09; Table 2), about the dates of 23–28 May, with most occurring in the spring season within the quarter-century before 1906.

Table 1 Earthquakes (≥5.5 mag) in coastal central California from 36°30′ N to 39°30′ N, 1855–1982

Year	Date	Time	Latitude	Longitude	Magnitude
1856*	2 15	13:25	37°36′ N	122°24′ W	5.9
1858*	11 26	8:35	37°36′ N	122°00′ W	5.9
1864*	2 26	8:40	36°30′ N	121°30′ W	5.8
1865*	10 8	20:46	37°06′ N	121°54′ W	6.2
1866	3 26	20:12	37°06′ N	121°30′ W	5.8
1866	7 15	6:30	37°18′ N	121°12′ W	5.7
1868*	10 21	15:53	37°42′ N	122°06′ W	6.7
1870*	2 17	20:12	37°12′ N	122°00′ W	5.5
1881*	4 10	10:00	37°18′ N	121°18′ W	6.0
1882	3 6	21:45	36°42′ N	121°12′ W	5.5
1883*	3 30	15:45	36°54′ N	121°36′ W	5.7
1885*	3 31	7:56	36°42′ N	121°18′ W	5.6
1885	4 2	15:25	37°00′ N	121°24′ W	5.5
1889*	5 19	11:10	38°00′ N	121°54′ W	5.6
1890*	4 24	11:36	36°54′ N	121°36′ W	5.9
1891*	10 12	6:28	38°18′ N	122°18′ W	5.6
1892	4 19	10:50	38°30′ N	122°00′ W	6.8
1892	4 21	17:43	38°36′ N	121°54′ W	6.3
1897*	6 20	20:14	36°54′ N	121°24′ W	6.0
1898*	3 31	7:43	38°12′ N	122°24′ W	6.3
1898*	4 15	7:07	39°12′ N	123°48′ W	6.7
1899*	4 30	22:41	36°54′ N	121°36′ W	5.7
1899*	6 2	7:19	37°48′ N	122°36′ W	5.5
1899	7 6	20:10	36°42′ N	121°18′ W	5.5
1902*	5 19	18:31	38°18′ N	121°54′ W	5.5
1903*	6 11	13:12	37°36′ N	121°48′ W	5.6
1903	8 3	6:49	37°18′ N	121°48′ W	5.6
1906*	4 18	13:12	37°42′ N	122°30′ W	8.3
1910*	3 11	6:52	36°54′ N	121°48′ W	5.7
1911*	7 1	22:00	37°15′ N	121°45′ W	6.0
1914*	11 9	2:31	37°10′ N	122°00′ W	5.5
1926*	10 22	12:35	36°37′ N	122°20′ W	6.2
1926	10 22	13:35	36°34′ N	122°20′ W	6.2
1926	10 24	22:51	37°01′ N	122°12′ W	5.6
1927	2 15	23:54	36°57′ N	122°15′ W	5.6
1955*	9 5	2:01	37°22′ N	121°47′ W	5.6
1969*	10 2	4:56	38°28′ N	122°41′ W	5.5
1979*	8 6	17:05	37°05′ N	121°28′ W	5.9
1980	1 24	19:00	37°50′ N	121°48′ W	5.8
1980	1 27	2:33	37°51′ N	121°47′ W	5.5

Data are from the catalogue in ref. 1, Appendix, Table A1. All magnitudes before 1900 and some during 1900–27, are intensity magnitudes (M_I) estimated from published felt-area data on the basis of empirical relations between local magnitude (M_L) and extent of isoseismal areas for twentieth-century earthquakes in California and western Nevada[14]. A test of M_I against M_L for twientieth-century earthquakes in coastal central California[1] suggests that, for this subregion, M_I is slightly conservative and may underestimate M_L by about 0.2 ± 0.2 units. Thus, the intensity magnitudes listed are presumed to be not greatly biased relative to the instrumental magnitudes. Magnitude listed for the 1906 event is surface-wave magnitude (M_S), M_L recently determined at 6.9 (ref. 15).

* 'Independent' events, which were separated by more than 1 yr or 1 geocentric degree.

Possible explanations for the observed earthquake seasonality include (1) incompleteness of the ≥5.5 mag earthquake catalogue, (2) fortuitous sampling of the catalogue by the assumed criteria of event independence, (3) fortuitous sampling of a limited geographical segment of the SAF system, (4) a real, though improbable, random variation in the earthquake occurrence rate and (5) a fundamental geological process. Because the catalogue is believed[1] to be complete for events down to 5.5 mag, it is unlikely that the observed high spring seismicity is due to the omission of numerous large earthquakes in other seasons. Likewise, the pattern probably is not an artefact of fortuitous sampling of the ≥5.5 mag catalogue, inasmuch as assuming the least conservative definition of event independence (Fig. 1*c*) also results in a disproportionately high frequency of

© 1984 Macmillan Journals Ltd

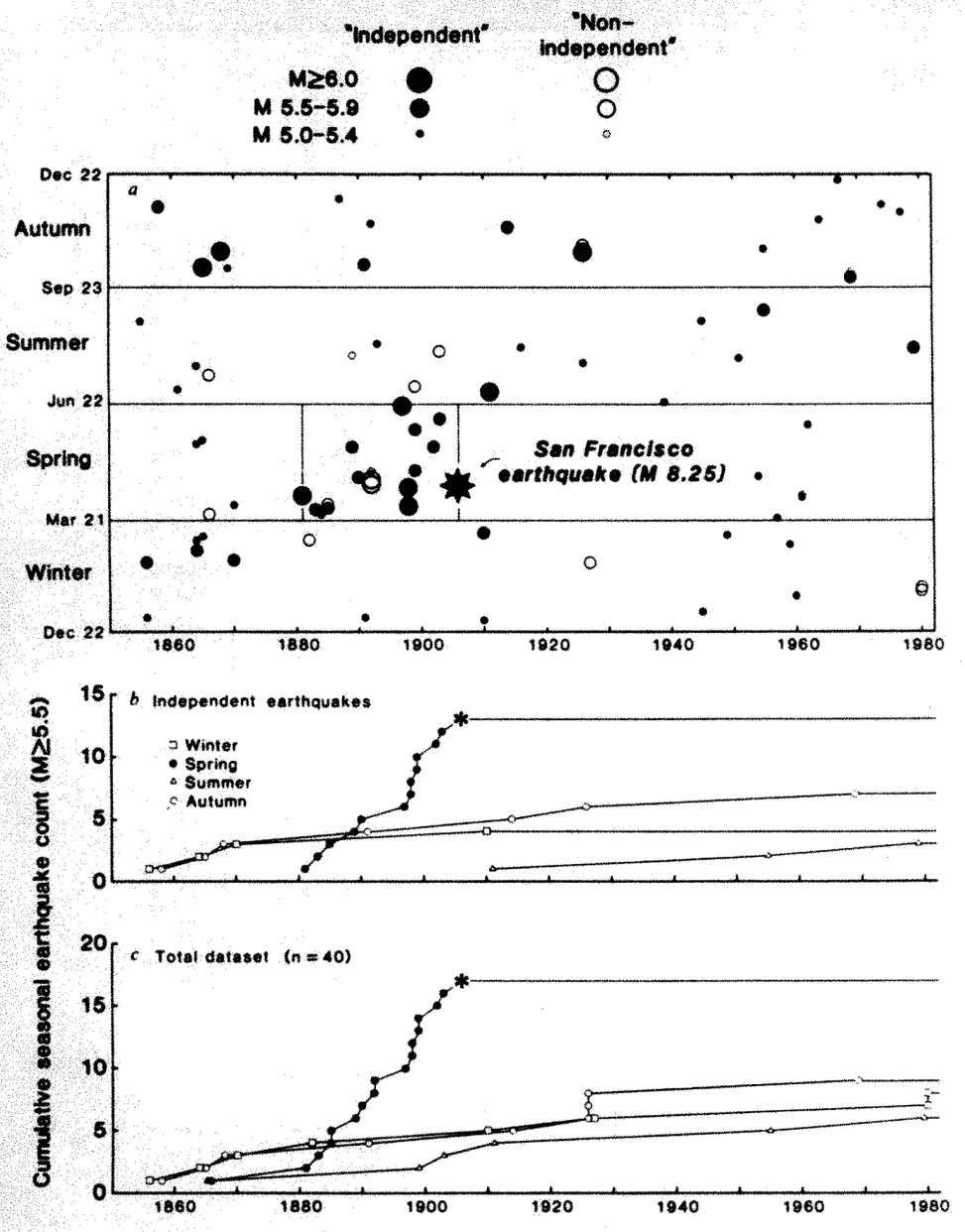


Fig. 1 *a*, Year-season plot of moderate to large (≥5.5 mag) earthquakes in coastal central California (36°30′–39°30′ N) from 1855 to 1983. Independent ≥5.5 mag events are defined as those separated by more than 1 yr or 1 geocentric degree. Seasons are defined by the conventional dates of the winter solstice (22 December), vernal equinox (21 March), summer solstice (22 June) and autumnal equinox (23 September). *b*, Cumulative count by season of independent ≥5.5 mag earthquakes plotted in *a* ($n = 27$). *c*, Cumulative count by season of total ≥5.5 mag earthquake set plotted in *a* ($n = 40$).

spring earthquakes. A more conservative assumption than was used seems unreasonable.

Regarding the geographical limitation of the earthquake sample, Schuster analysis of independent strong earthquakes (≥6.0 mag) on the southern SAF system (33–36° N, $n = 13$)[6], beginning with the great 1857 earthquake there to the present time, show that those events also are correlated ($P = 0.04$) about a date in the spring (31 March), as are those earthquakes in combination with the 10 strong earthquakes since 1855 in central California (36°30′–39°30′ N; Table 1) plus 12 other post-1855 events of about ≥6.0 mag which occurred on the SAF system between 33–36.5° N (Table 3)[7], but were not included in the list in ref. 6. This total set of ≥6.0 mag earthquakes ($n = 35$) is correlated ($P = 0.04$) about the date of 20 April, and suggests that the observed earthquake seasonality in coastal central California is stable against geographical extensions of the earthquake sample, at least for the strongest historical events over the extent of the SAF system. (Smaller earthquakes throughout the system were not analysed.) Finally, although the 25-yr-long sequence of spring earthquakes in central California may have been a mere coincidence of random events, the termination of the sequence with the unique 1906 earthquake circumstantially suggests otherwise. Thus, coastal central California appears to have been tectonically predisposed to the frequent occurrence of moderate to large earthquakes during the spring season from 1881 to 1906.

A physical basis for the observed earthquake seasonality on the northern segment of the SAF system can only be conjectured at present. Nevertheless, the fundamental mechanism almost certainly involves the manner in which elastic strain accumulates in the crust along that portion of the North American–Pacific plate boundary. Crustal strain accumulating there is released primarily in ground-rupturing earthquakes on the SAF that are probably similar in size to the 1906 earthquake and are believed to occur there at intervals averaging from 150 yr[1] to perhaps 230 yr[8] in length. This range of estimated recurrence intervals approximates the average of 145 yr (range 100–225 yr) between large or great ground-rupturing earthquakes on the south-central SAF, like the 1857 event, during the past 17 centuries (K. E. Sieh, personal communication). In addition, in coastal central California, overall historical seismicity above the 5.0 mag level was generally higher during the half-century before the 1906 earthquake than in the half-century afterward[1], not unlike a pattern, called the 'seismic cycle'[9,10], observed before and after great earthquakes in the Wadati–Benioff zones of the western Pacific basin. However, an unequivocal precursory increase in seismicity, as characterizes 'Stage II' of this cycle[10], has not hitherto been identified in pre-1906 earthquake data

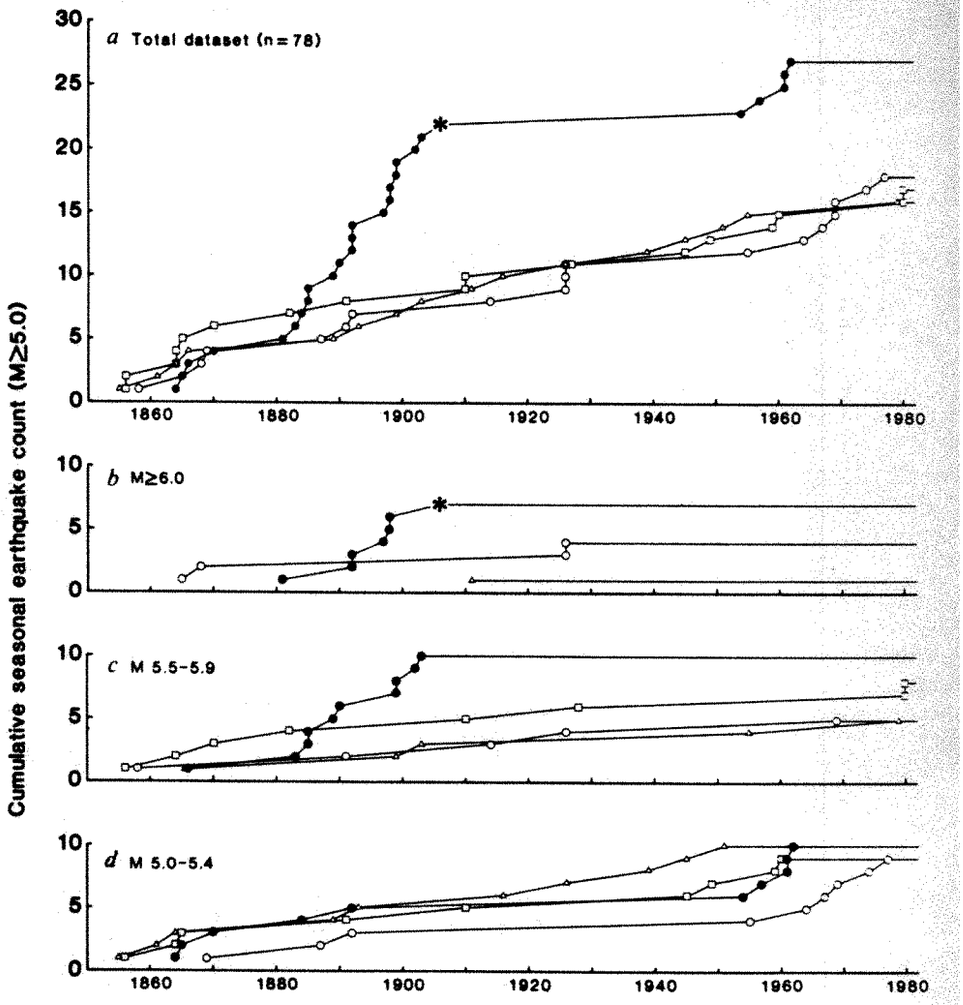


Fig. 2 *a*, Cumulative count by season of ≥5.0 mag earthquakes in coastal central California from 1855 to 1983, listed in ref. 1 ($n = 78$). *b*, Total ≥6.0 mag earthquake subset in *a*. *c*, Total 5.5–5.9 mag earthquake subset in *a*. *d*, Total 5.0–5.4 mag earthquake subset in *a*.

from central California[1,11]. Nevertheless, the quasiperiodic recurrence of great earthquakes on the northern SAF is suspected[1] to control a seismic cycle there also.

Conjectural mechanisms which might have selectively triggered earthquakes in the spring on faults in the SAF system necessarily presuppose that, before triggering, those faults were already tectonically strained to near the point of shear failure. It is not unreasonable, in retrospect, to assume that such a pre-strained condition became increasingly prevalent in the 25 yr leading up to 1906. Possible seasonal triggering mechanisms may be classified on the basis of how they might affect the three terms in the stress ratio:

$$\frac{\tau}{s_n - p} = K$$

where τ is shear stress, s_n is normal stress, p is pore-fluid pressure along a pre-existing fault, with K the critical ratio above which shear failure will occur in the given medium[12]. In theory, on a near-critical fault, any seasonal process which might relatively increase shear stress and/or pore pressure, or relatively decrease normal stress, could potentially destabilize the fault and trigger rupture, and thus account for earthquake seasonality before a great earthquake. Although limited space prevents a detailed discussion of candidate seasonal processes, Table 4 outlines several that might be most effective in the spring. Those listed are ordinarily nominal ambient processes which clearly would not be expected to trigger slip on faults at low strain levels. On faults at high strain levels, however, such as the subparallel faults in the San Andreas system late in a seismic cycle, the listed triggering mechanisms conceivably could introduce a seasonal bias into the time-distribution of earthquakes. In the absence of high pre-stress, such as early in a seismic cycle, or of a seasonal triggering influence, earthquakes would be expected to distribute themselves randomly throughout the year, as they have been distributed in coastal central California since 1906 (≥5.0 mag: Schuster $P = 0.70$).

Table 2 Schuster test results for selected subsets of earthquakes in coastal central California

	1855–1982	1855–1905	1907–1982	1808–1905*	1808–1982*
	≥6.0 mag (Independent events)				
n	10	7	2	10	13
ϕ	15 May	30 April	27 August	23–28 May	28 May–2 June
R	3.06	2.48	1.13	5.11–4.93	5.87–5.75
P	0.39	0.42	0.53	0.07–0.09	0.07–0.08
	≥5.5 mag (independent events)				
n	27	19	7		
ϕ	30 April	15 April	15 Sept		
R	6.50	8.20	3.37		
P	0.21	0.03	0.20		
	≥5.0 mag (independent events)				
n	61	34	26		
ϕ	11 April	15 April	24 October		
R	7.33	9.33	3.05		
P	0.41	0.08	0.70		

n, Number of earthquakes ('annual phases') in subset.
ϕ, Time of year (corresponding to net phase angle) near which earthquakes in subset tended to occur.
R, Vector sum over subset of earthquake phase angles.
P, Probability of observing a vector sum greater than R if n phases were random (valid for $n \geq 10$)[5].
* Subset includes three largest pre-1855 central California earthquakes: in 1808, 1836 and 1838 (Table 3). For the undated June 1838 event, extreme dates of 1 June and 30 June were assumed in calculating possible ranges for ϕ and P.

The above observations provide evidence for precursory seasonal seismicity leading up to the great San Francisco earthquake. Although the observations may be statistically significant, whether they are also physically significant remains to

Table 3 Other strong (≥6.0 mag) earthquakes in coastal central and southern California (see text)

Date	Latitude	Longitude	Maximum modified Mercalli intensity	Magnitude
1808 6 21	37°48′ N	122°30′ W	VIII	?
1836 6 10	37°48′ N	122°12′ W	VIII	6.8
1838 6 --	37°36′ N	122°12′ W	VIII	≥7.0
1885 4 12	36°24′ N	121°00′ W	VII	6.2
1890 2 9	33°24′ N	116°18′ W	VI+	6.3
1892 5 28	33°12′ N	116°12′ W	V–VI	6.3
1899 7 22	34°18′ N	117°30′ W	VIII	6.5
1907 9 20	34°12′ N	117°06′ W		6.0
1910 5 15	33°42′ N	117°24′ W		6.0
1916 10 23	34°54′ N	118°54′ W		6.0
1922 3 10	35°48′ N	120°18′ W		6.5
1927 11 4	34°54′ N	120°42′ W		7.5
1934 6 8	35°48′ N	120°18′ W		6.0
1952 11 22	35°42′ N	121°12′ W		6.0
1983 5 2	36°14′ N	120°17′ W		6.7

Pre-1900 earthquake data from ref. 7; post-1900 data from ref. 16; 1983 data (Coalinga earthquake) from University of California Seismographic Station, Berkeley. Pre-1900 magnitudes are M_I; post-1900 magnitudes are M_L, except for 1927 event, which is M_S.

Table 4 Conjectural mechanisms for springtime seismicity

1. *Pore-fluid pressure increase*
   - *a*, Downward-propagating pulse of above average crustal pore pressure due to increased infiltration of water from surface and subsurface reservoirs refilled by winter rainfall.
   - *b*, Static unloading of crust in coastal region by seasonal decline in monthly mean sea level of the order of 20 cm, leaving overpressures on pre-stressed faults at depth; minimum occurs in springtime off central California and follows a decline which may exceed 30 cm over a 2-month period (if after a winter-long high sea level anomaly, or 'El Nino')[17–19].
2. *Increase in shear stress and/or decrease in normal stress*
   - *a*, Springtime increase in northwestward force vector on northwestward-moving Pacific plate due to increasing inclination of Northern Hemisphere toward Sun. (As this notion might predict, strong earthquakes on the 1,100-km long SAF system tend to occur near a date earlier in the spring in the south (31 March) than in the north (late May).)
   - *b*, Interaction at the plate boundary of torques on the Pacific and North American plates resulting from the springtime deceleration of Earth's rotational spin (manifested by springtime increase in the length of day)[20].

be demonstrated. Nevertheless, the evidence corroborates, if rather oddly, the suspicion that a seismic cycle governs the long-term behaviour of the northern SAF system. If the historical pattern of earthquake seasonality is characteristic of that long-term behaviour, several interesting inferences are possible.

(1) Based on the absence since 1906 of frequent spring earthquakes of ≥5.5 mag in coastal central California, (or, perhaps, of high seismicity in another season), a repeat of the 1906 earthquake on the San Andreas fault is probably at least a quarter-century away (as others have concluded[1]). (2) Should a seasonal seismicity pattern re-emerge, the contemporary practice of estimating cumulative and annual probabilities of expected major earthquakes (see, for example, refs 11, 13) may be refined to the precision of seasonal probabilities. (3) Seasonal analysis of historical seismicity on shallow faults elsewhere in the world may yield similarly predictive results. (4) Seasonal analysis of other kinds of solid-Earth geophysical data, such as ground tilt, strain, creep, gravity, tectonomagnetism, microseismicity, well-water level and frequent data from land-based and astrometric geodesy obtained by radio and laser interferometric surveys, may likewise identify informative reltionships to earthquake activity. In this manner, some seasonal variations in instrumental data hitherto attributed to meteorological 'noise' may prove to have a deeper significance.

I thank W. Thatcher, A. G. Lindh, R. Wesson, W. H. Prescott and D. H. Peterson for discussions, T. R. Bruns for the loan of his calculator, and F. W. Klein and J. S. Derr for assistance in the preparation of the manuscript. I also thank D. S. McCulloch, G. W. Hill, J. S. Derr and W. L. Ellsworth for their support. Ideas and opinions expressed herein are the sole responsibility of the author, except where otherwise cited, and do not represent official views and conclusions of the US Geological Survey or the Minerals Management Service.

Received 13 May; accepted 31 October 1983.

1. Ellsworth, W. L., Lindh, A. G., Prescott, W. H. & Herd, D. G. in *Earthquake prediction—An International Review* (eds Simpson, D. W. & Richards, P. G.) 126–140 (American Geophysical Union, Washington DC, 1981).
2. Schuster, A. *Proc. R. Soc.* **61,** 455–564 (1897).
3. Shlien, S. *Geophys. J. R. astr. Soc.* **28,** 27–34 (1972).
4. Heaton, T. H. *Geophys. J. R. astr. Soc.* **43,** 307–326 (1975).
5. Klein, F. W. *Geophys. J. R. astr. Soc.* **45,** 245–295 (1976).
6. Kilston, S. & Knopoff, L. *Nature* **304,** 21–25 (1983).
7. Toppozada, T. R., Real, C. R. & Parke, D. L. *Calif. Div. Mines Geol. Open File Rep.* 81-11 SAC (1981).
8. Hall, N. T., Cotton, W. R. & Hay, E. A. in *AGU Chapman Conf. on Fault Behaviour and the Earthquake Generation Process* Abstr. (American Geophysical Union, Washington DC, 1982).
9. Fedotov, S. A. *Akad. Nauk. SSSR Inst. Fiz. Zeml: Trudy* **36,** 66–93 (1968).
10. Mogi, K. in *Earthquake Prediction—An International Review* (eds Simpson, D. W. & Richards, P. G.) 43–51 (American Geophysical Union, Washington DC, 1981).
11. Raleigh, C. B., Sieh, K. E., Sykes, L. R. & Anderson, D. L. *Science* **217,** 1097–1104 (1982).
12. Raleigh, C. B. in *Underground Waste Management and Environmental Implications* Mem. 18 (ed. Cook, T. D.) 273–279 (American Association of Petroleum Geologists, Tulsa, Oklahoma, 1972).
13. *U.S. geol. Surv. Open-File Rep.* 81–115 (1981).
14. Toppozada, T. R. *Bull. seism. Soc. Am.* **65,** 1223–1238 (1975).
15. Jennings, P. & Kanamori, H. *Earthquake Inform. Bull.* **13,** 49–51 (1981).
16. Toppozada, T. R., Real, C. R. & Parke, D. L. *California Division of Mines and Geology Map Sheet* 39 (1978).
17. Pattullo, J., Munk, W., Revelle, R. & Strong, E. *J. mar. Res.* **14,** 88–155 Appendix I and Chart 1 (1955).
18. Enfield, D. B. & Allen, J. S. S. *J. phys. Oceanogr.* **10,** 557–578 (1980).
19. Price, J. M. *J. phys. Oceanogr.* **11,** 1375–1302 (1981).
20. Lambeck, K. & Hopgood, P. *Geophys. J. R. astr. Soc.* **71,** 581–587 (1982).

Localized uplift in the Scottish Dalradian

T. J. Dempster

Grant Institute of Geology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JW, UK

Rb–Sr mica ages are reported here, from the Dalradian metasediments of the Angus region in the Scottish Highlands (Fig. 1), in a study of the uplift history of this classic metamorphic terrain. The samples come from across the Barrovian metamorphic zones[1], and provide evidence of diachroneity in both metamorphism and later cooling. Thus, the oldest mineral ages, and hence earliest cooling, are found in the highest grade metamorphic rocks to the north. The results show the importance of tectonically-controlled, localized uplift processes. Given that erosion is an important influence on the thermal development of metamorphic rocks[2], the constant or steadily decaying isostatic uplift rates, often assumed in models of orogenic belts[2,3], may be a marked oversimplification of the real events.

The major structure of the Dalradian is dominated by pre-metamorphic nappe structures[4], represented by the Tay and Tarfside Nappes in the present area[5] (Fig. 1). The rocks considered in this study come from the roughly flat-lying, right way-up limb of the Tarfside Nappe, along the line A–B in Fig. 1. Essentially, the area is considered to show a continuation of the flat belt and Highland Border Steep Belt of Perthshire, with the axial trace of the post-metamorphic Highland Border Downbend dividing these two domains. The flat belt region, in the area of the A–B traverse, also shows the complication of the late Tarfside Culmination[6] in the north of the area (Fig. 1). Regional metamorphic grade increases away from the Highland Boundary Fault, from the chlorite zone[7] to the sillimanite zone in the north[1]. Estimates of the age of peak metamorphism in the Dalradian, range from 515 to 490 Myr (refs 8, 9), although

0028–0836/84/020156—04$01.00

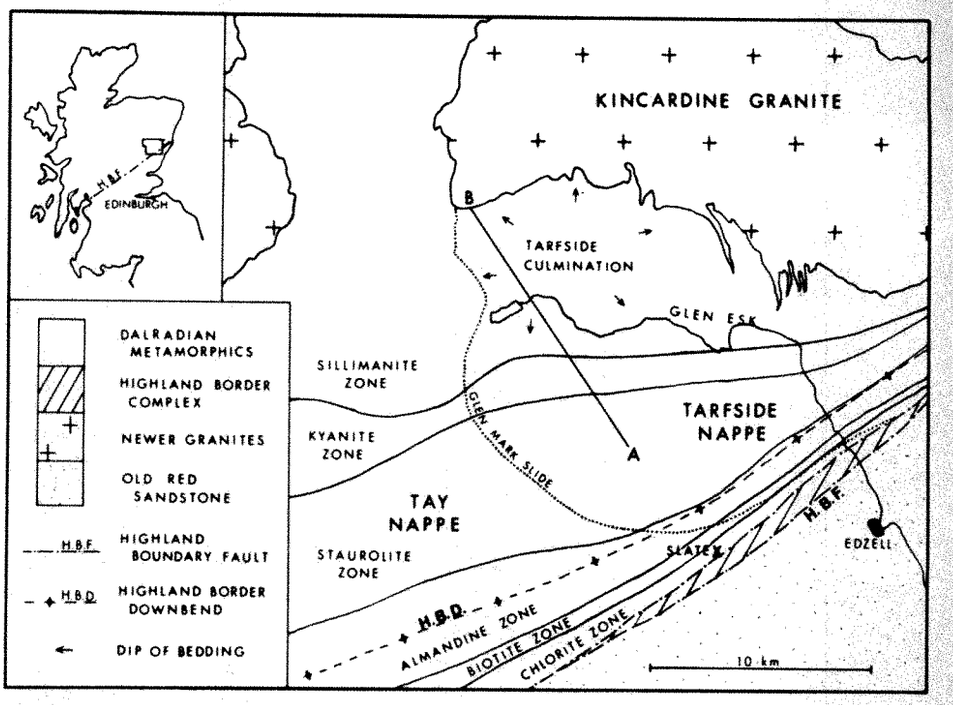


Fig. 1 Structural and metamorphic map of the Angus region in the Scottish Highlands (partly based on Barrow[1] and Harte[5]), showing the location of traverse A–B.

the Lower Palaeozoic metamorphism over the Scottish Highlands as a whole may show considerable diachronism[10,11]. Metamorphism in this traverse is roughly contemporaneous with other areas in the Dalradian, with the metamorphic climax approximately synchronous with the third regional deformation event (D3). However, slightly earlier garnet and staurolite porphyroblast growth, relative to the D3 mica fabric, is seen in the lower grade rocks, in the south of the traverse.

The interpretation of isotopic mineral ages in slowly cooling metamorphosed rocks is largely based on the theory of closure temperatures[12,13]. This predicts that minerals will record the times at which they cool through characteristic temperatures, below which the mineral is closed to the relevant isotopic exchange. Thus, biotites, with approximate Rb–Sr closure temperatures of 300 °C (ref. 12), record younger ages than coexisting muscovites, with higher Rb–Sr closure temperatures of about 500 °C (ref. 12). Dewey and Pankhurst[14] examined K–Ar mica ages from the Scottish Highlands and showed a broad relationship between the mineral ages and metamorphic grade, with younger ages and delayed cooling in the hotter, more deeply buried rocks. Thus, it might be expected that with roughly simultaneous metamorphism and uniform uplift, earlier cooling to mica closure temperatures, and hence older ages, would be seen in the lower grades to the south, along the present profile.

In this detailed study along the traverse A–B (Fig. 1), biotite and muscovite were separated from biotite–muscovite–plagioclase-bearing schists and analysed, together with whole-rock samples by Rb–Sr isotope dilution methods described elsewhere[15]. Details of all mineral age results, presented in Fig. 2, are available on request, and a selection of isotopic data is given in Table 1. The grain sizes and chemical compositions of each mica are relatively constant in the samples of this study and hence probably have little control on closure temperatures[17] or age variation. Thus, the isotopic age pattern (Fig. 2) largely reflects variation in the time of cooling to approximately uniform closure temperatures. It should be emphasized that the relative mica ages observed are more critical to any interpretation than the absolute ages and values of closure temperatures adopted.

The Rb–Sr mineral ages are corrected for initial Sr using the whole-rock analysis, and if the mica ages in the narrow thermal aureole of the Kincardine Granite are examined, this approach seems to be justified. Thus, mica ages are reset to an age of intrusion (~410 Myr), consistent with other independent estimates of the timing of Newer Granite emplacement[18]. So, the micas must exchange with a low $^{87}Sr/^{86}Sr$ phase, and any uncertainty in the exact composition of this phase, is more than outweighed by the large spread in Rb–Sr ratios of the exchanging phases.

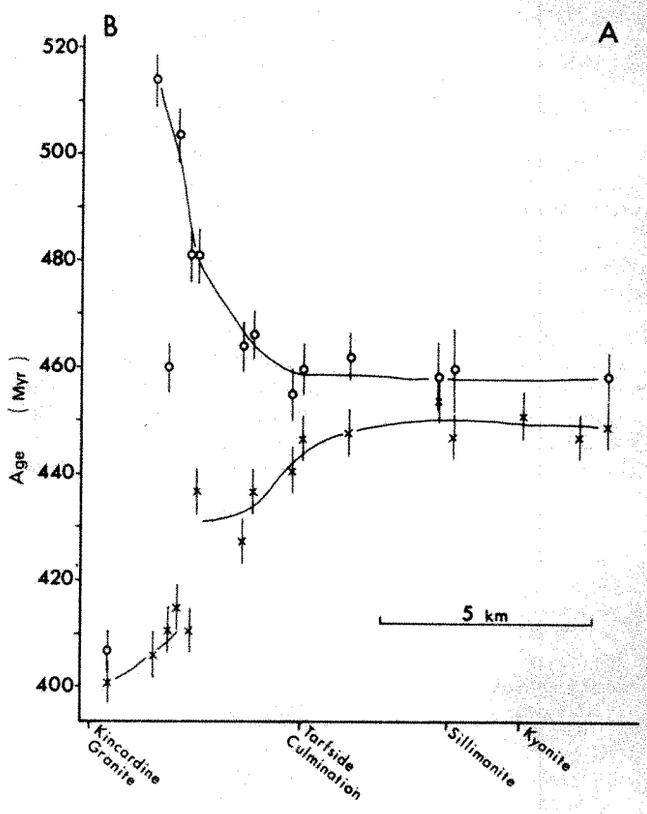


Fig. 2 Mineral age results from traverse A–B shown in Fig. 1. ○, Rb/Sr muscovite; ×, Rb/Sr biotite.

The whole-rock K–Ar dating of a well-crystallized slate, containing muscovite as its only major K-bearing phase and only one generation of white-mica, gives an estimate of the timing of metamorphism[9] in the chlorite zone (Fig. 1) of 489 ± 10 Myr (Table 1). Surprisingly, the oldest Rb–Sr muscovite age of 514 ± 5 Myr is found in the north of the traverse, which gives a minimum estimate of the timing of metamorphism in the high grades and supports textural evidence of diachroneity of

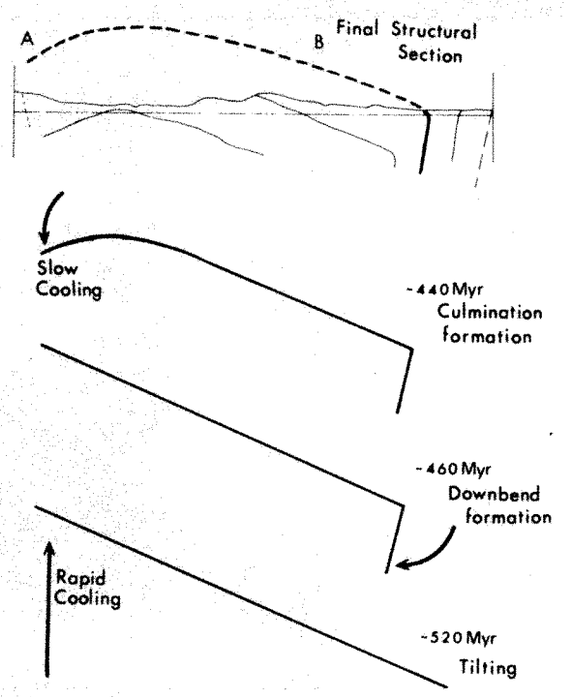


Fig. 3 Schematic sections showing the proposed structural evolution and cooling history that results in the mineral ages shown in Fig. 2.

metamorphism in the region. The old muscovite cooling ages in the north of the transect (Fig. 2) must indicate extremely rapid cooling, from the estimated peak metamorphic temperatures of ~650 °C (ref. 18), to below the approximate Rb–Sr closure temperatures of muscovite of ~500 °C (ref. 12) (although this rapid cooling itself may slightly increase the closure temperatures[16]). No evidence of this early rapid cooling is seen immediately to the south, where, despite a roughly similar (D3) textural age of metamorphism, initial slow cooling is indicated by Rb–Sr muscovite ages of ~460 Myr. However, a gradual transition occurs from the north towards the centre and to the south of the Tarfside Culmination occurs, as shown by the steady southerly decrease in the muscovite ages. After the initial rapid cooling in the northern area, very slow cooling must have followed as shown by the relatively young biotite Rb–Sr ages of ~430 Myr. Again contrasting behaviour is seen to the south of the Tarfside Culmination, in that the similarity of muscovite (~460 Myr) and biotite (~450 Myr) ages implies relatively rapid cooling and uplift for this area at a late stage.

Neither of the prominent post-metamorphic structures, the Highland Border Downbend or the Tarfside Culmination, seem to be capable of causing the early uplift, which is necessary for the rapid cooling in the very north. The Downbend structure must postdate the cooling and uplift in the sillimanite zone, as it deforms the Arenig fossil-bearing[19] (~480 Myr)[20] Highland Border Complex[21], and also folds the metamorphic zones of the lower grade areas[18], which are developed after cooling in the north. Similarly, the Tarfside Culmination is a young dome-structure and the old ages come from the northwestern rather than the central parts of the dome (Fig. 2). A striking feature of the Angus region is the significant dip of the 'flat belt' (to the south of the Tarfside Culmination) at angles of ~30° to the south-east, compared with the virtually horizontal beds of Perthshire[4]. It is suggested, as shown in Fig. 3, that an early tilting of originally flat-lying beds could result in rapid uplift, erosion and cooling in the most northerly rocks, which before the Culmination formation would be the highest level rocks (Fig. 3). This suggests that the Culmination forms subsequently by relative downwarping of the beds to the north, as a consequence of which, the once coolest rocks to the north, become the slowest cooling and show the youngest biotite ages (Fig. 2).

Thus, tectonic activity may be largely responsible for the cooling and uplift pattern in this part of the Highlands. A syn- to post-metamorphic tilting would also explain the diachronous metamorphism in this area, with convection of heat by rapid uplift[2], hastening the approach to peak metamorphic conditions. Thus, earlier-metamorphosed, higher-temperature rocks will be exposed in more rapidly uplifted areas. The approximately horizontal dip of the flat belt elsewhere in the Southern Dalradian, suggests that not only is the rapid early uplift localized in the north of the area considered, but is probably not seen elsewhere along the belt. This may account for the exposure of low level Tarfside Nappe rocks at the expense of the inverted Tay Nappe rocks to the south-west[5] (Fig. 1).

Clearly, such major cooling and uplift rate variations cannot be accommodated into regional (on the scale of the Grampian Highlands) block uplift events with steady isostatic return to normal crustal thicknesses[2,22]. It is apparent that localized uplift relating to structural activity and possibly decoupling and movement of small blocks in the basement is an important late tectonic

Table 1 Analytical data

Sample	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}Rb/^{86}Sr$ (atomic)	$^{87}Sr/^{86}Sr \pm 2\sigma$ (atomic)	Rb–Sr mineral age (Myr)
GL 771					
Whole-rock	97.8	158.6	1.7882	0.73450±20	
Muscovite	183.2	86.2	6.1847	0.76332±30	460±8
Biotite	342.4	30.9	32.721	0.93166±50	447±4
GL 813					
Whole-rock	63.1	126.6	1.4460	0.74034±60	
Muscovite	240.9	31.7	22.388	0.87942±30	466±5
Biotite	484.3	9.4	163.89	1.7506 ±4	437±4
GL 831					
Whole-rock	114.7	75.4	4.4275	0.75899±20	
Muscovite	250.3	47.9	15.299	0.83865±30	514±5
Biotite	475.5	9.0	167.43	1.7024 ∓2	406±4
GL 841					
Whole-rock	84.4	46.0	5.3377	0.76528±4	
Muscovite	260.0	16.9	45.849	0.99995±8	406±4
Biotite	510.4	7.1	236.12	2.0838 ±4	401±4

	K (wt%)	$^{40}Ar_{rad}$ (p.p.m.)	%Rad	K–Ar Age (Myr)
GL 691				
Whole-rock Slate	3.46	0.13473	98.2	489±10

Decay constants and isotopic abundances of Steiger and Jäger[25] are used.

feature of the Dalradian evolution. Given that there seems little reason to believe that the Lower Palaeozoic metamorphic belt of the Scottish Highlands should be markedly different from other orogenic belts, thermal models of metamorphic evolution in thickened crust, involving steadily decaying uplift processes[3,22], are probably an oversimplification.

These results must also cast doubt on the technique of assessing closure temperature variation, by comparing mineral ages over large tracts of metamorphic rocks on the assumption of uniform uplift and metamorphism[23]. The occurrence of localized uplift phenomena, within a regional metamorphic terrain, will also influence the variation in metamorphic grade or facies series or piezothermic arrays[24] (metamorphic geotherms[2]) across the terrain. Thus, a change in facies series[18] may partly reflect localized lateral (rather than vertical) differences in P–T-time paths for individual rock units. One further consequence of localized uplift is that it may also cause lateral redistribution of heat, thus, small uplifting areas may act like syn-metamorphic intrusions with respect to surrounding non-uplifting areas.

The support of a NERC grant is acknowledged and I thank Ben Harte for his encouragement and supervision, and John Hutchinson for assistance with mass spectrometry performed at the Scottish Universities Research and Reactor Centre.

Received 12 July; accepted 4 October 1983.

1. Barrow, G. *Proc. geol. Ass.* **23,** 268–290 (1912).
2. England, P. C. & Richardson, S. W. *J. geol. Soc. Lond.* **136,** 489–495 (1977).
3. England, P. C. *Tectonophysics* **46,** 21–40 (1978).
4. Bradbury, H. J., Harris, A. L. & Smith, R. A. in *The Caledonides of the British Isles—Reviewed* (eds Harris, A. L., Holland, C. H. & Leake, B. E.) 213–220 (Scottish Academic, Edinburgh, 1979).
5. Harte, B. in *The Caledonides of the British Iseles—Reviewed* (eds Harris, A. L., Holland, C. H. & Leake, B. E.) 221–228 (Scottish Academic, Edinburgh, 1979).
6. Read, H. H. *Trans. R. Soc. Edinb.* **55,** 755–772 (1928).
7. Tilley, C. E. *J. geol. Soc. Lond.* **81,** 100–112 (1925).
8. Pankhurst, R. J. & Pidgeon, R. T. *Earth planet. Sci. Lett.* **22,** 256–266 (1974).
9. Harper, C. T. *Scott. J. Geol.* **3,** 46–66 (1967).
10. van Breemen, O., Aftalion, M., Pankhurst, R. J. & Richardson, S. W. *Scott. J. Geol.* **15,** 49–62 (1979).
11. van Breemen, O. & Piasecki, M. A. J. *J. geol. Soc. Lond.* **140,** 47–62 (1983).
12. Purdy, J. W. & Jäger, E. *Mem. Instn. Geol. Miner. Univ. Padova* **30,** 1–32 (1976).
13. Desmons, J., Hunziker, J. C. & Delaloye, M. *Contr. Miner. Petrol.* **80,** 386–390 (1982).
14. Dewey, J. F. & Pankhurst, R. J. *Trans. R. Soc. Edinb.* **68,** 361–389 (1970).
15. Blaxland, A. B., van Breemen, O., Emeleus, C. H. & Anderson, J. G. *Bull. geol. Soc. Am.* **89,** 231–244 (1978).
16. Dodson, M. H. *Contr. Miner. Petrol.* **40,** 259–274 (1973).
17. Pidgeon, R. T. & Aftalion, M. *Geol. J. Spec. Iss.* **10,** 183–220 (1978).
18. Harte, B. & Hudson, N. F. C. in *The Caledonides of the British Isles—Reviewed* (eds Harris, A. L., Holland, C. H. & Leake, B. E.) 323–337 (Scottish Academic, Edinburgh, 1979).
19. Curry, G. B., Ingham, J. K., Bluck, B. J. & Williams, A. *J. geol. Soc. Lond.* **139,** 451–454 (1982).
20. Bluck, B. J., Halliday, A. N., Aftalion, M. & Macintyre, R. M. *Geology* **8,** 492–495 (1980).
21. Booth, J. E. thesis, Univ. Edinburgh (1983).
22. Wells, P. R. A. *J. geol. Soc. Lond.* **136,** 663–671 (1979).
23. O'Nions, R. K., Smith, D. G. W., Baadsgaard, H. & Morton, R. D. *Earth planet. Sci. Lett.* **5,** 339–345 (1969).
24. Richardson, S. W. & England, P. C. *Earth planet. Sci. Lett.* **42,** 183–190 (1979).
25. Steiger, R. H. & Jäger, E. *Earth planet. Sci. Lett.* **36,** 359–362 (1977).

Period multupling-evidence for nonlinear behaviour of the canine heart

Amy L. Ritzenberg*, Dan R. Adam† & Richard Jonathan Cohen*†

* MIT Department of Physics and † Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Although there has recently been considerable interest in applying the theory of nonlinear dynamics to the analysis of complex systems[1,2], as yet applications of the theory to biological systems *in vivo* have been very limited. We report here evidence of nonlinear behaviour in the electrocardiogram and arterial blood pressure traces of the noradrenaline-treated dog. Noradrenaline produces variations in these traces that repeat themselves with regular periods of integral numbers of heart-beats (period multupling), an effect that resembles the 'period-doubling' and other 'bifurcative' behaviour[3–5] observed when the driving frequency of a nonlinear oscillator is increased above a critical value[6–9]. The simplest type of periodic variation that we report is the so-called 'electrical alternans', which has long been known as one response of cardiac electrical activity to certain stresses and disease states[10–13].

Ten dogs were anaesthetized with sodium pentobarbitol and ventilated with a respirator at a fixed rate (to distinguish electrocardiogram (ECG) modulation due to respiration). Saline-filled catheters were introduced into the femoral artery and vein, and arterial blood pressure (ABP) tracings obtained. Unipolar leads were placed precordially and the surface ECG recorded. Noradrenaline bitartrate was given intravenously in a dosage sufficient to intoxicate (indicators being substantial sinus tachycardia and arterial hypertension). Dosages ranged from 0.02 to 0.05 mg kg^{-1}. To confirm the presence of period multupling more rigorously than via a simple visual inspection of the traces, ECG and ABP records were digitized and then fast Fourier transformed and the spectra plotted. According to Fourier theory, the Fourier spectrum of a record with no period multupling should contain sharp peaks only at the heart rate and at integral multiples (the Fourier harmonics) of that frequency. Period doubling (alternans) should appear in the spectrum as an isolated peak (subharmonic) at a frequency equal to one-half the heart rate and additional peaks at integral multiples of this frequency; period tripling should appear as peaks at integral multiples of the frequency equal to one-third the heart rate, and so on (hence 'period multupling' corresponds to frequency dividing).

The results of this means of analysis are summarized in Table 1. One of the 10 dogs fibrillated spontaneously upon injection with noradrenaline and is thus excluded from the study. Of the nine remaining, seven displayed modulations of clearly discernible order. There were several instances of period doubling, tripling, quadrupling and quintupling. Figure 1 shows typical episodes of tripling and quadrupling; ECG, ABP and their respective Fourier spectra are displayed. The time scale for these observations is as follows: episodes of 4.25 s or more were considered stable. Intervals of apparent stability ranged from approximately 6 s (an instance of quadrupling) to tens of seconds (instances of doubling). Inter-episode (apparently aperiodic) times varied from tens of seconds to minutes. In this study, multupling was always found in both ECG and ABP, but never in only one. Thus, we would expect that this phenomenon is not only electrical (periodic alterations of the pattern of conduction through the heart) but mechanical as well (affecting the contractile action of the heart as a whole). Since the frequency of the ECG modulation was unrelated to the frequency of respiration, the effect is not respiratory in origin.

If the analogy with a driven, nonlinear oscillator is appropriate, the heart rate should correlate strongly with the period multupling regime[14]. For example, in our experience of oscillators doubling precedes either tripling or quadrupling, quadrupling precedes quintupling, but quadrupling precedes tripling, due to the particular way in which the new regimes are generated (beyond the scope of this letter), as the driving frequency of

Table 1 Period multupling regimes observed

Animal	Regime	Ventricular rate	Animal	Regime	Ventricular rate
1	Doub.	211	7	Quad.	183
	Quad.	228		Trip.	198
2	Doub.	253		Quin.	225
	Quad.	267	9	Doub.	105
3	Doub.	225		Trip.	112
	Quad.	253	10	Doub.	112
4	Doub.	190			
	Trip.	295			

Experimental animals are numbered 1–10. For the seven animals in whose ECG and ABP traces regimes of period multupling were observed, the various regimes are displayed.

© 1984 Macmillan Journals Ltd

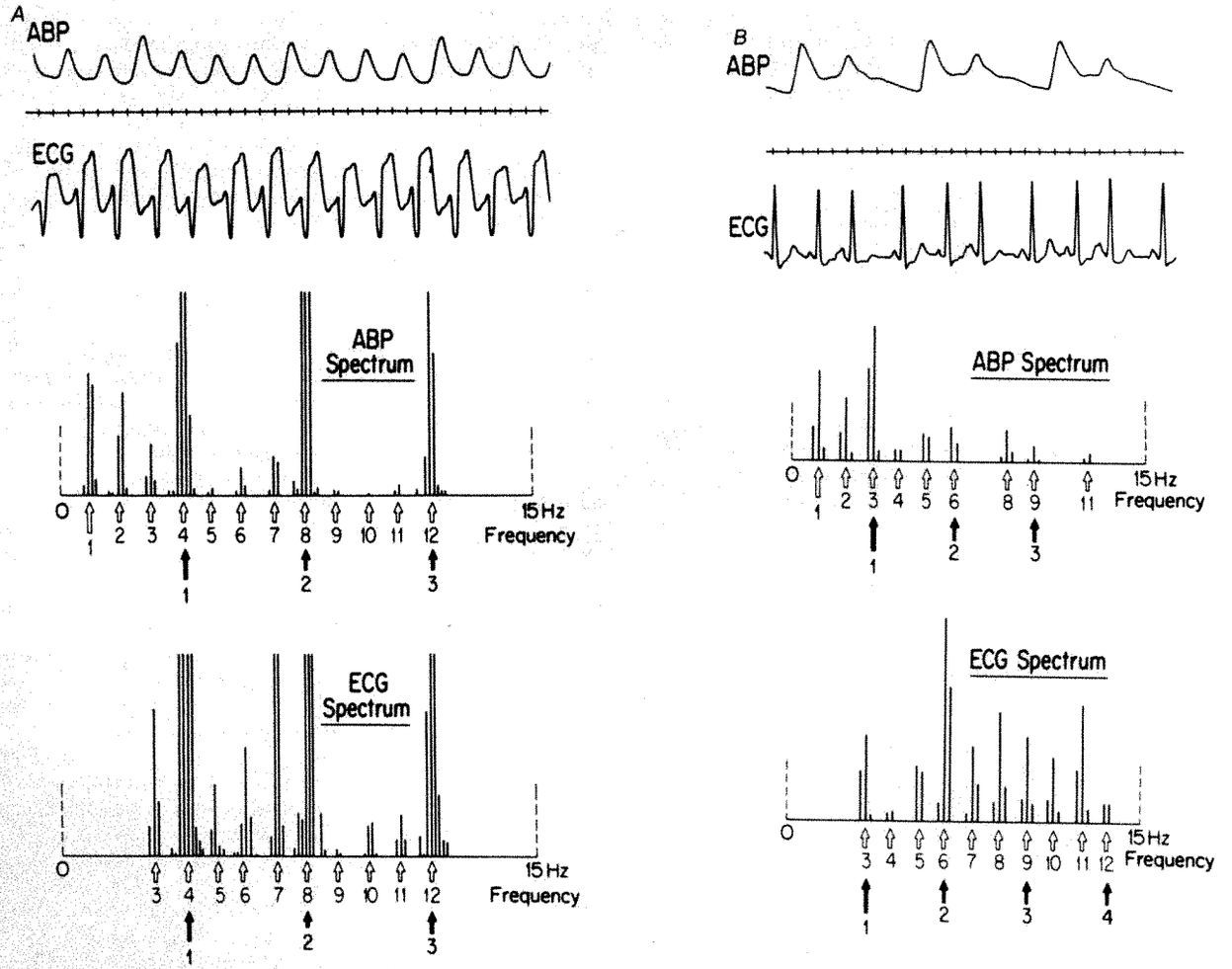


Fig. 1 ABP and ECG traces are displayed, followed by their respective fast Fourier transforms. In *A*, the heart rate is 228 beats per min, making the beat frequency 3.8 Hz (large black arrow). Subharmonics (large white arrow for the first, at 0.95 Hz) appear at quarters of the beat frequency, indicating quadrupling. In *B*, the heart rate is 198 beats per min making the fundamental frequency of atrial beating 3.3 Hz. This appears (large black arrow) as a major peak in both spectra. Subharmonics (large white arrow for the first, at 1.1 Hz) appear at thirds of this frequency, indicating period tripling. In both *A* and *B*, higher Fourier harmonics are shown with smaller arrows; black for higher harmonics of the beat frequency and white for higher harmonics of the multupling frequency. The number below the arrow gives the order of harmonic. (The amplitudes of the first few subharmonics in the ECG spectra are too low to appear on these plots; their higher Fourier harmonics show their presence.)

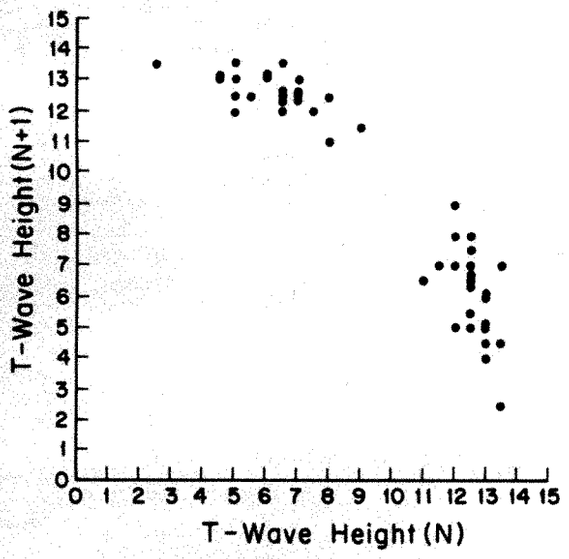


Fig. 2 The height of the $N+1$st T-wave plotted against the height of the Nth T-wave during 48 beats of an episode of period doubling (animal 1).

the nonlinear oscillator is raised. This precedence of period multupling regimes with increasing heart rate was obeyed in all cases (Table 1).

The period multupling behaviour described here may arise in different instances from different electrophysiological mechanisms. For example, in Fig. 1*A*, the intraventricular beat interval is fixed. Period quadrupling in the ECG record is comprised of modulation of the shape of the QRS and T-wave complexes. This suggests that the electrical pattern of depolarization and repolarization varies on a beat-to-beat basis. In Fig. 1*B*, the period tripling shown in ECG and ABP spectra reflects the timing of atrial excitations (which effectively penetrate the atrioventricular junction to activate the ventricles) as well as the sort of excitation pattern variability of Fig. 1*A*. Other mechanisms of period multupling include medically well known bigeminy, trigeminy and quadrigeminy, in which every second, third or fourth ventricular beat originates from a ventricular focus; and a variety of forms of second-degree atrioventricular block. We suggest here that period multupling is a common response of the electrical and mechanical activity of the stressed heart, even when apparently diverse mechanisms are operative.

The observation of period multupling in the canine heart upholds the naive expectation that this system is nonlinear, since a strictly linear system can never exhibit period doubling[3,14]. Recent work on the driving and resetting of neural oscillators,

including excised heart tissue, supports this[15–19]. One simple way to analyse such a system for linearity is to explore the functional dependence of the shape of one beat on the previous beat. We have generated 'scatter diagrams' (Fig. 2) of the T-wave peak height or T-wave area for the $N+1$st beat against the Nth. The degree of 'scatter' (range of ordinates corresponding to one abscissa) is a measure of how closely the $N+1$st beat depends for its shape solely on the shape of the Nth. Our plots show both the strong causal link between the Nth T-wave amplitude and the $N+1$st (that is, a small degree of scatter) and the nonlinearity of that causal relationship.

Further, from Li and Yorke's proof[20] the existence of tripling indicates that the cardiac system is sufficiently nonlinear to produce responses to atrial driving of all periodicities plus pseudostochastic sequences of waveforms. This leads us to ask why we saw no orders of period multupling higher than that of quintupling. A related question is why signals of a relatively low order of multupling alternate chronologically in our records (corresponding to similar heart rates and other system parameters) with aperiodic stretches. One answer is that there are several scenarios for the period multupling of noiseless nonlinear oscillators which predict the existence of intercalated bands of chaotic and periodic activity; in particular, the phenomenon of 'intermittency' is relevant[21,22]. Alternatively, noise disrupts the bifurcation structure of an idealized, non-linear system[8,9]. Higher order orbits are empirically more fragile (due to their smaller parameter space) and subject to effacement, so they are simply less likely to be observed. For example, Duffing's oscillator, when subjected to white noise, is found to develop a symmetrical gap in its bifurcation sequence. Higher-order orbits can even be induced solely by increasing the amplitude of the noise. In the heart, there are many sources of noise, in the form of stochastically varying parameters governing cardiac function; such as intrinsic fluctuations in the atrial rate, and variation in conduction and contractility due to local areas of ischaemia that develop in the stressed heart.

Our results support the model of the noradrenaline intoxicated heart as a highly nonlinear, driven oscillator subject to noise thus usefully linking many seemingly disparate dysrhythmias as bifurcative responses to variation in system parameters.

This research was supported by NSF grants 7922091-ECS and 8121571-ECS, ONR grant N00014-80-C-0520, a grant from the Whitaker Health Sciences Fund, and a grant from the R. J. Reynolds Industries. A.L.R. received support from a Whitaker Health Sciences Fund Fellowship and R.J.C. from a Hartford Foundation Fellowship.

Received 26 July; accepted 14 September 1983.

1. Wolf, A. *Nature* **305**, 182–183 (1983).
2. Holden, A. V. *Nature* **305**, 183 (1983).
3. May, R. & Oster, G. *Am. Nat.* **110**, 573–599 (1976).
4. Feigenbaum, M. J. *J. statist. Phys.* **19**, 25–52 (1978).
5. Hofstader, D. R. *Scient. Am.* **245**, 22–43 (1981).
6. Huberman, B. A. & Crutchfield, J. P. *Phys. Rev. Lett.* **43**, 1743–1747 (1979).
7. Guevara, M. R. & Glass, L. *J. math. Biol.* **14**, 1–23 (1982).
8. Glass, L., Graves, C., Petrillo, G. & Mackey, M. *J. theor. Biol.* **86**, 455–475 (1980).
9. Huberman, B. A. & Crutchfield, J. P. *Phys. Lett.* **A77**, 407–410 (1980).
10. Hellerstein, H. K. & Liebow, I. M. *Am. Heart J.* **160**, 366–374 (1949).
11. Hoffman, B. F. & Suckling, E. E. *Am. J. Physiol.* **179**, 123–130 (1954).
12. Kleinfield, M. & Stein, E. *Am. Heart J.* **75**, 528–530 (1968).
13. Adam, D. R., Akselrod, S. & Cohen, R. J. *Comp. Cardiol.* **8**, 307–310 (1981).
14. Feigenbaum, M. J. *Los Alamos Sci.*, 2–27 (1980).
15. Moulopoulos, S. D., Kardaras, N. & Sideris, D. A. *Am. J. Physiol.* **208**, 154–157 (1965).
16. Winfree, A. T. *Science* **197**, 761–762 (1977).
17. Moe, G., Jalife, J., Mueller, W. & Moe, B. *Circulation* **56**, 968–979 (1977).
18. Jalife, J. & Antzelevitch, C. *Science* **206**, 695–697 (1979).
19. Guevara, M., Glass, L. & Shrier, A. *Science* **214**, 1350–1353 (1981).
20. Li, T. & Yorke, J. *Am. math. Mon.* **82**, 985–992 (1975).
21. Metropolis, N., Stein, M. L. & Stein, P. R. *J. Comb. Theory* **A15**, 25–43 (1973).
22. Eckmann, J. P. *Rev. mod. Phys.* **53**, No. 4, Pt I, 643–654 (1981).

Brain potentials during reading reflect word expectancy and semantic association

Marta Kutas & Steven A. Hillyard

Department of Neurosciences, M-008, University of California, La Jolla, California 92093, USA

The neuroelectric activity of the human brain that accompanies linguistic processing can be studied through recordings of event-related potentials (e.r.p. components) from the scalp. The e.r.ps triggered by verbal stimuli have been related to several different aspects of language processing[1]. For example, the N400 component, peaking around 400 ms post-stimulus, appears to be a sensitive indicator of the semantic relationship between a word and the context in which it occurs. Words that complete sentences in a nonsensical fashion elicit much larger N400 waves than do semantically appropriate words or non-semantic irregularities in a text[2,3]. In the present study, e.r.ps were recorded in response to words that completed meaningful sentences. The amplitude of the N400 component of the e.r.p. was found to be an inverse function of the subject's expectancy for the terminal word as measured by its 'Cloze probability'. In addition, unexpected words that were semantically related to highly expected words elicited lower N400 amplitudes. These findings suggest N400 may reflect processes of semantic priming or activation.

Late negative e.r.ps resembling the N400 have been observed in experiments that required subjects to make decisions about words based on their semantic attributes,[4–7] and Stuss *et al.*[8] noted an N400-like component following isolated, single words or pictures that required naming. One likely interpretation of these findings would assume that N400 amplitude reflects the extent to which a word is unpredictable or unexpected, regardless of whether or not it is incongruous with a preceding context. Since word expectancy influences how rapidly and accurately words are accessed, recognized and understood[9], a physiological index of this process would have considerable usefulness for revealing the structure of language comprehension mechanisms.

We examined this relationship by recording e.r.ps to words that completed sentences in a meaningful way but varied systematically in the degree to which they were expected. The sentences were selected from a set in which the degree of expectancy for alternative terminal words had been determined using the 'Cloze' procedure; that is, by requiring a large group of subjects to fill in the missing terminal word[10]. A word's Cloze probability is defined as the proportion of subjects using that word to complete a particular sentence. The experimental design called for words having different Cloze probabilities (hi, med, or lo) to be placed at the ends of sentences having one of three levels of contextual constraint (hi, med, or lo). Highly constrained sentences were those that led to very predictable endings, while sentences of low constraint did not induce such strong expectations (for examples see Fig. 1*A*).

A total of 321 sentences were presented, one word at a time, on a video terminal controlled by a microcomputer. Words were presented once every 700 ms for a duration of 132 ms. Fourteen subjects were instructed to read the sentences silently in order to answer a questionnaire about their contents at the end of the experiment. Scalp electrical activity was recorded using non-polarizable electrodes from frontal (Fz), central (Cz), parietal (Pz), and occipital (Oz) midline locations and from symmetrical sites over the anterior temporal (AT) and posterior temporal (PT) regions of the left (L) and right (R) hemispheres, each referred to linked mastoids. Eye movements and blinks were monitored via infraorbital and external canthal electrodes. The midline recordings were amplified with a bandpass of 0–40 Hz; the lateral recordings and electrooculogram with a 0.01–40 Hz bandpass.

The e.r.p. waveforms in Fig. 1*B* show that highly probable words at the ends of highly constrained sentences were followed by a broad, late positivity (hi/hi, solid tracing). In contrast, the low probability words elicited a posteriorly distributed negative

© 1984 Macmillan Journals Ltd

A

hi/hi	He mailed the letter without a stamp.
hi/lo	The bill was due at the end of the hour.
med/hi	She locked the valuables in the safe.
med/med	Too many men are out of jobs.
med/lo	The dog chased our cat up the ladder.
lo/hi	There was nothing wrong with the car.
lo/lo	He was soothed by the gentle wind.

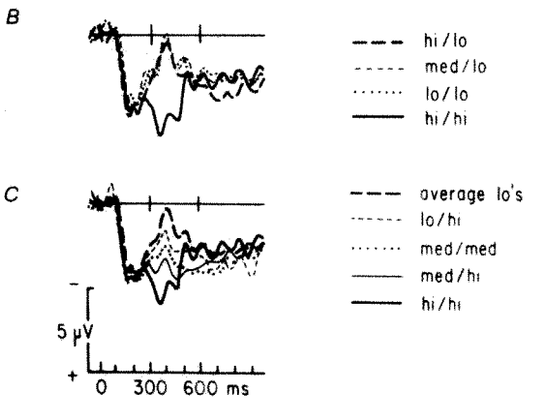


Fig. 1 *A*, An example from each of seven classes of sentences that varied in degree of contextual constraint and in the Cloze probability of the terminal word. There were 40–50 of each sentence type. On the left are shown the levels of contextual constraint/Cloze probability for each class. These two dimensions are not wholly independent, since only the more highly constrained sentences have the possibility of being terminated by words of very high Cloze probability. Thus, the level of Cloze probability considered to be 'hi' was relative to the level of contextual constraint, having average values of 0.92, 0.63 and 0.29 for the hi/hi, med/hi and lo/hi sentences, respectively. All of the low Cloze probability sentences, however, had average values of less than 0.03. *B*, Superimposition of grand average e.r.ps (across 14 subjects) from the Pz electrode to the low Cloze probability words terminating sentences of high, medium and low contextual constraint, together with the e.r.p. to high Cloze probability words completing highly constrained sentences. *C*, Grand average e.r.ps to terminal words of varying Cloze probability terminating sentences of high, medium and low contextual constraint. The e.r.p. to low Cloze probability words was averaged across the three levels of contextual constraint shown in *B*.

A

(best)	Don't touch the wet paint.
(unrelated)	Don't touch the wet dog.
(best)	He liked lemon and sugar in his tea.
(related)	He liked lemon and sugar in his coffee.

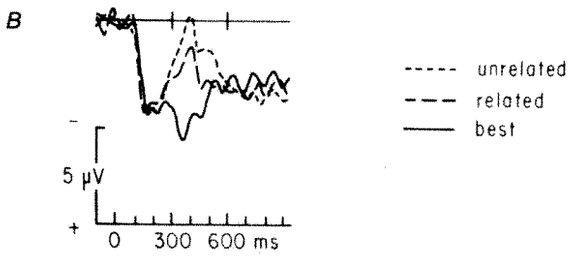


Fig. 2 *A*, Two examples of sentences with high contextual constraints completed by low Cloze probability words. Above each experimental sentence is the same sentence terminated by its 'best completion', which was or was not semantically related to the word that was actually presented in the sentence below. *B*, Grand average e.r.ps from Pz for the best completions (solid waveform), the semantically related (large dashed waveform) and the semantically unrelated (small dashed waveform) low Cloze probability words.

component (N400) that was superimposed upon the positive shift. The N400 amplitude to the low probability endings did not vary significantly over the three levels of contextual constraint (compare dotted and dashed tracings).

The N400 was measured as the mean amplitude over 300–500 ms post-stimulus, relative to a 50 ms prestimulus baseline. In general, the N400 amplitude was more sensitive to Cloze probability than to the degree of contextual constraint. For example, the e.r.ps to lo/hi versus med/med words, which were very similar in average Cloze probability (0.29 versus 0.23) but completed sentence fragments of low and medium constraint, respectively, did not differ significantly in N400 amplitude. On the other hand, comparisons of the e.r.ps to high, medium and low probability words terminating sentences of medium constraint revealed larger N400 amplitudes to the less probable words [main effect of ending Cloze probability $F(2,26) = 12.94$, $P < 0.001$; ending × electrode $F(14,182) = 6.07$, $P < 0.001$].

Inspection of the e.r.ps to all types of terminal words (Fig. 1*C*) revealed a gradient of potential, with the greatest positivity following hi/hi words and a progressively larger negativity (N400) elicited by words of decreasing Cloze probability. This relationship between Cloze probability and N400 amplitude was also evidenced by the product–moment correlations between the two measures across the seven classes of word endings [for the grand average e.r.ps, Fz, 0.80; Cz, 0.90; Pz, 0.92; Oz, 0.92; L.AT, −0.82; R.AT, 0.94; L.PT, 0.88; R.PT, 0.97]. At the parietal site, this correlation calculated from individual subjects' data averaged 0.70 (0.21) and reached significance in seven subjects (d.f. = 5, $P < 0.05$). This correlation also held when the e.r.p. data were collapsed across the different levels of contextual constraint and re-averaged according to the Cloze probability of the final words [Fz, 0.87; Cz, 0.91; Pz, 0.94; Oz, 0.92; L.AT, −0.60; R.AT, 0.75; L.PT, 0.91; R.PT, 0.93]. The negative correlation observed at the left anterior temporal site could result from this scalp region being located on the opposite side of the dipole field of the N400 generator.

The systematic decline in the N400 amplitude as a function of increasing Cloze probability indicates that semantic incongruity is not a necessary condition for N400 elicitation. Instead, N400 amplitude appears to vary systematically as an inverse function of word expectancy, operationally defined here in terms of Cloze probability.

The influence of context on word recognition has been attributed to the automatic priming or activation of semantic networks, as well as to slower, attention-directed processes[11–13]. Within such a framework, a sentence fragment primes (that is, activates for faster access) semantically related words whether or not they form acceptable sentence completions. In addition, sentence frames may result in the activation and retrieval of appropriate schemata[14–16]. If the N400 reflects some aspect of this semantic activation, its amplitude should vary according to whether or not an unexpected terminal words is semantically related to the most expected ending of that sentence (that is to the 'best completion' of the activated schema).

We tested this prediction by reanalysing the e.r.ps to low Cloze probability words that completed highly constrained sentences, now segregating them according to whether or not the terminal word was related to the best completion (BC) of the sentence in which it occurred. The degree of semantic relatedness had been determined by asking a different group of 25 subjects to rate each word pair on a 5-point scale. The mean ratings were 4.03 (0.70) and 1.95 (0.73) for the related and unrelated word pairs, respectively. Sample sentences are shown in Fig. 2*A*. N400 amplitude was indeed sensitive to the semantic relationship between the eliciting word and the expected best completion (Fig. 2*B*), with larger N400s following words that were unrelated to the BC [main effect of semantic relatedness

$F(1,13) = 17.37$, $P < 0.001$; relatedness × electrode $F(7,97) = 3.14$, $P < 0.001$].

These results are in agreement with the hypothesis that the N400 component reflects the extent to which a word is semantically primed, rather than its being a specific response to contextual violations. If the N400 amplitude proves to be a valid index of semantic priming, it should become possible to investigate the timing and spread of activation within semantic networks and knowledge schemata and to identify automatic and attentional components of processing.

We thank J. C. Phillips and J. Hansen for their help. This work was supported by NSF grant BNS 80-05525 and Sloan Foundation grant B1900-35. M. K. is supported by Research Scientist Development Award USPHS-1K02MH0322.

Received 4 August; accepted 10 October 1983.

1. Picton, T. W. & Stuss, D. T. in *Biological Perspectives in Language* (eds Caplan, D. N., Lecours, A. R. & Smith, A. M.) (MIT Press, in the press).
2. Kutas, M. & Hillyard, S. A. *Science* **207,** 203–205 (1980).
3. Kutas, M. & Hillyard, S. A. *Mem. Cognit.* **11(5),** 539–550 (1983).
3. Sanquist, T. F., Rohrbaugh, J. W., Syndulko, K. & Lindsley, D. B. *Psychophysiology* **17,** 568–576 (1980).
4. Boddy, J. & Weinberg, H. *Biol. Psychol.* **12,** 43–61 (1981).
5. Fischler, I., Bloom, P. A., Childers, D. A., Roucos, S. E. & Perry, N. W. Jr *Psychophysiology* **20,** 400–409 (1983).
7. Polich, J., Vanasse, L. & Donchin, E. *Psychophysiology* **18,** 142 (1981).
8. Stuss, D. T., Sarazin, F., Leech, E. & Picton, T. W. in *Brain Information: Event-related Potentials* Monogr. 12 (eds Karrer, R., Cohen, J. & Tueting, P.) (New York Academy of Sciences, 1983).
9. Lesgold, A. M. & Perfetti, C. A. (eds) *Interactive Processes in Reading* (Erlbaum, Hillsdale, New Jersey, 1981).
10. Bloom, P. A. & Fischler, I. *Mem. Cognit.* **8,** 631–642 (1980).
11. Stanovich, K. E. in *Interactive Processes in Reading* (eds Lesgold, A. M. & Perfetti, C. A.) 241–267 (Erlbaum, Hillsdale, New Jersey, 1981).
12. Posner, M. I. & Snyder, C. R. R. in *Information Processing and Cognition: The Loyola Symposium* (ed. Solso, R.) (Erlbaum, Hillsdale, New Jersey, 1975).
13. Posner, M. I. & Snyder, C. R. R. in *Attention and Performance V* (eds Rabbitt, P. M. A. & Dornic, S.) 669–682 (Academic, New York, 1975).
14. Thorndyke, P. W. & Hayes-Roth, B. *Cognit. Psychol.* **11,** 82–106 (1979).
15. Kleiman, G. M. *Mem. Cognit.* **8,** 336–344 (1980).
16. Foss, D. J. *Cognit. Psychol.* **14,** 590–607 (1982).

Cell determination and regulation during development of neuroblasts and neurones in grasshopper embryo

Paul H. Taghert, Chris Q. Doe & Corey S. Goodman

Department of Biological Sciences, Stanford University, Stanford, California 94305, USA

The embryonic development of the central nervous system (CNS) involves the generation of an enormous diversity of cellular types arranged and interconnected in a remarkably precise pattern. In each hemisegment of the grasshopper embryo, the ectoderm generates a stereotyped pattern of 30 neuronal precursor cells, called neuroblasts (Fig. 1)[1]. Each of these stem cells makes a stereotyped contribution of 6–100 progeny to the ~1,000 different neurones, each cell identifiable according to its unique morphology, physiology and biochemistry. What are the contributions of cell interactions and cell lineage to the generation of this diversity and specificity of identified neurones in the grasshopper CNS? Here we report on cell ablations with a laser microbeam at different stages of development. Our results suggest the importance of cell–cell interactions in the determination of ectodermal cells to become identified neuroblasts[2]. However, once a neuroblast begins to divide, then cell lineage appears to play an important role in the determination of its stereotyped family of neuronal progeny[3]. Furthermore, cell-specific interactions continue to play an important role as neurones, according to their mitotic ancestry, recognize and interact with other differentiating neurones in their environment[4–6].

Within the neuroepithelium in each segment of the grasshopper embryo, there arise 61 neuronal precursor cells, called neuroblasts (NBs), arranged in two symmetric plates of 30 NBs each and one median neuroblast (MNB) (Fig. 1)[1]. We can identify each NB by its position within the neuroepithelium; moreover, many NBs can now be uniquely identified by the highly stereotyped family of identified neurones they produce[3,7–10]. Each NB is a stem cell which divides repeatedly to generate a chain of ganglion mother cells. Each ganglion mother cell then divides once more to generate two ganglion cells in a chain of cell doublets that then differentiate into neurones. The 30 NBs in each hemisegment largely generate the ~1,000 neurones in each thoracic hemiganglion and the ~250 neurones in each abdominal hemiganglion; most if not all of these neurones can be uniquely identified according to their characteristic morphology, physiology and biochemistry. The number of neuronal progeny appears to be specific for individual NBs; for example, NB 7–3 produces 6 progeny[10] whereas the MNB produces ~100 progeny[6–8]. Furthermore, the identities of the progeny appear to be specific for individual NBs; the fate of individual neurones is highly correlated with their specific positions in the family trees of particular NBs[6–10].

The progeny of NBs form coherent families of neurones that are inserted into the developing CNS in characteristic positions. The determination of an individual neurone might be due to its position in the NB family tree, or alternatively to its position in the developing ganglion and thus its reproducible interactions with its neighbours. Selective ablation of identified cells with a laser microbeam can help to distinguish between these alternatives. For example, NB 7–3 gives rise to six progeny (Fig. 1). In the abdominal segments (A4–A6), the first two NB 7–3 progeny are called S1 and S2; following their differentiation they can be specifically stained with an anti-serotonin antibody (Fig. 2). They are the only prominent serotonin-immunoreactive neurones in these segments. Furthermore, S1 and S2 can also be individually identified by their distinctive morphologies after intracellular dye injections (Fig. 1)[1]. We ablated NB 7–3 *in ovo* just before it begins its first cell division to test if some other NB could produce neurones with either the biochemical or morphological phenotypes of S1 and S2 (Fig. 2). We also ablated other NBs at the same stage to test if interactions with neighbouring NBs or their progeny were necessary for NB 7–3 to produce neurones S1 and S2.

NBs were identified in a 30%[11] embryo through the dechorionated eggcase under brightfield optics, and ablated with a pumped-dye laser (Fig. 2). Experimental and control embryos were examined at three different stages with three different techniques: at 33% with Nomarski optics to look for NB 7–3; at 50% with intracellular dye injections of Lucifer Yellow to look for cells with the distinctive morphology of neurones S1 and S2; and at 70% with the anti-serotonin antibody to look for cells with the distinctive serotonin-immunoreactivity of neurones S1 and S2.

When NB 7–3 was ablated before it began its first cell division and the embryos were assayed at 33% (the following day) for the presence or absence of the NB, we found that in approximately 80% of the cases ($n > 20$) no regulation took place. When regulation does occur, a new NB 7–3 is present as are all neighbouring NBs. When embryos were assayed at 70%, in 7 of 10 cases no serotonin-immunoreactive neurones were present on the experimental side of the ganglion (Fig. 2). In these embryos, the homologous S1 and S2 neurones from the contralateral NB 7–3 were always present and had the normal morphological and biochemical features. These observations suggest that when a NB is ablated before it begins its first cell division, regulation does not usually take place. In addition, progeny of neighbouring NBs do not differentiate with either the morphological or biochemical properties of neurones S1 or S2. The partial (20–30%) regulation we observe following NB ablation is likely to result from the recruitment of a neighbouring ectodermal cell, as described in the *in vitro* experiments below. In three other cases in which NB 7–3 was ablated, exhaustive intracellular dye injections at 50% of every other cell body in the region where S1 and S2 normally appear revealed no neurones with the distinctive morphology of S1 and S2 on the experimental side; S1 and S2 were easily found on the control side, and did

© 1984 Macmillan Journals Ltd

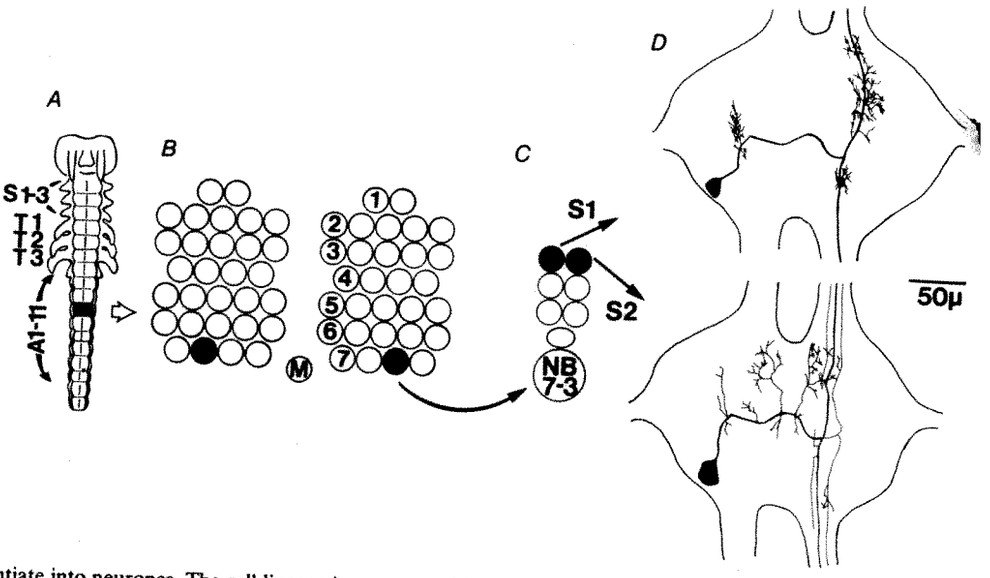


Fig. 1 *A*, Schematic diagram of a 30% grasshopper embryo[11] (hatching occurs at 100%) showing the segmental structure of the ectoderm. The middle of the ectoderm is a longitudinal strip of neuroepithelium which gives rise to the CNS. The location of the neuronal precursor cells for a single segment (A4) are drawn in black. *B*, Pattern of NBs for a single segment (A4) includes two plates of 30 NBs arranged in a precise pattern of seven rows, and one MNB making a total of 61 NBs. *C*, Cell lineage of the two identified serotonin immunoreactive neurones, S1 and S2, from NB 7–3 in segment A4. Cells S1 and S2 arise by an invariant cell lineage from the first division of NB 7–3. NBs generate neuronal progeny by a series of asymmetric cell divisions that produce small, ganglion mother cells. Ganglion mother cells then each divide once more symmetrically to produce two cells that will both differentiate into neurones. The cell lineage is represented here with a ganglion mother cell closest to the NB and older neuronal progeny lying progressively farther away from the NB. *D*, The cell-specific morphologies of neurones S1 (upper) and S2 (lower) in the A4 ganglion at 70% of embryonic development, as revealed by intracellular injection of Lucifer Yellow followed by HRP immunocytochemistry with an anti-Lucifer Yellow antibody[18].

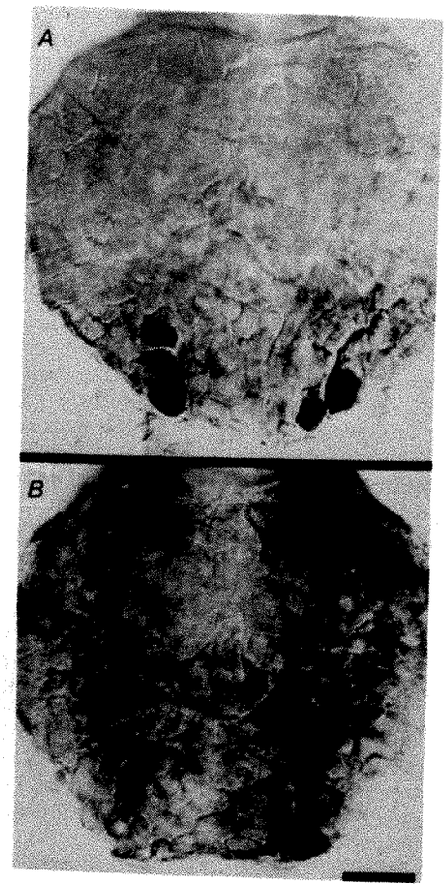


Fig. 2 Laser ablation of NB 7–3 *in ovo* before it began its cycle of cell divisions (30% of development) led to the absence of its identified progeny, neurones S1 and S2, in segment A4. *A*, Photomicrograph of an unoperated control ganglion stained in wholemount with the anti-serotonin antiserum at 70% of embryonic development. Note the bilaterally symmetrical immunoreactive cells, S1 and S2. *B*, Photomicrograph of a similarly stained ganglion in which NB 7–3 had been laser ablated earlier in development. Note the absence of immunoreactive cells on the operated side. Scale bar, 30 μm.

Methods: 30% Embryos were dechorionated in bleach, rinsed in saline and gently flattened with a coverslip to improve visibility of the NBs. NBs were identified through the dechorionated eggcase under brightfield optics with a Zeiss compound microscope using a Leitz 50× water immersion lens. Laser ablations were performed with a Chromaser pumped-dye laser system (Phase-R Corporation) using Coumarin 504 dye. Target cells were selected with a colinear helium-neon laser; 5 to 10 shots at ~250 mJ per shot over 5 to 10 min were used to kill NBs. Condensation of chromatin and subsequent loss of cellular integrity were seen within this time. Eggs were discarded given any sign of damage to neighbouring, non-target cells. Eggs with specific cellular ablations were removed from the slide and incubated. For serotonin immunostaining, the isolated embryonic CNS was fixed in 2% paraformaldehyde in Millonig's buffer and then processed according to the peroxidase–anti-peroxidase method of Sternberger[19]. Tissues were preincubated in 10% normal goat serum, 2% bovine serum albumin (BSA), and 1% Triton X-100 in phosphate-buffered saline (PBS) for 1 h. Tissues were then incubated in anti-serotonin antibody (Immunonuclear) at a 1:500 dilution, 1% normal goat serum, 1% bovine serum albumin and 1% Triton X-100 in PBS overnight at 4 °C. Following incubations in linking antibody and the peroxidase–anti-peroxidase antibody complex for 1 h each at room temperature, the tissues were developed for the horseradish peroxidase reaction product, cleared and examined.

not dye couple (as they normally do) to their homologues on the experimental side.

Not only does regulation not usually take place, but NB 7–3 appears determined to produce a particular family of neurones. Ablation of neighbouring NBs [for example, NB 6–5 ($n = 20$) which produces a small family, and NBs 7–2 and 7–4 ($n = 4$) which produce large families] did not appear to alter the morphological or biochemical differentiation of these first two NB 7–3 progeny as revealed with the anti-serotonin antibody. Furthermore, there was no apparent increase in the size of the NB 7–3 family to compensate for the loss of neighbouring families. Thus, cell lineage does appear to determine some aspects of cell fate in the insect CNS; NBs appear determined to produce particular families of neurones which differentiate with particular phenotypes.

But in what fashion does the precise pattern of identified NBs arise? Do they arise as a consequence of their cell lineage, as occurs for neuronal precursor cells in the nematode[12] and leech[13], or do they arise by cell–cell interactions, as occurs for the sensory neurone precursor cells in the epidermis of insects[14]?

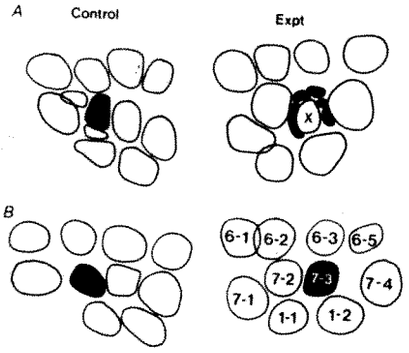


Fig. 3 *In vitro* laser ablation of ectodermal cell enlarging to become NB 7–3. Camera lucida drawings of NB rows 6 and 7 in segment T1, and NB row 1 in adjacent segment T2. *A*, A 26% embryo was removed from its eggcase under sterile conditions and viewed with a Zeiss compound microscope using Nomarksi optics and a Leitz ×50 water immersion lens for cell identifications. At the future position of NB 7–3 on the control side, the enlarging ectodermal cell (shaded) was allowed to develop normally, whereas its contralateral homologue (marked by X) was killed with three shots of the laser microbeam at 200 mJ per shot. This ablation left 5 smaller ectodermal cells (shaded) in the presumptive NB 7–3 position. The embryo was then cultured for 24 h in an RPMI 1640 culture medium containing glutamine (0.29 g l^{-1}), sodium pyruvate (0.11 g l^{-1}), penicillin (50 IU l^{-1}), streptomyocin (50 μg ml^{-1}), sodium biocarbaonate (2 g l^{-1}), horse serum (10%), glycine (6.0 g l^{-1}), bovine insulin (7.6 mg l^{-1}), 20-hydroxyecdysone (15 μg ml^{-1}), and juvenile hormone I (0.5 μg ml^{-1})[5]. Cultures were maintained in a moist incubator in a 5% CO_2 atmosphere at 29 °C. *B*, Camera lucida drawing of the same NB rows as in *A*, but after 24 h in culture. NB 7–3 (shaded) developed on both the control and experimental sides.

In insect embryos, gastrulation leads to an inner layer of mesodermal cells and an outer layer of ectodermal cells. It is from the seemingly uniform sheet of ectodermal cells between the midline and limb buds that the stereotyped pattern of NBs develops. Not all NBs form simultaneously. Initially, NBs arise from ectodermal cells that enlarge and delaminate inwards. Later, NBs arise from ectodermal cells that have delaminated to form clusters of small cells between the older NBs (Fig. 3). Ultimately, only one cell within each cluster enlarges and becomes a NB; the remaining cells either die or form ensheathing cells around the NB and sometimes its progeny. There are between three and six ectodermal cells in the region between NBs 7–2 and 7–4 from which NB 7–3 arises. To distinguish between cell lineage and cell-cell interactions in NB determination, we used a laser microbeam *in vitro* to ablate individual cells within the cluster of ectodermal cells from which NB 7–3 normally arises (Fig. 3)[2].

When we ablated the entire cluster of small cells, one of which would normally form NB 7–3, the NB did not appear ($n = 2$). When we ablated only a portion of the cluster, however, NB 7–3 formed. In particular, when we killed only the cell that was enlarging to become NB 7–3, regulation did take place ($n = 6$); one of the remaining cells became NB 7–3 (Fig. 3)[2]. The new NB 7–3 generated progeny by stereotyped asymmetric divisions, but embryos cultured *in vitro* did not develop far enough to determine the morphological or biochemical identity of these progeny. Apparently NB 7–3 can form from any one of the cells within the cluster, even after one cell has begun enlarging. Thus, cell–cell interactions within the cluster appear to determine which cell becomes the NB.

Our results demonstrate that at early stages no one cell is uniquely determined to be NB 7–3, but rather NB 7–3 develops from a restricted group of cells that share a common potential. Cell–cell interactions appear to assure that only one cell acquires the NB 7–3 fate. We do not know if the specification of individual NBs occurs in one step, or alternatively if an ectodermal cell first acquires a general NB determination and subsequently acquires a unique NB fate. By the time NB 7–3 begins its first cell division, however, it appears uniquely committed to generate a specific family of neurones; no other cell, including ectodermal cells adjacent to the ablated NB, can take its place. The NB 7–3 progeny cannot be replaced by progeny of neighbouring NBs. The final differentiation of each individual neurone depends on its mitotic ancestry and its later cell-specific interactions with other neurones in its environment[4–6].

These results suggest that the mechanisms giving rise to precursors for motoneurones and interneurones in the CNS are similar to those giving rise to precursors for sensory neurones[14] in the periphery of insects. Both types of precursors are derived from the ectoderm. Both undergo stereotyped patterns of cell divisions, the peripheral precursors typically generating four cells, one of which becomes a neurone, while the central precursors each generate 6–100 cells, all of which become neurones. The determination of both types of precursors appears to be based on position and not lineage, and involves cell–cell interactions that lead to an inhibition of surrounding cells from assuming similar fates. The differences are simply in the precision and complexity of the pattern. The central precursors develop in a highly stereotyped array while the peripheral precursors are more sparsely and often less precisely arranged. Moreover, while the peripheral precursors produce only a few different types of lineages (bristle, campaniform sensillum and so on), each central precursor appears to be uniquely determined to produce a specific lineage of neurones, each NB differing from the others in both the number and phenotypes of its neuronal progeny. Thus, the pattern formation in the CNS may involve a more complex form of the same mechanisms underlying pattern formation in the epidermis. Whether making the CNS involves more complex cell–cell interactions, or subdivisions of the ectodermal compartments[15–17] into smaller units than those required to generate the epidermis, remains to be tested.

We thank John Thomas for criticism of the manuscript. This work was supported by a NIH postdoctoral fellowship to P.H.T., NSF predoctoral fellowship to C.Q.D., and a NIH grant and McKnight Scholars Award to C.S.G.

Received 8 August; accepted 3 October 1983.

1. Bate, C. M. *J. Embryol. exp. Morph.* **35**, 107–123 (1976).
2. Doe, C. Q. & Goodman, C. S. *Soc. Neurosci.* **9**, 899 (1983).
3. Taghert, P. H. & Goodman, C. S. *J. Neurosci.* (in the press).
4. Raper, J. A., Bastiani, M. J. & Goodman, C. S. *Soc. Neurosci.* 1044 (1983).
5. Raper, J. A., Bastiani, M. J. & Goodman, C. S. *Cold Spring Harb. Symp. quant. Biol.* **48**, (in the press).
6. Bastiani, M. J. & Goodman, C. S. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
7. Goodman, C. S. & Spitzer, N. C. *Nature* **280**, 208–214 (1979).
8. Goodman, C. S. & Bate, M. *Trends Neurosci.* **4**, 163–169 (1981).
9. Goodman, C. S. in *Neuronal Development* (ed. Spitzer, N. C.) (Plenum, New York, 1982).
10. Raper, J. A., Bastiani, M. & Goodman, C. S. *J. Neurosci.* **3**, 20–30 (1983).
11. Bentley, D., Keshishian, H., Shankland, M. & Toroian-Raymond, A. *J. Embryol. exp. Morph.* **54**, 47–74 (1979).
12. Sulston, J. E. & Horvitz, H. R. *Dev Biol.* **56**, 110–156 (1977).
13. Stent, G. S., Weisblat, D. A., Blair, S. S. & Zackson, S. L. in *Neuronal Development* (ed. Spitzer, N. C.) (Plenum, New York, 1982).
14. Lawrence, P. A. in *Developmental Systems in Insects* Vol. 2 (eds Counce, S. J. & Waddington, C. H.) (Academic, New York, 1973).
15. Garcia-Bellido, A., Ripoll, P. & Morata, G. *Nature* **245**, 251–253 (1973).
16. Crick, F. H. C. & Lawrence, P. A. *Science* **189**, 340–347 (1975).
17. Lawrence, P. A. *Cell* **26**, 3–10 (1981).
18. Taghert, P. H., Bastiani, M. J., Ho, R. H. & Goodman, C. S. *Devl Biol.* **94**, 391–399 (1982).
19. Sternberger, L. A. *Immunocytochemistry* (Wiley, New York, 1978).

Prostaglandin I_3 is formed *in vivo* in man after dietary eicosapentaenoic acid

Sven Fischer & Peter C. Weber

Medizinische Klinik Innenstadt der Universität München, Ziemssenstraße 1, 8000 München 2, FRG

Greenland Eskimos who live on a traditional marine diet rich in long chain ω3-polyunsaturated fatty acids have a low incidence of cardiovascular disease and myocardial infarction[1–3]. In their plasma and platelet lipids, arachidonic acid, the precursor of dienoic prostanoids, is partly replaced by eicosapentaenoic acid (C20:5, ω3; EPA), the precursor of trienoic prostanoids[4,5]. Studies with an Eskimo diet[6] or a

© 1984 Macmillan Journals Ltd

Western diet supplemented with sea fish or fish oil rich in EPA[7–14] resulted in an 'Eskimo-like' pattern of plasma and platelet lipids. Moreover, less reactive platelets, a reduced *ex vivo* formation of proaggregatory thromboxane A_2 and a blunted circulatory response to pressor hormones were reported. These favourable functional effects may be induced by a shift of prostanoid formation from the dienoic to the trienoic series[14,15]. We show here that the major urinary metabolite of endogenous prostaglandin I_3 is present in subjects that have ingested either cod liver oil (~4 g EPA per day) or mackerel (~10–15 g EPA per day). Our studies provide the first direct evidence for *in vivo* formation of prostaglandin I_3 in man.

In vitro experiments have shown that EPA-derived thromboxane A_3 (TXA_3), in contrast to arachidonic acid (C20:4, ω6; AA)-derived thromboxane A_2 (TXA_2)[16], does not[17,18] or only weakly[19] aggregate platelets, whereas EPA-derived prostaglandin I_3 (PGI_3)[15,18,20] is as antiaggregatory as prostaglandin I_2 (PGI_2)[21]. Consequently, a shift of prostanoid formation from the dienoic to the trienoic series may change the TXA/PGI balance[22] in a favourable way[15] (Table 1).

Previous studies have demonstrated formation of PGI_3 from exogenous EPA or endoperoxide PGH_3 in certain *in vitro* systems including rat arterial tissue[15], bovine and porcine aortic microsomes[18,23] and human umbilical arteries[24]. Tissue formation of trienoic prostaglandins has been found in bovine lung[25] and kidney homogenates from rats fed with fish oil[26]. The physiological significance of these findings has been uncertain because comparative studies using purified cyclooxygenase from sheep vesicular glands and AA and EPA as substrates[27] indicate that a high peroxide level, presumably not present *in vivo*, might be necessary for an appreciable formation of trienoic prostaglandins. Moreover, in other EPA feeding studies in the rat, tissue formation of PGI_3 was not detectable[28].

We addressed three major questions in this study: (1) Is there a substantial formation of PGI_3 *in vivo* in healthy man after a diet rich in EPA and is the intake of EPA associated with a decrease in PGI_2 formation? An analogous alteration has been found in platelet studies where EPA reduced formation of TXA_2 (refs 8, 14, 18). (2) Is there a quantitative relationship between the amount of EPA ingested, the induced changes of fatty acids in plasma phospholipids and the formation of PGI_2/PGI_3? (3) Are there changes in platelet aggregability, formation of TXA_2 and bleeding time after dietary EPA?

In our study we extracted, before and after dietary supply of EPA, dinor metabolites of PGI according to a highly selective method[29] from urine collected over 24-h periods. We used Δ17-2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ (PGI_3-M) as an index of total body production of PGI_3 in analogy to 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ (PGI_2-M), which has been shown to be the major urinary metabolite of PGI_2 (refs 29, 30). A prerequisite for identification and quantification of PGI_3-M by mass spectrometry was its separation from PGI_2-M. This was achieved by using a capillary gas chromatography column with high resolving power. A partial mass spectrum of endogenous PGI_3-M extracted from urine after dietary intake of EPA is shown in Fig. 1. For comparison a partial mass spectrum of PGI_2-M simultaneously extracted from the same urine sample is presented below. The characteristically different fragmentation of PGI_3-M is due to the additional Δ17-double bond in this molecule. Fragments m/z 530 (M^+-69(71)), 440 (M^+-90-69(71)) and 350 (M^+-2×90-69(71)) are common to both metabolites, but are more intense in PGI_3-M, due to preferred allylic cleavage of the methyl side chain (C_{16}–C_{20}). Fragments m/z 584 (M^+-15), 568 (M^+-31), 478 (M^+-90-31) and 388 (M^+-2×90-31) are characteristic of PGI_3-M, but are less intense than fragments 586 (M^+-15), 570 (M^+-31), 480 (M^+-90-31) and 390 (M^+-2×90-31) which are characteristic of PGI_2-M. Some unidentified peaks in the spectra result from biological contaminating compounds in the urinary lipid extract which co-elute from the capillary column.

The multiple ion-selection tracings shown in Fig. 2 illustrate that PGI_3-M elutes from the capillary column about 35 s after

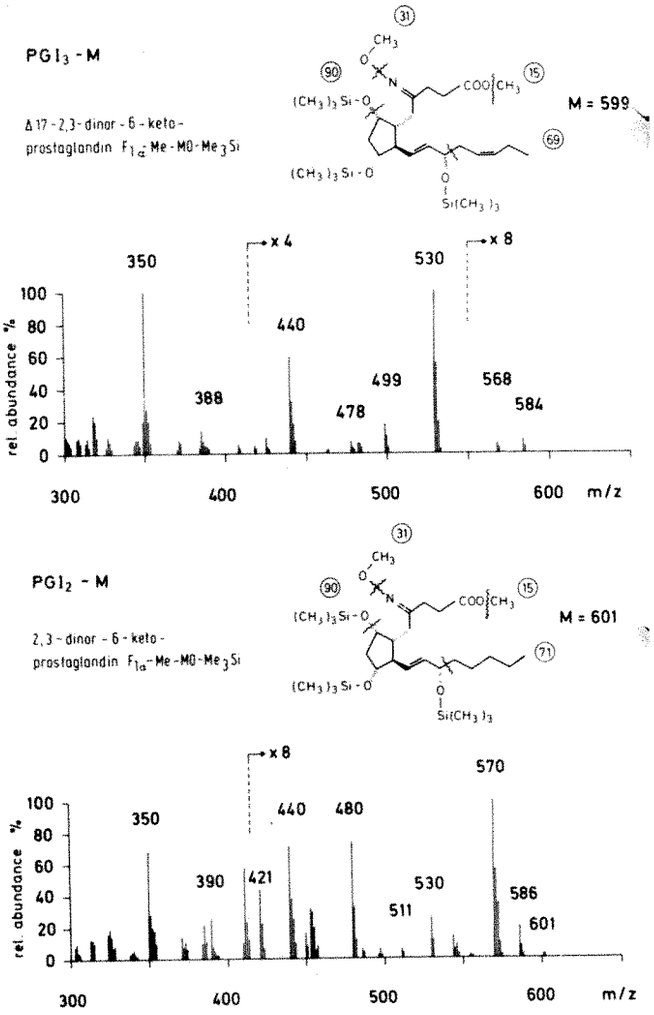


Fig. 1 Partial mass spectra (Me, MO, Me_3Si derivatives) of endogenous Δ17-2,3-dinor-6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$, the main urinary metabolites of PGI_3 and PGI_2, respectively. 800 ml of urine, collected after daily ingestion of 750 g mackerel for 3 days, were extracted according to the method of Falardeau *et al.*[29]. This method extracts the dinor metabolites of PGI_2 and PGI_3 highly selectively due to their ability to form γ-lactones in acidic solution. Extracts were methylated with ethereal diazomethane and further purified on silicagel TLC using the solvent system ethylacetate/isooctane/acetic acid/water = 110/50/20/100 (organic phase). The R_F value of authentic methylated 2,3-dinor-6-keto-$PGF_{1\alpha}$ was 0.24. The Me, MO, Me_3Si derivatives for GC-MS were prepared as described previously[29]. Prominent ions in the range above m/z 300 for Δ17-2,3-dinor-6-keto-$PGF_{1\alpha}$-Me,MO,Me_3Si are: 584 (M^+-15), 568 (M^+-31), 530 (M^+-69), 499 (M^+-69-31), 478 (M^+-90-31), 440 (M^+-90-69), 388 (M^+-2×90-31) and 350 (M^+-2×90-69); for 2,3-dinor-6-keto-$PGF_{1\alpha}$-Me,MO,Me_3Si: 601 (M^+), 586 (M^+-15), 570 (M^+-31), 530 (M^+-71), 511 (M^+-90), 480 (M^+-90-31), 440 (M^+-90-71), 421 (M^+-2×90), 390 (M^+-2×90-31) and 350 (M^+-2×90-71). The GC-MS system was a Finnigan MAT 44S equipped with a 50-m fused silica WCOT capillary column (Carbowax CP 51, 0.23 mm internal diameter; Chrompack), separating prostaglandins of the two and three series. Operation conditions of the GC-MS system were: injection port 280 °C; interface 270 °C; ion source 200 °C, electron impact energy 80 eV; current of emission 0.9 mA; electron multiplier voltage 1.8 kV.

PGI_2-M and is detectable only after dietary intake of EPA. Chemical reactions were performed to confirm the structure of PGI_3-M. Derivatization with methoxy-$^2H_3$-amine hydrochloride resulted in an increase of the molecular weight of PGI_2-M and PGI_3-M by 3 mass units and established the presence of one keto group in both molecules. Identity of PGI_3-M was confirmed by catalytic hydrogenation of the urinary extract.

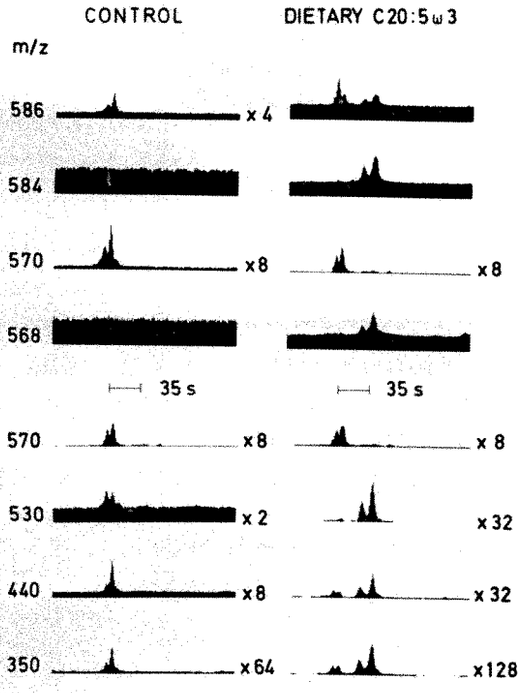


Fig. 2 Multiple ion-selection tracings (Me,MO,Me_3Si derivatives) of PGI_2-M extracted from a control urine (left) and of PGI_2-M and PGI_3-M extracted from a urine collected after daily ingestion of 750 g mackerel for 3 days (right). Qualitatively similar tracings were found after ingestion of cod liver oil. Double peaks are due to *syn*, *anti*-isomers formed by the methoxime group of the derivatives. A time axis indicates the elution time between both metabolites. Sensitivity of the tracings is lowered by the factors indicated and is adjusted to the highest occurring peak. Fragments 586 (M^+-15) and 570 (M^+-31) are specific for PGI_2-M, whereas analogous fragments 584 (M^+-15) and 568 (M^+-31) are specific for PGI_3-M. Fragments m/z 530 (M^+-69(71)), 440 (M^+-90-69(71)) and 350 (M^+-2×90-69(71)) are common to both metabolites. Deuterated internal standard (2,3-dinor-6-keto-19,19′,20,20′-^{2}H-$PGF_{1\alpha}$) for quantification of PGI_2-M has not been added to this sample.

Table 1 Physiological properties of the conversion products of arachidonic acid and eicosapentaenoic acid in vessel wall and platelets

Precursor acid	Arachidonic acid	Eicosapentaenoic acid
Vessel wall	PGI_2 Antiaggregatory	PGI_3 Antiaggregatory
Platelets	TXA_2 Proaggregatory	TXA_3 Weakly proaggregatory

Table 2 Polyunsaturated fatty acid metabolism after intake of cod liver oil or mackerel

	Control	Cod liver oil Day 25	Control	Mackerel Day 1	Mackerel Day 3
	$n=6$	$n=6$	$n=3$	$n=3$	$n=3$
Rel.%					
C20:5ω3	0.7±0.3	6.5±1.2*	1.1±0.7	7.5±1.8*	12.2±4.7*
C22:6ω3	4.0±0.8	8.7±1.3*	4.1±1.5	5.0±1.2	6.2±0.8
C20:4ω6	9.8±1.9	7.9±1.2	8.7±2.6	7.2±2.4	6.8±1.1
C18:2ω6	23.2±2.1	14.1±2.4*	21.1±6.0	7.9±1.4*	5.6±1.9*
ng per 24 h					
PGI_2-M	146±39	162±52	121±51	192±27†	236±32†
PGI_3-M	Not detectable	83±25	Not detectable	114±32	134±48

Patterns of polyunsaturated fatty acids (% of total fatty acids, mean ±s.d.) in plasma phospholipids and urinary excretion of PGI_2-M and PGI_3-M (ng per 24 h, mean ±s.d.) after intake of cod liver oil (40 ml ≙ 4 g EPA daily for 24 days; unchanged Western diet) or mackerel (750 g ≙ 10–15 g EPA daily for 3 days; no intake of other proteins or lipids). Fatty acids in plasma phospholipids were quantified by gas chromatography as described previously[8]. PGI_2-M and PGI_3-M were extracted simultaneously from urine according to the method of Falardeau *et al.*[29]. PGI_2-M was quantified by GC-MS using 2,3-dinor-6-keto-19,19′,20,20′-^{2}H-$PGF_{1\alpha}$ as the deuterated internal standard and monitoring the ion pair m/z 570/574 (M^+-31). The analysis was based on a standard curve which was shown to be linear in the observed range[34]. Excretion of PGI_3-M could not be quantified by GC-MS via a direct method due to lack of deuterated PGI_3-M or authentic unlabelled material. Quantification using deuterated PGI_2-M was also not possible because fragments suitable for multiple ion-selection represent different percentages of the total ion current of each molecule. Therefore, PGI_3-M excretion was quantified by an indirect method assessing at first the total excretion of PGI_2-M and PGI_3-M. This was achieved after catalytic hydrogenation of the urinary extract containing deuterated PGI_2-M as internal standard by monitoring the ion pair m/z 572/576 (M^+-31) of hydrogenated $PGI_{2,3}$-M and hydrogenated deuterium labelled internal standard (Me, MO, Me_3Si derivatives) which in their hydrogenated form represent compounds with identical fragmentation patterns. Urinary excretion of PGI_3-M was then calculated by subtracting the previously measured amount of PGI_2-M from the total amount of hydrogenated $PGI_{2,3}$-M. The method for quantification of urinary PGI_3-M was validated by a recovery experiment. Increasing amounts of PGI_2-M and a constant amount of deuterated PGI_2-M were added to a urine voided after mackerel ingestion. After hydrogenation of the urinary extract the added PGI_2-M was recovered quantitatively by GC-MS. Cod liver oil was manufactured by Møller A/S. All solvents and silicagel TLC plates were from Merck-Darmstadt. The 2,3-dinor-6-keto-$PGF_{1\alpha}$-lactone and the 2,3-dinor-6-keto-19,19′,20,20′-^{2}H-$PGF_{1\alpha}$ were a gift from Dr J. Pike (Upjohn). Methoxy-$^2H_3$-amine hydrochloride was prepared according to ref. 35. Catalytic hydrogenation was performed in the presence of Rh/charcoal in ethyl acetate.

* $P<0.01$; † $P<0.05$ compared with control values.

A single new compound was formed from PGI_2-M and PGI_3-M with a molecular weight of m/z 603, corresponding to an uptake of 1 and 2 equivalents of hydrogen, respectively. This substance co-chromatographed on capillary gas chromatography with authentic hydrogenated PGI_2-M and exhibited identical fragments with analogous intensities.

Table 2 summarizes the analyses of ω3 and ω6 polyunsaturated plasma phospholipid fatty acids and the urinary excretion rates of PGI_2-M and PGI_3-M after two separate studies with different amounts of dietary EPA. Polyunsaturated plasma phospholipid fatty acids indicated a characteristic change. Whereas EPA increased drastically during both dietary regimens, C22:6, ω3 was elevated significantly only after ingestion of cod liver oil due to the higher content of this fatty acid in cod liver oil as compared with mackerel. AA only decreased slightly after cod liver oil or mackerel ingestion, whereas C18:2, ω6 was strongly reduced after these dietary regimens. The more pronounced decrease of C18:2, ω6 in plasma phospholipids during the mackerel diet probably resulted from the complete substitution of animal and vegetable ω6-fatty acids by ω3-fatty acids of the mackerel. Excretion of PGI_3-M was not detectable in control conditions, but was clearly measurable after intake of cod liver oil and increased with increasing EPA incorporation into plasma phospholipids, reaching levels one-half of those

measured for PGI_2-M. The content of EPA in plasma phospholipids and the urinary excretion rates of PGI_3-M were elevated within 1 day of mackerel ingestion and increased only slightly thereafter. This may indicate that endothelial cells rapidly equilibrate with the plasma lipids and thereby transform EPA to PGI_3, as has been shown for PGI_2 from AA[31]. Our results also show that dietary EPA does not inhibit *in vivo* formation of PGI_2 from AA, confirming similar results from *in vitro* studies using human unbilical vasculature and AA and EPA as substrates[32]. Excretion of PGI_2-M was unchanged (cod liver oil) or even increased (mackerel) under the influence of dietary EPA. The reason for the increase of PGI_2-M excretion after mackerel ingestion is unclear. According to observations in endothelial cell cultures[33], the drastic decline of C18:2, $\omega 6$ in plasma phospholipids after mackerel ingestion may contribute to an increased prostacyclin synthetase activity in endothelial cells.

We have previously shown that platelet aggregation induced by low concentrations of collagen and associated formation of immunoreactive TXB_2 are reduced after a diet of mackerel in healthy subjects[8]. In the present study also, we found that ingestion of cod liver oil (CLO) for 25 days resulted in a reduced platelet aggregation (measured as % light transmission) in platelet rich plasma on stimulation with low doses (0.75 $\mu g\ ml^{-1}$) of collagen (control, $70 \pm 5\%$ (s.d.); CLO, $53 \pm 15\%$; $P < 0.01$). Concomitantly, a reduced formation of immunoreactive TXB_2 in collagen stimulated platelets (control, $40 \pm 13\ ng\ ml^{-1}$; CLO, $15 \pm 2.5\ ng\ ml^{-1}$; $P < 0.01$) and an increase of bleeding time (control, 104 ± 34 s; CLO, 145 ± 52 s; $P < 0.01$) were observed. Furthermore, in recent experiments[36] we have traced by combined gas chromatography–mass spectrometry (GC-MS) *ex vivo* formation of weakly proaggregatory TXA_3 in human platelets after supplementation of the diet with cod liver oil. Thus, the reduced platelet aggregability, increased bleeding time and reduced blood pressure response to pressor hormones[14] after dietary EPA may be due to formation of PGI_3 and TXA_3 associated with a reduced synthesis of TXA_2 and an unchanged or even increased formation of PGI_2.

Our findings with a diet rich in EPA prove that it is possible in man to change the spectrum of biologically highly active prostanoids by nutritional means and alter it in a favourable direction. This may be a mechanism by which EPA-enriched diets induce a less thrombogenic state and hence may be beneficial for the prevention of atherothrombotic disorders.

We thank Drs B. Scherer, R. Lorenz and W. Meister for cooperation, Dr C. von Schacky for performing parts of the fatty acid analyses and U. Katzner for technical assistance. This study was supported by Deutsche Forschungsgemeinschaft.

Received 27 June; accepted 10 November 1983.

1. *Lancet* **i**, 1139–1141 (1983).
2. Bertelsen, A. *Medd. Grönland* **117**, 1–83 (1935).
3. *The State of Health in Greenland*, Annual reports 1973, 1974, 1975, 1976 (Godthab, Denmark, 1978).
4. Dyerberg, J., Bang, H. O. & Hjørne, N. *Am. J. clin. Nutr.* **28**, 958–966 (1975).
5. Dyerberg, J. & Bang, H. O. *Lancet* **ii**, 433–435 (1979).
6. Sinclair, H. M. in *Drugs Affecting Lipid Metabolism* (eds Fumagalli, R., Kritchevsky, D. & Paoletti, R.) 363–370 (Elsevier, Amsterdam, 1980).
7. v. Lossonczy, T. O., Ruiter, A., Bronsgeest-Schoute, H. C., van Gent, O. M. & Hermus, R. J. J. *Am. J. clin. Nutr.* **31**, 1340–1346 (1978).
8. Siess, W. *et al. Lancet* **i**, 441–444 (1980).
9. Sanders, T. A. B., Vickers, M. & Haines, A. P. *Clin. Sci.* **61**, 317–324 (1981).
10. Goodnight, S. H., Harris, W. S. & Connor, W. E. *Blood* **58**, 880–885 (1981).
11. Thorngren, M. & Gustafson, A. *Lancet* **ii**, 1190–1193 (1981).
12. Brox, J. H., Killie, J. E., Gunnes, S. & Nordøy, A. *Thromb. Haemostasis* **46**, 604–611 (1981).
13. Hirai, A. *et al. Thromb. Res.* **28**, 285–298 (1982).
14. Lorenz, R., Spengler, U., Fischer, S., Duhm, J. & Weber, P. C. *Circulation* **67**, 504–511 (1983).
15. Dyerberg, J., Bang, H. O., Stoffersen, E., Moncada, S. & Vane, J. R. *Lancet* **ii**, 117–119 (1978).
16. Hamberg, M., Svensson, J. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994–2998 (1975).
17. Needleman, P., Minkes, M. & Raz, A. *Science* **193**, 163–165 (1976).
18. Needleman, P., Raz, A., Minkes, M. S., Ferrendelli, J. A. & Sprecher, H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 944–948 (1979).
19. Gryglewski, R. J. *et al. Prostaglandins* **18**, 453–478 (1979).
20. Nidy, E. G. & Johnson, R. A. *Tetrahedron Lett.* **27**, 2375–2378 (1978).
21. Moncada, S., Gryglewski, R., Bunting, S. & Vane, J. R. *Nature* **263**, 663–665 (1976).
22. Moncada, S. & Vane, J. R. *Br. med. Bull.* **34**, 129–135 (1978).
23. Smith, D. R. *et al. Prostaglandins* **18**, 423–438 (1979).
24. Dyerberg, J., Jørgensen, K. A. & Arnfred, T. *Prostaglandins* **22**, 857–862 (1981).
25. Samuelsson, B. *Biochim. biophys. Acta* **84**, 707–713 (1964).
26. Ferretti, A., Schoene, N. W. & Flanagan, V. P. *Lipids* **16**, 800–804 (1981).
27. Culp, B. R., Titus, B. G. & Lands, W. E. M. *Prostaglandins Med.* **3**, 269–278 (1979).
28. Hornstra, G., Christ-Hazelhof, E., Haddeman, E., ten Hoor, F. & Nugteren, D. H. *Prostaglandins* **21**, 727–738 (1981).
29. Falardeau, P., Oates, J. A. & Brash, A. R. *Analyt. Biochem.* **115**, 359–367 (1981).
30. Rosenkranz, B. *et al. Biochim. biophys. Acta* **619**, 207–213 (1980).
31. Spector, A. A., Kaduce, T. L., Hoak, J. C. & Fry, G. L. *J. clin. Invest.* **68**, 1003–1011 (1981).
32. Dyerberg, J. & Jørgensen, K. A. *Artery* **8**, 12–17 (1980).
33. Spector, A. A. *et al. J. clin. Invest.* **65**, 1003–1012 (1980).
34. Fischer, S., Scherer, B. & Weber, P. C. *Biochim. biophys. Acta* **750**, 127–133 (1983).
35. Hamberg, M. & Samuelsson, B. *J. biol. Chem.* **246**, 6713–6721 (1971).
36. Fischer, S. & Weber, P. C. *Biochem. biophys. Res. Commun.* **116**, 1091–1099 (1983).

Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts

Suzanne T. Ildstad & David H. Sachs

Transplantation Biology Section, Immunology Branch, Cancer Institute, National Institutes of Health, Building 10, Room 4B17 Bethesda, Maryland 20205, USA

Clinical organ transplantation between genetically disparate individuals currently requires the use of chemotherapeutic agents to suppress the rejection reaction. The deleterious side effects of these reagents and their inability to prevent rejection completely has led to a continuing search for methods to induce specific transplantation tolerance in adult recipients. Numerous experimental animal models utilizing irradiation and bone marrow transplantation coincident with organ transplantation have been proposed[1–4]. Bone marrow transplantation, however, has its own major complications, including graft–versus-host reactions and immunoincompetence, probably resulting from a failure of appropriate immune cell interactions in the reconstituted host[5,6]. We have now attempted to overcome these difficulties by reconstituting the irradiated host with T-cell depleted bone marrow containing both host (syngeneic) and donor (allogeneic or xenogeneic) components. This technique leads to long-term survival of the reconstituted animals and specific prolongation of subsequent skin grafts of donor type. Animals reconstituted in this fashion are fully reactive to third-party allografts and xenografts and do not appear to manifest signs of graft–versus-host disease.

Inbred mice of the congenic strains C57BL/10Sn (B10) and B10.D2/nSn (B10.D2) (Jackson Laboratory, Bar Harbor) and rats of strains F344 and Wistar Furth (WF) (Charles River Laboratories) were used. Recipient B10 males were given a single dose of 950 rad from a caesium source. Donor bone marrow was treated with rabbit anti-mouse brain antiserum (RAMB) and guinea pig complement to remove mature T lymphocytes as previously described[7].

Titrations were performed to determine the optimal number of syngeneic and allogeneic or xenogeneic bone marrow cells needed to achieve consistent reconstitution and tolerance. The experiments described here were performed with a dose of 5×10^6 syngeneic cells and either 1.5×10^7 allogeneic B10.D2 bone marrow cells or 4×10^7 xenogeneic F344 rat bone marrow cells. Bone marrow preparations were treated separately *in vitro* with RAMB plus complement and were then washed, counted and mixed just prior to inoculation through the tail vein. Animals were subsequently housed in a specific pathogen-free facility at the National Institutes of Health.

The survival of B10 animals reconstituted with B10 plus B10.D2 bone marrow was 100% at 100 days and 88% at 200 days, which is essentially identical to the survival of B10 animals reconstituted with syngeneic bone marrow (data not shown). All irradiation control animals died within 2 weeks. Cytotoxicity

Table 1 Characterization of mice reconstituted with mixed allogeneic bone marrow (B10+B10.D2→B10)

Animal no.	Complement control	% Cytotoxicity B10.D2αB10 + complement*	% Cytotoxicity B10αB10.D2 + complement	% B10.D2 cells
1	3	9	75	92
2	4	80	27	23
3	7	26	80	79
4	7	18	79	87
5	8	32	75	74
6	8	80	25	19
7	4	19	75	82
8	8	70	38	33
9	5	39	38	49
10	8	45	75	64
11	8	57	44	42
12	1	22	52	70
13	5	40	37	48
14	3	55	27	32
15	6	45	43	49
16	4	27	45	64
17	5	92	10	5
Normal B10	4	99	4	0
Normal B10.D2	6	5	100	100

Complement-mediated cytotoxicity was determined on peripheral blood lymphocytes (PBL) by trypan blue exclusion.

* Maximum % lysis by antiserum plus complement. Totals for B10 and B10.D2 do not necessarily equal 100% because of inherent sampling error in the assay. % B10.D2 was calculated by normalizing total specific % kill by the two antisera to 100% after correction for % lysis by complement alone

$$(\%\ B10.D2 - C/(\%\ B10.D2 + \%\ B10) - 2(C)) \times 100$$

Table 2 Characterization of mice reconstituted with mixed xenogeneic bone marrow (B10+rat→B10)

Animal no	% Rat lymphocytes in PBL assessed by complement-mediated cytotoxicity assay*	% Rat lymphocytes in spleen assessed by FACS analysis lymphoid cells†
226	−2	+0.47
227	0	+0.92
228	+1	+0.11
229	+9	+0.87
230	0	−0.29
234	0	+0.94
235	+6	+2.26
250	+5	+2.12
214	0	NT
217	0	NT
240	+2	NT
241	−1	NT
Normal B10 control	0	+0.44±0.03

* % Lysis was that obtained by mouse anti-rat serum plus complement minus complement control.

† % Of cells with fluorescence intensity greater than 300 on log scale following staining with mouse anti-rat serum and fluorescein isothiocyanate conjugated goat $F(ab)_2$ anti-mouse IgG preparation (FITC-GAMIG) minus % staining with FITC-GAMIG alone. Animals were subjected to partial splenectomy, and splenic lymphoid suspensions were stained with mouse anti-rat antiserum and a FITC-GAMIG. NT, not tested.

typing of peripheral blood lymphocytes[8] of reconstituted animals demonstrated persistence of both host- and donor-type lymphoid cells at 60–120 days following reconstitution (Table 1). These animals therefore represent mixed allogeneic chimaeras.

Animals were grafted with full thickness skin grafts from donor and third-party allogeneic strains of mice approximately 12 weeks after reconstitution, using a modification of the method of Billingham[9]. Rejection was considered complete when no clearly viable graft could be detected. Eighty-six per cent of mixed allogeneic chimaeras were tolerant to donor-type skin grafts, showing complete survival of fully viable skin grafts for more than 145 days (Fig. 1). The tolerance was specific, since third-party allogeneic skin grafts were rejected with a median survival time essentially the same as that of unirradiated controls.

B10 recipient mice reconstituted with a mixture of syngeneic and xenogeneic F344 rat bone marrow cells also demonstrated long-term survival similar to that of syngeneically reconstituted animals (86% survival at 100 days). In contrast to the mixed allogeneic chimaeras, peripheral blood lymphocyte typing of mixed xenogeneically reconstituted animals at day 60–70 following reconstitution revealed low numbers, if any, of donor lymphoid cells in the peripheral blood of these animals (Table 2). Further analysis of these animals was performed by flow microfluorometry (FMF) using the fluorescence-activated cell sorter (FACS)[10]. Control experiments showed that 0.5% contaminating rat lymphoid cells could reliably be detected. The number of rat lymphoid cells detectable in spleens of mixed xenogeneically reconstituted mice was very small, ranging from 0–2.26% (Table 2).

Nevertheless, these animals appeared to be specifically hyporeactive to donor-type F344 rat skin grafts (Fig. 2). Fourteen B10 animals reconstituted with mixed syngeneic and xenogeneic F344 rat bone marrow cells were grafted with both F344 and B10.D2 skin grafts 75–85 days following irradiation and reconstitution. Rat skin grafts exhibited signs of inflammation beginning 2–10 weeks following grafting. This inflammation often subsided, but eventually recurred in all animals and led either to late rejection or contraction of the skin graft to a small but viable residual. However, all of the animals showed prolonged survival of the rat skin grafts (median survival time [MST] > 50 days) compared with normal B10 animals, which

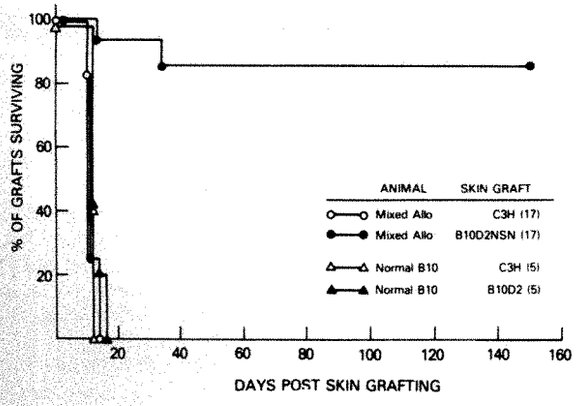


Fig. 1 Survival of B10.D2 and C3H full thickness tail skin grafts on lateral thoracic wall of mixed allogeneic chimaera (B10+B10.D2→B10) and controls calculated by life table method.

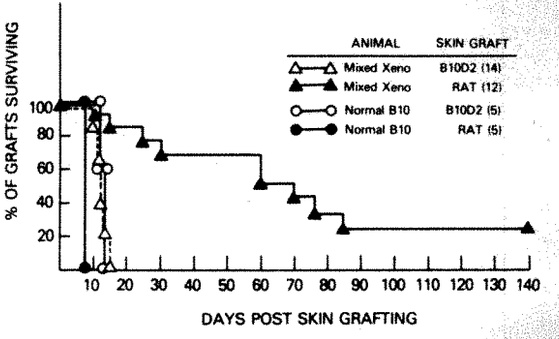


Fig. 2 Survival of F344 rat and B10.D2 full thickness tail skin grafts on lateral thoracic wall of mixed xenogeneic chimaeras (B10+F344 rat→B10) and controls calculated by life table method. Fourteen reconstituted animals were grafted with both rat and B10.D2 skin grafts, but only 12 of the rat skin grafts were technically successful and could be evaluated.

rejected F344 rat skin grafts in less than 9 days. Furthermore, these same animals rejected the third party allogeneic B10.D2 skin grafts with an MST identical to that of normal B10 controls. This result has subsequently been confirmed in two additional groups of mice. In one of these groups, an additional skin graft was performed from a WF rat, a rat strain differing from the F344 at both major and minor histocompatibility loci[11]. The WF skin grafts were all rejected promptly, while the F344 skin grafts again showed the same prolonged survival.

Numerous previous studies[7,12,13] have shown that elimination of mature T cells from donor bone marrow inocula can lead to long-term survival of fully allogeneic bone marrow chimaeras. However, the survival of such animals is generally not as good as that of syngeneically reconstituted controls, and the health of these animals is compromised, probably as the result of partial immunoincompetence[5,6]. The model for fully xenogeneic chimaeras[14] has been less extensively studied than that of allogeneic chimaeras. In our experience, such animals show prolonged survival over irradiation controls, but all succumb within a few weeks following reconstitution. It is not clear whether the death of these animals is due to a chronic graft–versus-host disease, or to infectious complications secondary to partial immunoincompetence.

In contrast, the studies presented here indicate that reconstitution with mixtures of syngeneic bone marrow and either allogeneic or xenogeneic marrow leads to long-term survival of recipients equivalent to that of syngeneically reconstituted animals. The animals have remained healthy and have shown no evidence of infections or of graft–versus-host disease for longer than 3 months.

Typing of mixed allogeneically reconstituted animals at 2–3 months following reconstitution showed them to be truly mixed chimaeras, bearing variable percentages of syngeneic and allogeneic lymphoid cells in their peripheral circulation. Such animals are immunocompetent[13] and specifically tolerant to donor skin grafts. In mice reconstituted with mixed syngeneic and xenogeneic bone marrow, we have found little evidence for persistence of the xenogeneic lymphoid compartment. However, these animals also appear to be specifically hyporeactive to subsequent skin xenografts. They may be more similar to neonatally induced allochimaeras[15], in which only very small numbers of persistent donor-type cells can be found[16]. Since the survival of skin grafts is frequently more difficult to prolong than that of most other foreign tissues[17], it seems likely that animals manifesting this level of hyporeactivity to skin grafts would be highly tolerant to xenografts of kidneys, hearts and other solid organs.

These data therefore indicate that reconstitution of lethally irradiated mice with mixtures of syngeneic and either allogeneic or xenogeneic bone marrow leads to the induction of specific immune unresponsiveness to subsequent donor skin grafts. The mechanism of this specific unresponsiveness may not be the same in these mixed allogeneic and xenogeneic models, and further mechanistic studies both *in vitro* and *in vivo* are presently in progress. Both types of reconstitution offer models which may be directly applicable to organ grafting in other species, including man.

We thank Dr Hugh Auchincloss Jr for helpful discussions, Mr David Stephany for performing the FACS analysis, Ms Judith Jaworek for manuscript preparation, and Ms Susan O. Sharrow and Drs David Tollerud, Richard Hodes and Mark Peskovitz for review of the manuscript.

Received 30 June; accepted 19 October 1983.

1. Rapaport, F. T. *et al. Transplantn Proc.* **9,** 891–894 (1977).
2. Slavin, S., Strober, S., Fuks, Z. & Kaplan, S. *J. exp. Med.* **146,** 34–48 (1977).
3. Dittmer, J. & Bennett, M. *Molec. cell. Biochem.* **21,** 83–94 (1978).
4. Myburgh, J. A., Smit, J. A., Browde, S. & Hill, R. R. H. *Transplantation* **29,** 401–404 (1980).
5. Zinkernagel, R. M., Althage, A., Callahan, G. & Welsh, R. M. *J. Immun.* **124,** 2356–2365 (1980).
6. Rayfield, L. S. & Brent, L. *Transplantation* **36,** 183–189 (1983).
7. Auchincloss, H. Jr & Sachs, D. H. *Transplantation* **36,** 436–441 (1983).
8. Arn, J. S., Riordan, S. E., Pearson, D. & Sachs, D. H. *J. immun. Meth.* **55,** 141–153 (1982).
9. Billingham, R. E. in *Transplantation of Tissues and Cells* (eds Billingham, R. E. & Silvers, W. K.) (Wistar Institute, Philadelphia, 1961).
10. Sharrow, S. O., Mathieson, B. J. & Singer, A. *J. Immun.* **126,** 1327–1335 (1981).
11. Gasser, D. L. *Adv. Immun.* **25,** 93–139 (1977).
12. Krown, S. E., Coico, R., Scheid, M. P., Fernandez, G. & Good, R. A. *Clin. immun. Immunopath.* **19,** 268–283 (1981).
13. Singer, A., Hathcock, K. S. & Hodes, R. J. *J. exp. Med.* **153,** 1286–1301 (1981).
14. Muller-Ruchholtz, W., Muller Hermelink, H. K. & Wottge, H. U. *Transplantn Proc.* **11,** 517–521 (1979).
15. Billingham, R. E., Brent, L. & Medawar, P. B. *Nature* **172,** 603 (1953).
16. Streilein, J. W. & Klein, J. *J. Immun.* **119,** 2147–2150 (1977).
17. Boyse, E. A., Lance, E. M., Carswell, E. A., Cooper, S. & Old, L. J. *Nature* **227,** 901–903 (1970).

Genetic evidence of X–Y interchange in a human XX male

Albert de la Chapelle*, Patricia A. Tippett†, Gunilla Wetterstrand‡ & David Page§

* Department of Medical Genetics, University of Helsinki, 00290 Helsinki 29, Finland
† MRC Blood Group Unit, Wolfson House, University College London, 4 Stephenson Way, London NW1 2HE, UK
‡ Jorv Hospital, 02740 Esbo 74, Finland
§ Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Of the hypotheses put forward to explain why occasional individuals with two X chromosomes are nonetheless male, the one that has attracted most attention is the possibility[1] that one of the X chromosomes has obtained a small piece of Y chromosome which is sufficient to produce 'maleness'. This hypothesis was based primarily on the observation that in two families with XX males[2–4] both fathers were Xg(a+) and both probands Xg(a−). (*Xg* shows X-linked dominant inheritance.) This theory holds that an anomalous X–Y interchange at meiosis in the father resulted in the paternal X chromosome's losing the *Xg* gene and acquiring a male-determining gene from the Y chromosome. While, for example, the frequencies of Xg phenotypes among XX males[5,6] and the cytogenetic observation of a structural abnormality in one X[7,8] are compatible with this hypothesis, direct evidence of it is lacking. Here we describe an XX male who expresses his father's allele for 12E7, a Y-linked marker, but fails to express his father's allele for Xg, an X-linked marker. These findings strongly suggest that anomalous X–Y interchange occurred in this case and perhaps in that of many other XX males. We suggest that a male-determining gene on the Y has also been translocated to the X and caused maleness in the proband. These results are discussed in the light of current models of X–Y chromosomal homology.

A boy born in 1980 had slightly dysmorphic facial features but was otherwise normal. He has remained in good health but his physical growth is greatly retarded (at 2 yr and 3 months old, height = 75 cm, weight = 7.4 kg). His height development is well below the 2.5 percentile for boys and even for girls. Clinical and laboratory investigations had failed to disclose anything abnormal to account for the retarded growth until a karyotype showed him to be 46, XX; there was no evidence of mosaicism in the two tissues karyotyped (lymphocytes and skin fibroblasts). The parents (born in 1953) are non-consanguineous. All four grandparents are alive. The proband has a brother born in 1978 and a sister born in 1983. All family members are healthy.

All family members (Fig. 1) were tested for the following blood groups: ABO, MNSs, P_1, Rh, Lu^a, Kell, Lewis, Duffy, Kidd, Dombrock and Co^b. That II-1 is indeed the father of III-2 is supported by the inheritance of these markers (data not shown). Figure 1 shows the segregation of the blood group Xg, the antigen 12E7, and the polymorphic X–Y homologous DNA locus designated *DXYS1* (ref. 9) in the members tested. Both parents, II-1 and II-3, are Xg(a+); the mother is heterozygous Xg^aXg as her father, I-3, is Xg(a−). Both the proband, III-2, and his brother, III-1, are Xg(a−) so they have received the maternal *Xg* gene. All Xg(a+) individuals express high levels

0028–0836/84/020170—02$01.00

© 1984 Macmillan Journals Ltd

of 12E7 (ref. 10). However, in families with Xg(a−) members, the quantitative polymorphism of 12E7 expression shows Y-linked inheritance[11]. For example, 77 Xg(a−) high-level 12E7 males (first tested member of sibship) have 110 Xg(a−) younger (or tested later) brothers of whom 107 have high-level 12E7 and 3 have low-level 12E7 expression; 18 Xg(a−) low-level 12E7 males have 19 Xg(a−) younger brothers of whom 17 have low-level 12E7 and 2 have high-level 12E7 expression. These comparisons give $\chi^2 = 20.51$ and 15.81 respectively if 12E7 were not Y-controlled. The five exceptional brothers could be the result of X–Y interchange, or of difficulties in testing travelled red cells, or of a different father (the father of only one of the exceptions has been tested)[11].

In the family reported here, both the paternal uncle, II-2, and elder brother, III-1, are Xg(a−) and high expressors of 12E7, suggesting that the Y chromosome in the paternal family carries Yg^a. (The Y-linked *12E7* locus has been designated *Yg* on the basis of its hypothesized homology to *Xg*[10]. The allele for high expression of 12E7 has been designated Yg^a.) The maternal grandfather, I-3, is Xg(a−) low 12E7 and, therefore, *XgYg*.

The proband does not carry the paternal Xg^a allele but has received a gene producing high 12E7 expression. As this could not have come from the mother, the most straightforward explanation is an interchange of the *Xg* and *Yg* loci in paternal meiosis. As a consequence of this, the X chromosome of paternal origin has lost its Xg^a but acquired Yg^a. The presence of testes and male differentiation in the proband is probably the consequence of a sex-determining gene having been exchanged as well. Perhaps a male-determining gene normally located in proximity to *Yg* on the Y chromosome has been translocated to the X. Alternatively, an X-borne gene involved in sex determination may have been lost or inactivated.

Our interpretation assumes that one of the proband's X chromosomes is indeed paternal. The *Taq*I restriction fragment length polymorphism at the *DXYS1* locus shows normal segregation of the Y-specific 15-kilobase (kb) fragment with the Y chromosome in the father, II-1, and brother, III-1 (Fig. 1). The proband has a double dose of the X-specific 11-kb allele (one from the father and one from the mother). This is not formal proof of the paternal derivation of one X, as it is also consistent with the proband having two copies of the maternal X carrying the 11-kb allele (and *Xg*). However, the latter possibility is inconsistent with the 12E7 data. The segregation of eight additional X-linked DNA polymorphisms was examined in this family but none of the polymorphisms proved to be informative with respect to a paternal contribution (data not shown).

It has been shown recently that many XX males have one paternal and one maternal X chromosome. When the segregation of the *DXYS1* alleles was tested in six families, four were informative. In each of these four cases the XX male proband was heterozygous[11,12] while the mother was homozygous for one of the alleles and the father hemizygous for the other (D.P. and A. de la C., unpublished results). This supports the notion that one X is paternal in many, if not most, XX males.

The proposed interchange explains three findings (loss of Xg^a, acquisition of Yg^a, maleness) as the consequence of one event. The alternative—non-disjunction at maternal meiosis plus non-disjunction at the first paternal meiotic division or zygotic loss of the Y—does explain the chromosomal and Xg data but fails to explain the level of 12E7 expression and the maleness of the proband. Hence, the interchange mechanism must be considered the most likely one.

At present we do not know what proportion of XX males arises by the type of interchange that occurred in this case. That it may be common is suggested by the fact that at least nine families are known in which the XX male proband apparently did not inherit his father's *Xg* allele[6]. Furthermore, the frequency of Xg phenotypes in XX males is close to that of XY males and is significantly different from that of females (R. Sanger and P.A.T., unpublished observations on 94 northern European XX males).

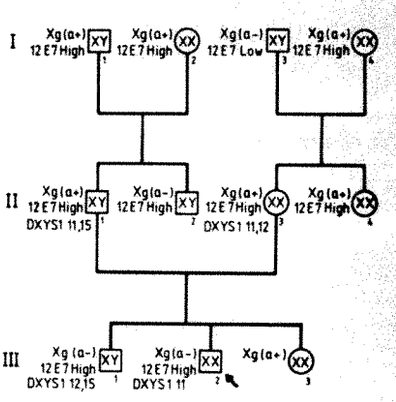


Fig. 1 Pedigree of the family showing the sex chromosome constitution and segregation of the blood group Xg, the antigen 12E7, and the X–Y homologous DNA locus *DXYS1*.

In contrast, at least five families are known in which the XX male proband did inherit his father's *Xg* allele[6]. These cases either arose by another mechanism or the interchange involved different regions of those chromosomes. Light and electron microscopic evidence[12,13] indicates that terminal portions of the short arms of the human X and Y chromosomes normally pair at male meiosis. It has been suggested that crossing-over within this 'pairing region' occurs as a normal event[1,14–16], but there is no genetic evidence for this. Here we have described a clearly anomalous meiotic interchange of normally X-linked and Y-linked markers. Of note is the fact that the *Xg* gene maps to the 'pairing region' on the X[17] while a *12E7* structural gene maps to the euchromatic portion of the Y[18], which includes the pairing region. Thus, the X–Y interchange reported here may be an example of a common event, but one which is abnormally proximal to the centromere.

The aetiology of XX males is probably heterogeneous. The underlying mechanisms may include errors of X-chromosome inactivation, mutations in autosomal or X-chromosomal genes, and X–Y interchange (probably with different breakpoints in different cases). A somewhat analogous finding is that the sex reversal mutation in the mouse (*sxr*) is now known to be due to a complicated Y–X exchange[19,20].

This study was aided by grants from the Sigrid Jusélius Foundation, the Nordisk Insulinfond, the Academy of Finland, the NIH (USA), and the Wills Foundation. We thank Dr Peter Goodfellow for the anti-12E7 antibody and Drs David Botstein and Arlene Wyman for critical reading of the manuscript.

Received 1 August; accepted 1 November 1983.

1. Ferguson-Smith, M. A. *Lancet* **ii,** 475–476 (1966).
2. de la Chapelle, A., Hortling, H., Niemi, M. & Wennström, J. *Acta med. scand. Suppl.* **412,** 25–38 (1964).
3. de la Chapelle, A., Hortling, H., Wennström, J., Niemi, M. & Johansson, C.-J. *Acta endocr. Suppl.* **100,** 90 (1965).
4. de la Chapelle, A., Similä, S., Lanning, M., Kontturi, M. & Johansson, C.-J. *Hum. Genet.* **11,** 286–294 (1971).
5. Race, R. & Sanger, R. *Blood Groups in Man* 6th edn (Blackwell, Oxford, 1975).
6. de la Chapelle, A. *Hum. Genet.* **58,** 105–116 (1981).
7. Evans, H. J., Buckton, K. E., Spowart, G. & Carothers, A. D. *Hum. Genet.* **49,** 11–31 (1979).
8. Magenis, R. R. *et al. Hum. Genet.* **62,** 271–276 (1982).
9. Page, D. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 5352–5356 (1982).
10. Goodfellow, P. N. & Tippett, P. *Nature* **289,** 404–405 (1981).
11. Tippett, P., Shaw, M.-A., Daniels, G. L. & Green, C. A. *Human Gene Mapping* Vol. 7 (in the press).
12. Pearson, P. C. & Bobrow, M. *Nature* **226,** 959–961 (1970).
13. Moses, M. J., Counce, S. J. & Paulson, D. F. *Science* **187,** 363–365 (1975).
14. Polani, P. E. in *Mechanisms of Sex Differentiation in Animals and Man* (eds Austin, C. R. & Edwards, R. G.) 465–488 (Academic, London, 1981).
15. Polani, P. E. *Hum. Genet.* **60,** 207–211 (1982).
16. Burgoyne, P. S. *Hum. Genet.* **61,** 85–90 (1982).
17. Ferguson-Smith, M. A., Sanger, R., Tippett, P., Aitken, D. A. & Boyd, E. *Cytogenet. Cell Genet.* **32,** 273–274 (1982).
18. Goodfellow, P. *et al. Nature* **302,** 346–349 (1983).
19. Singh, L. & Jones, K. W. *Cell* **28,** 205–216 (1982).
20. Evans, E. P., Burtenshaw, M. D. & Cattanach, B. M. *Nature* **300,** 443–445 (1982).

Human XX males with Y single-copy DNA fragments

Georges Guellaen*, Myriam Casanova†, Colin Bishop*†, Danielle Geldwerth*, Gabriel Andre‡, Marc Fellous† & Jean Weissenbach*

* Unité de Recombinaison et Expression Génétique, INSERM U 163, CNRS LA 271 and † Unité d'Immunogénétique Humaine, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France
‡ Hospices Civils, 67000 Strasbourg, France

In humans, XX maleness is the best known example of a sex reversal syndrome occurring with an incidence of one XX male among approximately 20,000 to 30,000 newborn boys[1–4]. The karyotypes of the majority of these individuals are apparently normal, with respect to the numbers and structure of the chromosomes, but is in contradiction with the phenotypic sex which they display. XX maleness may be either a non Y-related mechanism triggered by a mutation on another chromosome[3–5] or could be the result of the expression of some cytogenetically undetectable Y chromosome material present in the genome of such individuals. Recently, a number of human Y-specific single copy probes have been isolated[6,7]. In this study, using several of these Y-specific probes we definitively demonstrate the presence of Y-chromosomal material in the genome of some 46,XX human males. These XX males carry only a fraction of the human Y chromosome. In the three positive cases reported here, presence of inclusive overlapping chromosomal fragments has been detected, implying a genetic heterogeneity of these patients.

The 4 cases of XX male analysed are sporadic with no familial incidence of genetic intersexuality. Their clinical and biological features are summarized in Table 1. DNAs from cloned lymphoblastoid cell lines or lymphocytes originating from the four different human XX males have been digested with *Eco*RI, *Hin*dIII or *Taq*I, fractionated and blotted onto DBM-cellulose paper[8]. The DBM papers were successively hybridized to different probes detecting Y-specific DNA fragments. Under stringent hybridization conditions these probes detected single copy DNA fragments whereas under less stringent conditions additional bands are sometimes observed that are not strictly homologous. In order to detect a maximal number of Y-specific bands, low stringency washing conditions have been used in this survey whenever possible. Figure 1 shows the hybridization pattern of two probes 52d and 50f2 to *Taq*I digests. Probe 52d (Fig. 1*A*) detects three Y-specific bands in normal XY DNAs and a small X-located fragment[7] not present in this figure. Two out of the three Y-specific fragments are also present in XX males 1 and 4 indicating the presence of Y chromosomal DNA in some XX males. Since the third Y-specific band does not appear in these cases (1 and 4), we conclude that the Y chromosome is not present as a whole. Confirmation of this result is obtained with probe 50f2 (Fig. 1*B*). This probe detects 1 autosomal band at 12 kilobases (kb), in both male and female DNA, and in addition, four Y-specific fragments. Here again, only 2 out of 4 of the Y specific bands are present in the same patients 1 and 4.

The data on the Y-specific sequences, tested as probes on genomic blots of XX males, are summarized in Table 2. Out of the four individuals, only three reacted with the probes used (case 1, 3 and 4). The three positive cases did not react to the same extent with the probes tested. Although case 1 and 4 reacted with six and five probes respectively, case 3 reacted only with probe 47c. This observation implies that these XX male patients each contain a different amount of Y-chromosomal DNA and thus exhibit a genetic heterogeneity. In all three cases, the same subset of Y-specific bands was detected, implying presence of overlapping chromosomal fragments. The genome

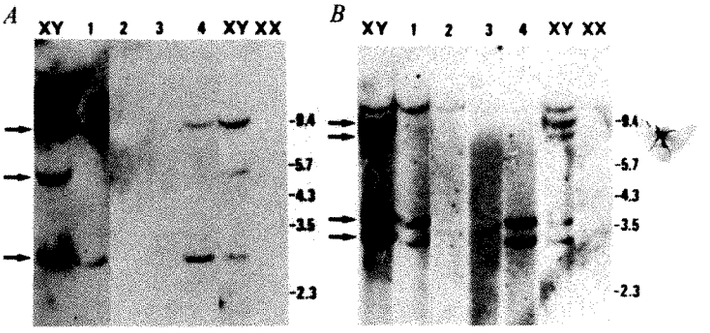


Fig. 1 DNA was extracted as previously described[6,7] from lymphocytes of normal individuals or from cloned lymphoblastoïd cell lines derived by Epstein–Barr virus transformation of lymphocytes from XX males. The probes were derived from a partial Y chromosome library as previously reported[6,7]. The genomic blot used in *A* and *B* was prepared by digesting genomic DNA to completion with *Taq* (Bio Labs) used according to manufacturers' specifications, separating on 1% agarose gels and transferring to DBM paper. Normally 15 μg of DNA was used per lane. The DBM paper blots were hybridized[8] with 30×10^6 c.p.m. per blot of the probes 52d (*A*) or 50f2 (*B*) (10^8 c.p.m. μg^{-1}) and washed under non-stringent conditions (2×SSC; 68 °C; 2×60 min). Exposure of the film was done for various times at −70 °C in the presence of an intensifying screen. XY: normal male; XX: normal female; 1–4: different XX males. The male specific bands are arrowed on the left of the two panels. The size of the markers is indicated in kilobase pair units on the right.

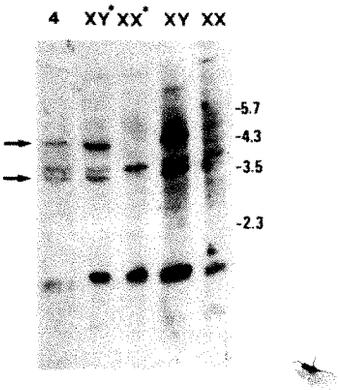


Fig. 2 The experiment was performed as for Fig. 1. The five DNAs were hydrolysed with *Hin*dIII, the genomic blot hybridized with probe 47c and washed under non-stringent conditions. XY* and XX* are the parents of case 4, XY and XX: normal male and female.

of case 1 contains the most numerous Y DNA fragments. These fragments completely overlap with the fragments present in case 4 which themselves overlap with the fragments of case 3. This totally inclusive overlapping could be observed by chance, but it is also consistent with an involvement of one of the telomeric parts of the Y chromosome. If, in addition, one assumes that only a single Y-chromosomal piece is present in the genome of these XX males, blocks of overlaps can be positioned with reference to a common origin and the distance of a DNA fragment from this origin is inversely related to the frequency of this fragment in XX males. This would represent the first attempt to order Y DNA fragments by deletion mapping.

Since the probes described here also hybridize to non-Y material, the possibility exists that the fragments detected in XX males are due to a restriction fragment length polymorphism of this non-Y material. This possibility can be rejected in view of the following arguments: (1) the non-Y bands are still present in the expected amount in the XX males according to hybridization intensity, (2) all the putative Y bands in the XX males appear exactly at the same level as in normal males (Fig. 1) and in different restriction digests (not shown). It is practically impossible that, by chance, restriction length polymorphism could have resulted in numerous bands all of the exact same size as the Y-specific ones.

The parental origin of the Y-specific fragment could be assessed further for case 4. Figure 2 represents a genomic blot

0028-0836/84/020172—02$01.00

Table 1 Clinical and biological summary of the 4XX males analysed

	Case 1	Case 2	Case 3	Case 4
Age (yr)	21	11	22	20
Sterility	Azoospermy	NT	Azoospermy	Azoospermy
Testes	Small	Extremely reduced	Small	Small
Gynaecomasty	Absent	Absent	Absent	Absent
Histopathology of the gonad	NT	Testicular structure without germ cells, with Sertoli cells	Testicular structure without germ cells, with Sertoli cells	Testicular structure without germ cells, with Sertoli cells
FSH level	High	High	High	High
Testosterone level	Low	Very low	Low	Low
Karyotype	XX	XX	XX	XX
H-Y	Present	Present	Present	Present

Karyotype and H-Y determination[15] were performed on lymphoblastoïd cell lines and peripheral lymphocytes. Similar results were obtained with both types of cells. NT, not tested. In all the four cases, at least 200 mitoses were analysed both on peripheral lymphocytes and lymphoblastoïd cell line obtained by Epstein–Barr virus transformation of B lymphocytes. Trypsin band (G banding) and Q banding were performed. No Y material could be detected and both X chromosomes appear of normal size. Familial blood groups, including HLA antigen were performed. No exclusion of paternity was observed. Xg anomalous segregation were found for cases 2 and 3 and will be described elsewhere. For the two other cases, the Xg phenotype was not informative.

of *Hin*dIII restriction enzyme hydrolysis of case 4 along with DNA from his parents (asterisk) and normal male and female DNA. Hybridization to probe 47c reveals an identical pattern between case 4, XY* and XY, and a total absence of male specific bands on the DNA of the mother(XX*). This result excludes maternal inheritance of the Y-chromosomal DNA in this case.

It is generally thought that the mammalian Y chromosome is the bearer of a male sex determining locus[1,2]. It is reasonable to conclude that this putative locus is present in the piece of Y chromosome detected in XX male patients. In case 3 only a single Y specific fragment has been detected. This suggests that only a small part of the Y chromosome is present and that this part is sufficient for controlling essential steps of the male gonadal sex determination.

This study represents the first unambiguous molecular evidence for the presence of Y DNA material in some human XX male genomes. Furthermore, it also proves that only a part of this chromosome is present and this is probably the reason for the absence of its detection by cytogenetical means. These results can be related to the recent explanation of the sex reversal mutation in mouse[9,10]. However, it should be pointed out that in man this syndrome is mainly sporadic, whereas in mouse, the *Sxr* mutation is genetically transmissible by affected XY males[11]. In the aetiology of human XX maleness, the Y chromosome is mainly involved in two hypotheses: translocation of a piece of Y chromosome and hidden mosaïcism. Since the number of mitoses usually examined in karyotyping studies is relatively important, the cytogenetical approaches would certainly remain more sensitive for the detection of a mosaïcism.

The form in which this Y chromosome DNA is present in XX males is at present unknown. The most probable explanation involves a translocation event and as reported elsewhere, the X chromosome is the most likely candidate[12–14]. X–Y interchange can even be suspected in case 3 where an anomalous segregation of the Xg blood marker has been observed (M. Fellous, unpublished results).

One patient (case 2) remained negative in this survey, it is not known if this is related to the fact that this case displayed the most severe hypogonadism. Since it is very difficult to know if all the regions of the Y chromosome are covered by our probe collection, presence of Y material cannot therefore be excluded in negative cases for the moment. In the near future, it should be possible, with the availability of more Y-specific probes, to discriminate unambiguously between putative true XX males and XX males bearing Y DNA material.

We thank Professor P. Tiollais for his support, C. Johnsson and A. Abadie for technical assistance, F. Latron for the karyotyping and H-Y studies; DNA samples and DBM blots have been shared with J. L. Mandel whose help is gratefully acknowledged; we are also very grateful to Professor Roy and Dr Caporal for their help and collaboration; C.E.B. was in receipt of a Joliot-Curie grant. This work was supported by INSERM grant PRC 135012 and CRL 814030, DGRST 870346 and by the Fondation de la Recherche Médicale.

Table 2 Male specific bands in the genome of XX males

Probe	Normal male	XX males 1	3	4
47c	2(T)	2	2	2
50f2	4(T)	2	—	2
52d	3(T)	2	—	2
48	1(H)	1	—	1
118	8(T)	4	—	4
27	1(T)	1	—	—
64a7	1(E)	—	—	—
37c	1(E)	—	—	—
49f	3(T)	—	—	—
12f3	1(T)	—	—	—
Male repetitive* probe	1(E)	—	—	—

DNA, genomic blots and hybridization procedures are as depicted in the legend of figure 1. The results refer to the number of male specific bands present on a *Taq* (T), a *Hin*dIII (H) or an *Eco*RI (E) blot. Following hybridization, a low stringency of washing was used (see Fig. 1) except for probes 64a7, 37c, 49f, and the repetitive probe which were hybridized under high stringency (0.1×SSC instead of 2×SSC).

* Repetitive probe refers to male specific 3.4 kb *Hae*III fragment[16].

Received 15 August; accepted 19 October 1983.

1. Haseltine, F. P. & Ohno, S. *Science* **211,** 1272–1278 (1981).
2. Gordon, J. W. & Ruddle, F. H. *Science* **211,** 1265–1271 (1981).
3. Polani, P. E. in *Mechanisms of Sex Differentiation in Animals and Man* (eds Austin, C. R. & Edwards, R. G.) 465–529 (Academic, New York, 1981).
4. de la Chappelle, A. *Hum. Genet.* **58,** 105–116 (1981).
5. Wolf, U. *Hum. Genet.* **58,** 25–28 (1981).
6. Bishop, C. E. *et al. Nature* **303,** 831–832 (1983).
7. Bishop, C. E., Guellaen, G., Geldwerth, D., Fellous, M. and Weissenbach, J. *J. mol. Biol.* (in the press).
8. Alwine, J. C., Kemp, D. J. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5350–5354 (1977).
9. Singh, L. & Jones, K. W. *Cell* **28,** 205–216 (1982).
10. Evans, E. P., Burtenshaw, M. D. & Cattanach, B. M. *Nature* **300,** 443–445 (1982).
11. Cattanach, B. M., Evans, E. P., Burtenshaw, M. D. & Barlow, J. *Nature* **300,** 445–446 (1982).
12. Ferguson-Smith, M. A. *Lancet* **11,** 475–476 (1966).
13. Evans, H. J., Buckton, K. E., Spowart, G. & Carothers, A. D. *Hum. Genet.* **49,** 11–31 (1979).
14. Magenis, R. E. *et al. Hum. Genet.* **62,** 271–276 (1982).
15. Casanova-Bettane, M., Latron, F., Jakob, H. & Fellous, M. *Hum. Genet.* **58,** 21–24 (1981).
16. Cooke, H. *Nature* **262,** 182–186 (1976).

The establishment and maintenance of pregnancy using *in vitro* fertilization and embryo donation in a patient with primary ovarian failure

Peter Lutjen, Alan Trounson, John Leeton, Jock Findlay*, Carl Wood & Peter Renou

Department of Obstetrics and Gynaecology, Monash University, Queen Victoria Medical Centre and *Medical Research Centre, Prince Henry's Hospital, Melbourne, Australia 3000

Ovarian steroid replacement therapy in the ovariectomized ewe, given in the correct sequence to mimic endogenous steroid changes in the normal ovulatory cycle, allows the development of embryos transferred *in utero*[1-3]. A similar type of sequential therapy was designed for steroid replacement in women with primary ovarian failure. This produces the histological changes in uterine endometrial morphology and plasma oestradiol and progesterone similar to those observed in the normal ovulatory cycle[4,5]. We now report that in one of these women a donated oocyte[6], fertilized by her husband's spermatozoa[7] and cultured to the two-cell stage *in vitro*, was transferred *in utero*, resulting in a normal pregnancy and the delivery of a healthy child. Oestrogen therapy was withdrawn at 12 weeks and progesterone at 19 weeks gestation. This technique allows the treatment of human infertility due to primary ovarian failure.

The recipient was a 25-year-old married woman complaining of 5 years of amenorrhoea following withdrawal from contraception. Endocrine investigations throughout this period had shown continually depressed urinary oestradiol excretion on 24 separate occasions and continually elevated plasma gonadotropins in the postmenopausal range (follicle stimulating hormone, luteinizing hormone > 30 IU l^{-1}), indicative of primary ovarian failure. Treatment with both human chorionic gonadotropin (β-HCG, 3,000 IU per day for 3 days) and a combination of ethinyloestradiol and progesterone failed to elicit any detectable ovarian response in terms of oestradiol production. The patient presented with characteristic 'hot flushes' and vaginal dryness, indicative of her hypo-oestrogenic state. Secondary sexual development appeared normal and her karyotype (G banded) was normal (46 XX). Both thyroid and adrenal functions were assessed and were normal. Diagnostic laparoscopy at 20 years of age revealed marked ovarian atrophy and complete absence of follicular development. An ovarian biopsy showed an absence of primordial follicles. At 25 years of age the patient commenced ovarian steriod replacement with the intention of receiving a donated embryo.

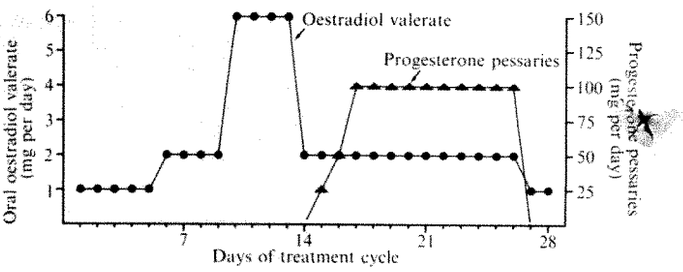


Fig. 1 Dose regime for the cycle replacement of oestrogen and progesterone. Oestradiol valerate was taken orally at 24-h intervals, at doses of 1 and 2 mg per day (days 1–9 and 14–28) or at 8-h intervals at a dose of 6 mg per day (days 10–13). Progesterone was administered as a single 25 or 50 mg intravaginal pessary once a day (days 15–16) or two pessaries introduced separately at 12-h intervals (days 17–26).

The regimen of ovarian steroid replacement with oral oestradiol valerate (Progynova, Schering) and intravaginal progesterone pessaries is given in Fig. 1. The treatment was formulated to mimic as closely as possible the ovarian steroid profile throughout a normal 28-day ovulatory cycle[5]. On the completion of the 28th day of steroid replacement, the patient recommenced therapy at day 1 and repeated this treatment in a cyclic manner.

Peripheral plasma oestradiol and progesterone levels were monitored during the third treatment cycle and on the 16th day a single two-cell embryo was transferred to the patient[8]. Oestrogen and progesterone replacement was continued, as shown in Figs 2 and 3. Following embryo transfer, progesterone pessaries were replaced by intramuscular progesterone injections (Proluton, Schering) for the remaining period of steroid therapy.

Using procedures described previously for embryo donation[6], a single oocyte was inseminated with spermatozoa prepared from a semen sample from the recipient's husband[7]. The donor of the oocyte was a 29-yr-old woman whose infertility was due to bilateral tubal blockage. Five oocytes were obtained from the donor during an *in vitro* fertilization treatment cycle and

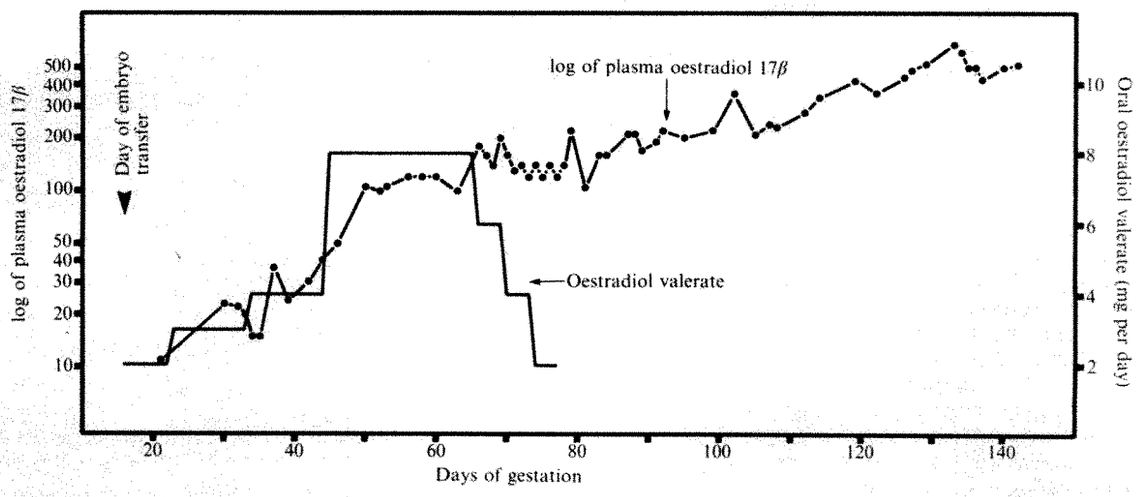


Fig. 2 Oestradiol valerate replacement therapy following embryo transfer and plasma oestradiol-17β levels (●) during pregnancy. Doses of 2 mg per day were taken at 24-h intervals, 3 and 6 mg at 8-h intervals, and 4 and 8 mg per day taken at 6-h intervals.

0028-0836/84/020174—02$01.00

© 1984 Macmillan Journals Ltd

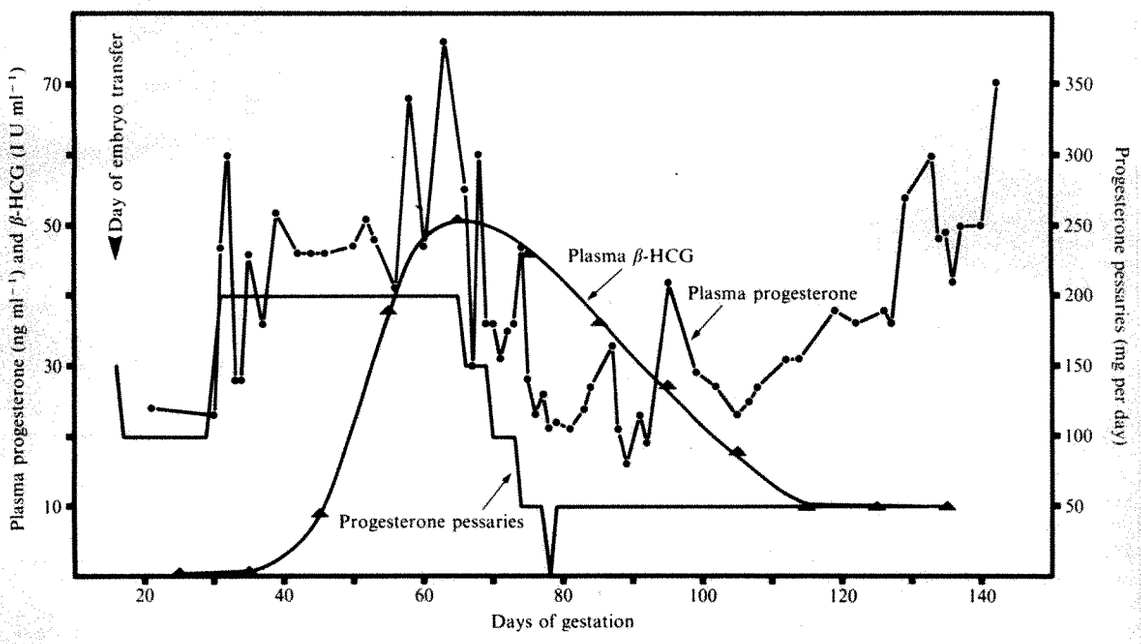


Fig. 3 Intramuscular progesterone (Proluton, Schering) replacement therapy following embryo transfer and plasma progesterone (●) and endogenous β-HCG (▲) levels during pregnancy. Doses of 50 mg per day were given at 24-h intervals, 100 mg per day at 12-h intervals, 150 mg per day of 8-h intervals and 200 mg per day at 6-h intervals.

she and her husband consented to donate one oocyte to the recipient couple. The donor failed to become pregnant on the cycle of IVF treatment after transfer of four of her own two-cell embryos *in utero*.

Pregnancy was confirmed in the recipient by normal changes in β-HCG (Fig. 3). The dosage of oestradiol valerate and progesterone administered was altered to keep the patient's peripheral plasma levels of these hormones in the normal range for pregnancy (Figs 2, 3). Ultrasonic reports on the fetus and placenta at 8, 12, 17 and 28 weeks of gestation were normal. Oestrogen therapy was withdrawn at 12 weeks' gestation. Progesterone therapy was unable to be completely withdrawn until 19 weeks because plasma progesterone levels declined to below the normal levels expected in pregnancy when withdrawal was attempted at 12 weeks.

The pregnancy continued normally until 38 weeks of gestation when a healthy baby boy was delivered by elective caesarean section. HLA-AB typing using peripheral blood lymphocytes in a standard microlymphocytotoxicity assay excluded the recipient as the biological mother and verified the association of the baby with the donor. The establishment and maintenance of pregnancy in the present report confirms our hypothesis[6] that in women with primary ovarian failure, progesterone and oestradiol can be used successfully as replacement therapy for the establishment and maintenance of pregnancy following transfer of donor embryos. In these women, there is no other treatment for their infertility. Prior to this successful case, we had transferred embryos to six patients, given similar or slightly modified steroid hormone treatments without success.

Shortly after the pregnancy was established, the Premier of the state of Victoria requested the cessation of all work involving donated oocytes to allow consideration of the social, ethical and legal issues of gamete donation in IVF by a state government appointed committee. The committee's recommendations in favour of continuing the work were reported in September 1983[9]. However, the state government has not revoked the moratorium and, as a consequence, we have not been able to continue the research programme and repeat the successful procedure described.

During the preparation of this paper a report was published on the establishment and maintenance of pregnancy in ovariectomized monkeys given ovarian steroid implants[10]. Future studies in this area need to determine whether sequential therapy designed to mimic normal endocrine levels in the ovulation cycle is essential for the establishment of pregnancy, as studies in sheep have demonstrated[1-3], or whether a simplified steroid therapy regimen may be used. The use of synthetic progestagens instead of progesterone would also simplify hormone replacement therapy for the patients. Pregnancy may be limited to the synchronous transfer of particular cleavage stage embryos on specific days of the treatment cycle. More flexibility of the timing of oocyte donation and embryo transfer would be achieved by embryo cryopreservation[11]. Legal and ethical difficulties encountered with embryo donation[6] will be satisfied if governments adopt recommendations such as those proposed in Victoria[9].

We thank all the members of the Monash-Epworth Infertility Unit and Department of Obstetrics and Gynaecology for helpful advice and assistance, and Dr Brian Tait of the Royal Melbourne Hospital for HLA typing. Progesterone and Proluton were provided by Schering Ltd.

Received 9 December; accepted 16 December 1983.

1. Trounson, A. O. & Moore, N. W. *Aust. J. biol. Sci.* **21**, 511–517 (1974).
2. Miller, B. G. & Moore, N. W. *Aust. J. biol. Sci.* **29**, 563–573 (1976).
3. Moore, N. W., Miller, B. G. & Trappl, M. N. *J. Reprod. Fert.* **68**, 129–135 (1983).
4. Conti, A. *et al. Proc. Aust. Soc. Reprod. Biol.* **14**, 116 (1982).
5. Lutjen, P. J. *et al. Proc. Endocrine Soc. Aust.* **26**, 112 (1983).
6. Trounson, A., Leeton, J., Besanko, M., Wood, C. & Conti, A. *Br. med. J.* **286**, 835–838 (1983).
7. Mahadevan, M. M., Trounson, A. O. & Leeton, J. F. *Fert. Steril.* **40**, 340–343 (1983).
8. Leeton, J., Trounson, A. O., Jessup, D. & Wood, C. *Fert. Steril.* **38**, 156–161 (1982).
9. *Law Reform Commission Report on Donor Gametes in IVF* (Victoria Law Offices, Melbourne, 1983).
10. Hodgen, G. D. *J. Am. med. Ass.* 2167–2171 (1983).
11. Trounson, A. & Moir, L. *Nature* **305**, 707–709 (1983).

Similar level of polyteny in bands and interbands of *Drosophila* giant chromosomes

Anne Spierer & Pierre Spierer

Department of Molecular Biology, University of Geneva, 30, quai Ernest-Ansermet, 1211 Geneva 4, Switzerland

The giant polytene chromosomes of *Drosophila melanogaster* have long been of interest to the geneticist because of the visible map of the genome provided by their characteristic banding patterns[1]. An issue in the understanding of the molecular basis of chromosome banding has been whether the chromosomal DNA is replicated to the same extent in bands and interbands. Although various suggestions have been put forward the point has remained controversial[2–5]. We have isolated 315 kilobases (kb) of contiguous *Drosophila* genomic DNA[6] which spans an interval of approximately 13 bands and interbands of the polytene chromosomes[7]. We report here the measurement of the relative level of DNA replication in polytene chromosomes of 84 adjacent restriction fragments of our cloned DNA. We conclude that there are no significant differences in the level of polyteny within the large band and between bands and interbands of this region. This result supports the 'folded fibre' model of polytene chromosome organization[8], rather than models involving disproportionate replication along the banding pattern[2–4].

The 315 kb of chromosomal DNA sequence that we have isolated includes 13 ± 1 chromomeric units (band and adjacent interband) of sizes ranging from about 3 to 160 kb, and 12 recessive lethal complementation groups[7]. To detect any local variation in polyteny we decided to measure the relative DNA replication level in polytene chromosomes of 84 fragments representing this interval by quantitative Southern blot hybridizations[9,10]. Two control experiments checked that this method can quantitate single copy sequences in genomic DNA. A reconstruction experiment demonstrated that the autoradiographic signal is indeed proportional to the relative abundance of a unique restriction fragment in a genomic background (Fig. 1). In a second control, the sensitivity of the method was tested *in vivo*, with a heterozygous deletion ending within the chromosomal walk. Single copy fragments within the deletion produced about half the signal that was seen with wild-type DNA or when compared with probes outside the deletion (Table 1). From these experiments and from tests of reproducibility (not shown), we felt confident that any local change in polyteny larger than twofold from the mean could be detected.

Table 1 Quantitative Southern blot hybridizations distinguish one dose from two doses of a single-copy sequence within genomic DNA

Probe[7]	Fragment size (kb)	Signal ratio (wt/ry^{614})	Mean of ratios
2189	3.1	0.42	0.46
	1.9	0.39	
	1.5	0.56	
2849	1.7	1.0	0.93
	1.4	0.78	
	1.1	1.0	

Wild-type chromosome: 2849 — 2189

Df(3R)ry^{614}/wt

2 μg of wild-type genomic DNA and 4 μg of DNA from the heterozygous deletion Df(3R)ry^{614} (refs 7 and 13) were digested with *Eco*RI, size-separated on agarose gels and transferred to nitrocellulose. Filters were hybridized to the recombinant 2189[6] or 2849[6]. The autoradiographic signals were measured by scanning densitometry. The position of the two probes relative to the deletion breakpoint is shown schematically under the table.

DNA was prepared from polytene tissue (salivary glands from third instar larvae) and from diploid tissues (embryos). Parallel tracks of equal amounts of 'polytene' and 'diploid' DNA digested with restriction enzymes were transferred to nitrocellulose paper and hybridized to a set of probes representing the walk. The signal could thus be expressed for each fragment as a 'polytene/diploid' ratio. A second calibration step was to hybridize all the filters to the same probe, to correct them for unequal loading or differences in transfer efficiency. This probe (2148)[6] was arbitrarily chosen as providing a polytene/diploid ratio of 1.0. The filters were then recycled and re-used with other probes to span the whole chromosomal walk. Figure 2 shows autoradiographs of eight filters hybridized to eight different segments of the walk and to the calibration segment. We also took the precaution not to quantitate fragments larger than 5 kb, for they are frequently under-represented in DNA preparations of salivary glands because of shearing (our unpublished observations). The complete results are shown diagrammatically in Fig. 3. Each of the 84 dots represents the level of replication of a restriction fragment and its position on the walk; an artist's view

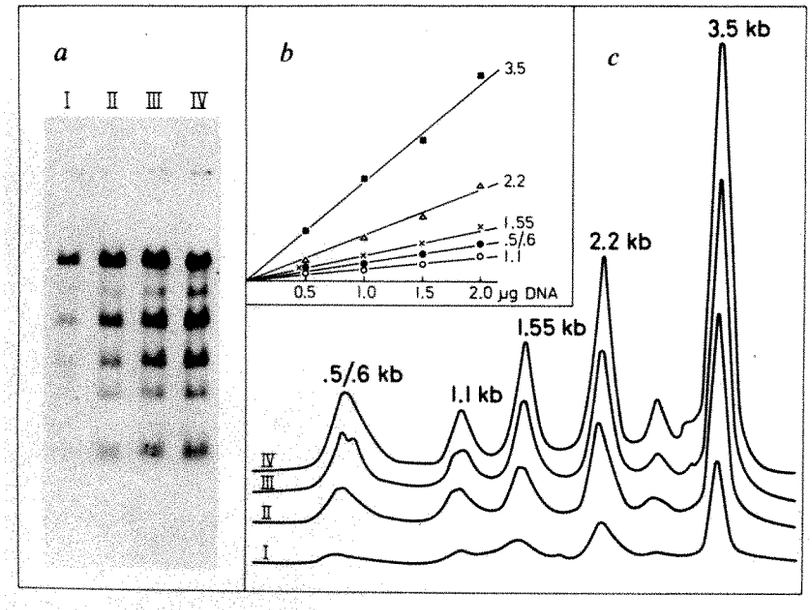


Fig. 1 Densitometry of autoradiographs of genomic Southern blot hybridizations does measure single copy restriction fragments. *a*, 0.5 μg (I), 1.0 μg (II), 1.5 μg (III) and 2.0 μg (IV) of *Drosophila* embryonic DNA brought to 2.0 μg with salmon sperm DNA were digested with *Eco*RI, size separated on 0.9% agarose gels and transferred to nitrocellulose. Filters were hybridized to the nick-translated recombinant 2160[6], which comprises only single copy sequences, and autoradiographed. *b*, Plot of the heights of peaks [cm] obtained by scanning microdensitometry (*c*) of autoradiographs in *a*, as a function of *Drosophila* DNA concentration.

0028-0836/84/020176—03$01.00

© 1984 Macmillan Journals Ltd

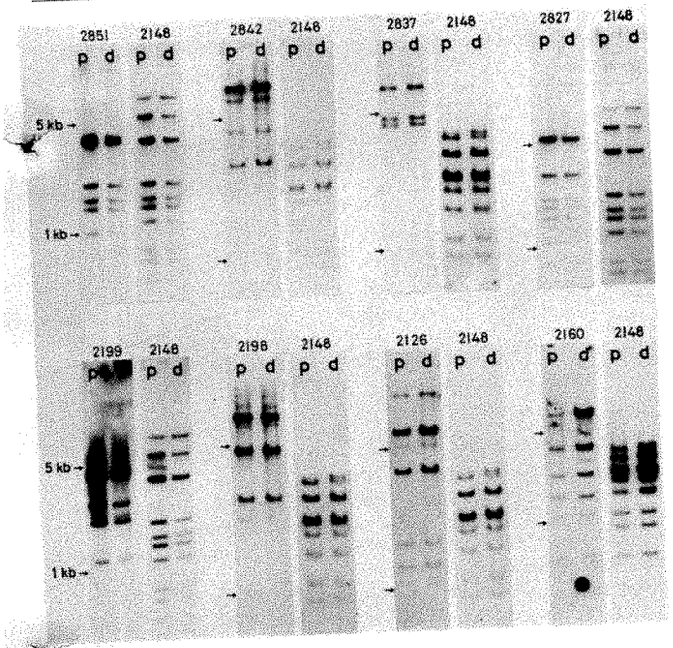


Fig. 2 Autoradiographs of genomic Southern blot hybridizations of polytene and diploid DNA with different segments of the walk. Polytene DNA was prepared from hand-dissected salivary glands of third instar larvae while diploid DNA was from embryos (0–16-h old). DNAs were digested with *Eco*RI (2842, 2837, 2198, 2126, 2160) or with *Bam*HI and *Hin*dIII (2851, 2827, 2199). Polytene (p) and diploid (d) DNAs were size-separated on parallel tracks of agarose gels and transferred to nitrocellulose. Filters were hybridized firstly to 2148[6] for calibration and then to other probes whose positions on the walk are indicated in Fig. 3. The arrows indicate the 5-kb size (top) and 1-kb size (bottom). For each pair of panels, the panel on the right shows the calibration hybridization with 2148[6] (equivalent to 2146 in Fig. 3); the panel on the left is the hybridization of the same filter to one of the segments of the walk. Filters were recycled by soaking in 0.1 M NaOH for 3 min at room temperature. The importance of this calibration is visible in the 2851 measurement: the signal is fainter in the diploid track to the same extent in the 2851 and in the 2148 hybridizations. Intensities and sizes of bands are as expected for unique sequences. Fainter bands are seen when the probe overlaps only partially with the fragment (5-kb and 11-kb fragments in 2126, for example) or in a few cases because of partial digests. Partial digestion, however, represents always less than 10% of the full digest (as seen on scans) and, hence, does not contribute significantly to the results. Heavier than expected bands are due to superposition of fragments of the same size (bands of about 4.5 kb in 2851, and of about 2.3 kb in 2148 digested by *Eco*RI for example).

of the polytene chromosome banding pattern is aligned with the DNA sequence map. The mean of the ratios for the 81 unique fragments is 0.97. The values range from 0.55 to 1.56 with a standard deviation of 0.25.

We see no local change in the level of polytenization, for no value goes beyond the 50% over or under-replication which we would consider significant. The large band 87E1,2 extends over 160 kb in the central part of the walk[7]. The relative abundance of about 35 fragments was measured in this interval, thus showing that there is no significant variation of level of DNA replication across this large band. The left part of the walk covers 9 ± 1 bands and interbands in 85 kb. The resolution at which we can place bands and interbands on the DNA map in this region does not allow us to tell whether particular restriction fragments are made of band or interband DNA[7]. However, cytological measurements of interband length led Beermann[5] to estimate that their average DNA sequence content is about 2 kb. Therefore, the 9 ± 1 interbands of the region spans about 18 out of the 85 kb of sequence and should be largely represented in the set of 26 fragments (mean size 2.6 kb) which were measured. We conclude that there is no large variation of polyteny among bands and interbands, and within a large band in this interval. Finally replication models in which bands and interbands have different levels of replication require that dormant replication forks are aligned at the band/interband boundaries. This should change the appearance of restriction fragments on genomic Southern blots. The genomic restriction pattern, however, was identical in polytene and diploid DNA all along the walk (not shown except the eight examples in Fig. 2).

Our study of a typical segment of the chromosome comprising bands of all sizes suggest that monotonous polyteny along the euchromatic arms of polytene chromosomes is a general feature. This is supported by the recent observation that a set of cloned sequences arising from different chromosomal locations have a similar level of replication in polytene chromosomes[11]. Chromosomal constrictions may, however, be the unavoidable exceptions to the rule. Lyfschitz[11] found that the cluster of histone genes close to or within the constriction at 39D is under-replicated. We have determined that the bithorax complex of homeotic genes[12] which spans the 89DE constriction is also under-replicated in polytene chromosomes (P.S., A.S. and W. Bender, unpublished results).

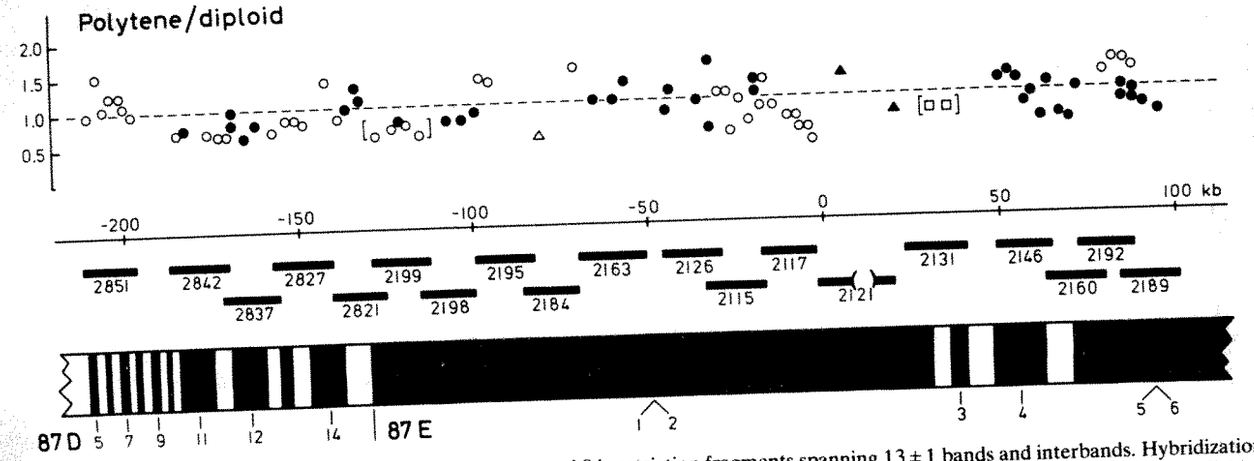


Fig. 3 Relative level of replication in polytene chromosomes of 84 restriction fragments spanning 13 ± 1 bands and interbands. Hybridizations were as in Fig. 2. The position of the probes is indicated with solid bars, and their numbers refer to ref. 6. Polytene/diploid ratios of individual restriction fragments are plotted as a function of their DNA map position. Calibration steps are explained in the text. The banding pattern is schematically aligned with the DNA map as described in ref. 7. ○, Fragments resulting from *Bam*HI and *Hin*dIII combined digestion; ●, fragments from *Eco*RI digestion; △, ▲, fragments larger than 5 kb, digested with *Bam*HI/*Hin*dIII or *Eco*RI respectively, when no smaller fragment could be used; □, fragments from *Bam*HI/*Hin*dIII which may contain moderately repeated sequences (not shown). Order of fragments in parentheses has not been determined. When two fragments co-migrated in gels, they were plotted at their respective positions on the walk with a ratio equal to the ratio of the sum of the signals.

We thank Professor Alfred Tissières for his interest and support, and we acknowledge the help of Mrs M. Vollenweider and Mrs C. H. Tonka in the task of hand-dissecting salivary glands. This work was supported by a grant from the Swiss National Science Foundation.

Received 17 June; accepted 19 October 1983.

1. Painter, T. S. *Genetics* **19**, 179–188 (1934).
2. Sorsa, V. *Hereditas* **78**, 298–302 (1974).
3. Laird, C. D. *Cell* **22**, 869–874 (1980).
4. Laird, C. D., Wilkinson, L., Johnson, D. & Sandström, C. *Chromosomes Today* Vol. 7 (eds Bennet, M., Bobrov, M. & Hewitt, G.) 74–83 (George, Allen & Unwin, Hemel Hempstead, 1981).
5. Beermann, W. (ed.) *Results and Problems in Cell Differentiation* Vol. 4, 1–33 (Springer, New York, 1972).
6. Bender, W., Spierer, P. & Hogness, D. S. *J. molec. Biol.* **168**, 17–33 (1983).
7. Spierer, P., Spierer, A., Bender, W. & Hogness, D. S. *J. molec. Biol.* **168**, 35–50 (1983).
8. DuPraw, E. J. & Rae, P. M. M. *Nature* **212**, 598–600 (1966).
9. Lis, J. T., Prestidge, L. & Hogness, D. S. *Cell* **14**, 901–919 (1978).
10. Endow, S. A. & Glover, D. M. *Cell* **17**, 597–605 (1979).
11. Lyfschitz, E. *J. molec. Biol.* **164**, 17–34 (1983).
12. Bender, W. *et al. Science* **221**, 23–29 (1983).
13. Hilliker, A. J., Clark, S. H., Chovnick, A. & Gelbart, W. M. *Genetics* **95**, 95–110 (1980).

Human hepatitis B vaccine from recombinant yeast

William J. McAleer, Eugene B. Buynak, Robert Z. Maigetter, D. Eugene Wampler, William J. Miller & Maurice R. Hilleman*

Division of Virus and Cell Biology Research, Merck Institute for Therapeutic Research, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, USA

The worldwide importance of human hepatitis B virus infection and the toll it takes in chronic liver disease, cirrhosis and hepatocarcinoma, make it imperative that a vaccine be developed for worldwide application[1]. Human hepatitis B vaccines[2–6] are presently prepared using hepatitis B surface antigen (HBsAg) that is purified from the plasma of human carriers of hepatitis B virus infection. The preparation of hepatitis B vaccine from a human source is restricted by the available supply of infected human plasma and by the need to apply stringent processes that purify the antigen and render it free of infectious hepatitis B virus and other possible living agents that might be present in the plasma. Joint efforts between our laboratories and those of Drs W. Rutter and B. Hall led to the preparation of vectors carrying the DNA sequence[7,8] for HBsAg and antigen expression in the yeast *Saccharomyces cerevisiae*[9]. Here we describe the development of hepatitis B vaccine of yeast cell origin. HBsAg of subtype adw was produced in recombinant yeast cell culture, and the purified antigen in alum formulation stimulated production of antibody in mice, grivet monkeys and chimpanzees. Vaccinated chimpanzees were totally protected when challenged intravenously with either homologous or heterologous subtype adr and ayw virus of human serum source. This is the first example of a vaccine produced from recombinant cells which is effective against a human viral infection.

Several alternative approaches to a hepatitis B vaccine are being developed. HBsAg has been expressed by several transformed mammalian cell lines, such as the human hepatoma line, PLC/PRF/5 (refs 10, 11), simian virus 40-infected monkey kidney cells[12] and mouse L cells[13]. These sources are of some concern, however, because the cell lines may be neoplastic. Although HBsAg has been cloned in bacteria[7,14], expression was very weak. Other laboratories[15–19] have described the synthesis of oligopeptides that carry antigenic determinants of HBsAg but their potency in animals is low and much work will need to be done to potentiate antigenicity. Smith and collaborators[20] have described the construction of a recombinant vaccinia virus which expresses HBsAg and have proposed its use as a live attenuated vaccine; its antigenic potency has been demonstrated but whether such a vaccine would be safe and effective in man is still unknown.

Table 1 Antigenic potency in mice of HBsAg purified from yeast and from human plasma

Vaccine source	Antigen dose per injection (μg protein)	Anti-HbsAg response after vaccination No. positive/total	GMT
Human plasma (lot 799-2)	10	9/10	563
	2.5	10/10	2,235
	0.625	4/9	32
	0.156	0/10	4
ED_{50}	0.639		
Yeast (lot 81-4)	40	10/10	5,432
	10	10/10	3,400
	2.5	8/10	673
	0.625	8/10	967
ED_{50}	<0.625		

Groups of 10 5-week-old ICR/Ha mice propagated in our laboratories were given a single 1-ml injection intraperitoneally of serial fourfold dilutions of yeast or human plasma vaccine in alum diluent. The mice were bled individually and tested for serum antibody level 4 weeks later. Human plasma vaccine, lot 799-2, was prepared in these laboratories[2–4]. Yeast-derived vaccine, lot 81-4, was purified as described in Fig. 1 legend and adsorbed to alum. GMT, geometric mean titre, expressed in AUSAB units; ED_{50}, dose required to seroconvert 50% of the mice.

Valenzuela *et al.*[9] originally reported that yeast cells are able not only to express the HBsAg gene but also to assemble the polypeptides into particles that have much the same appearance as particles isolated from human plasma and which are immunogenic in mice. Since then, other laboratories[21,22] have shown that HBsAg produced in yeast is antigenic in rabbits and guinea pigs. With such progress, recombinant yeast has become an attractive alternative to human plasma as a source of antigen for hepatitis B vaccine.

For vaccine preparation, the HBsAg used was of subtype adw and was produced in fermentation cultures of *S. cerevisiae* carrying an expression vector using yeast alcohol dehydrogenase I as a promoter. The yeast strain used in these studies was obtained from G. Ammerer (University of Washington) and is similar to the strain described by Valenzuela *et al.*[9] in which the production of HBsAg in yeast was first reported.

Cells were collected by centrifugation and broken by homogenization with glass beads[23]. HBsAg particles were purified from the clarified extract by immune affinity chromatography using goat antibody to human HBsAg. Electron microscopy (Fig. 1) revealed a homogeneous array of particles free of extraneous morphological entities. The UV absorption pattern was the same as for the plasma antigen, with an $E^{1\%}$ of 45. SDS-polyacrylamide gel electrophoresis (Fig. 2) in reducing conditions revealed a major band at molecular weight 23,000 (23K) corresponding to the non-glycosylated polypeptide which is the major polypeptide of the viral envelope. In this respect it differs from the plasma antigen which has, in addition to the 23K polypeptide, a glycosylated derivative which migrates at 27K. The yeast and plasma antigens differ also in their reactivity in the radioimmunoassay (RIA) (AUSRIA II, Abbott). RIA reactivity of purified yeast-derived HBsAg varied from preparation to preparation in the range 20–50% of the reference human antigen.

Because of this reduced radioimmune reactivity, and because the yeast antigen is not glycosylated, it was important to determine whether the antigen was immunogenic. To test both antigenicity and immunogenicity in animals, purified antigen was formulated into a vaccine by adsorbing on alum adjuvant to contain 40 μg HBsAg protein and 0.5 mg aluminium (hydroxide) per 1 ml dose.

* To whom reprint requests should be addressed.

0028–0836/84/020178–03$01.00

Table 2 Antigenic potency in grivet monkeys of HBsAg purified from yeast and from human plasma

Vaccine source	Antigen dose per injection (μg protein)	Anti-HBsAg response after initial vaccine dose (geometric mean titre) Week 4	Week 8	Week 12	Week 52
Human plasma (lot 86016)	10	36	213	170	127
	2.5	343	6,227	17,348	9.924
	0.625	53	4,642	3,164	5,688
	0.156	15	128	83	358
Yeast (lot 81-4)	40	88	1,078	7,103	11,554
	10	184	877	8,489	4,984
	2.5	225	1,168	6,361	10,868
	0.625	109	925	518	313

A group of four initially seronegative grivet monkeys (*Cercopithecus aethiops*), weighing 3–5 kg, were each given two 1-ml intramuscular (i.m.) doses of yeast or human plasma vaccine 4 weeks apart. Dilutions of antigen were made in alum placebo of the same composition as the vaccine. Animals were bled at biweekly intervals for 1 yr and tested for antibody to HBsAg by using a commercial RIA kit (AUSAB, Abbott). Protein was measured by the method of Lowry[29]. Human plasma lot 86016 was prepared in these laboratories[2–4].

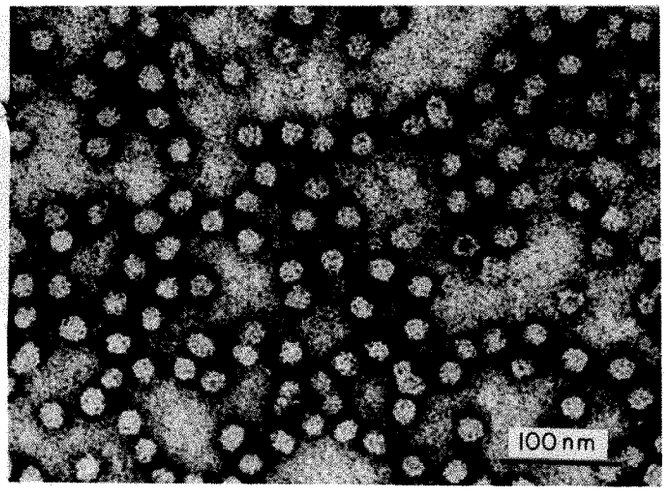


Fig. 1 Electron micrograph of HBsAg particles from recombinant yeast. Cells were grown in a 335-l fermentation vessel, collected by centrifugation, resuspended in an equal volume of 0.01 M sodium phosphate *p*H 7.5, containing 0.01% Triton X-100, and disrupted by rapid stirring with glass beads in a Dyno-Mill (Impandex; see ref. 23). The resulting extract was clarified by centrifugation for 90 min at 10,000*g*. The clarified yeast extract was applied to a column of Sepharose 4B to which had been attached goat antibody to human HBsAg. The column was developed at a flow rate of 2 column vol per h. Extraneous protein was washed away with 5 column vol of buffer A and the HBsAg was eluted with 3 M NH_4SCN. Fractions containing HBsAg were pooled and thiocyanate was removed by dialysis against 0.01 M sodium phosphate *p*H 6.8, containing 0.15 M NaCl. Dialysed antigen was diluted to 40 μg ml^{-1} and visualized by negative staining with 2% phosphotungstic acid.

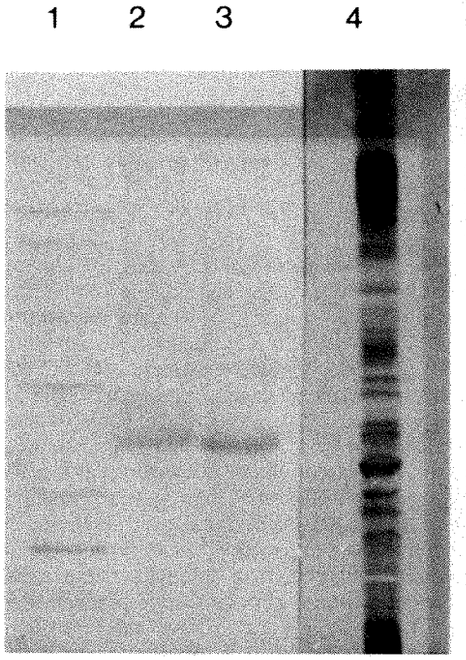


Fig. 2 SDS-polyacrylamide gel electrophoresis of cell culture and yeast-derived HBsAg. All samples were reduced, denatured and electrophoresed as described by Laemmli[30]. After electrophoresis, polypeptides were visualized with Coomassie brilliant blue (lanes 1–3) or with the silver stain procedure described by Morrissey[31] (lane 4). Lane 1, molecular weight standards (3 μg each): phosphorylase *b* (94K), bovine serum albumin (68K), ovalbumin (43K), carbonic anhydrase (30K), soybean trypsin inhibitor (21K) and lysozyme (14.3K). Lane 2, 30 μg of HBsAg from the human hepatoma cell line PLC/PRF/5 (ref. 10), also purified from yeast as described in Fig. 1 legend. Lane 3, 30 μg of HBsAg purified from yeast as described in Fig. 1. Lane 4, 10 μg of clarified yeast extract as described in Fig. 1 legend.

Studies in mice (Table 1) showed the yeast-derived antigen to be at least as antigenic as the antigen purified from human plasma. Grivet monkeys also developed antibody following vaccination with the yeast-derived antigen (Table 2). A single injection of the vaccine at all dose levels resulted in seroconversion of all the animals in both vaccine groups. These results were important as they showed that high antibody titres were maintained for at least a year.

Protective efficacy was tested for by using susceptible chimpanzees. The four chimpanzees that received the recombinant vaccine developed antibody in substantial titre following vaccination (Table 3). Following challenge with infectious human plasma, all four vaccinated animals were protected. By contrast, all four unvaccinated animals developed hepatitis B virus infection with positive antigenaemia, antibody to hepatitis B core antigen (anti-HBcAg), elevation of serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), and liver histopathology. It is important to note that the animals were protected against both subtype adr and ayw challenge even though the vaccine is of the adw subtype.

Yeast fermentation technology is well established and we have shown that HBsAg can be isolated from yeast extracts in a highly purified form by a single application of immune affinity chromatography. Vaccine made from this antigen is equally as potent as human plasma-derived vaccine in stimulating antibodies in mice, and is protective in challenge experiments in chimpanzees. Antibodies raised by yeast-derived vaccine persisted for at least a year in monkeys, showing no important deviation from that of the plasma vaccine.

Table 3 Protective efficacy in chimpanzees of HBsAg purified from yeast and from human plasma

Injection	Chimp no.	Before challenge: Anti-HBsAg titre (at 12 weeks)	Before challenge: Antigen subtype	After challenge (week of onset or weeks of duration): HBsAg Onset	HBsAg Duration	Anti-HBcAg Onset	Anti-HBcAg Duration	SGOT elevation Onset	SGOT elevation Duration	SGPT elevation Onset	SGPT elevation Duration	Liver pathology onset
Yeast vaccine (lot 81-4)	110	1,830	adr	–	–	–	–	–	–	–	–	–
	138	540	adr	–	–	–	–	–	–	–	–	–
	103	18,300	ayw	–	–	–	–	–	–	–	–	–
	120	7,200	ayw	–	–	–	–	–	–	–	–	–
Unvaccinated controls	111	<8	adr	10	10	15	9	17	3	17	6	20
	128	<8	adr	8	11	12	12	17	3	16	5	20
	127	<8	ayw	6	14	12	12	13	3	13	7	16
	130	<8	ayw	6	18	10	14	22	1	14	10	24

Eight chimpanzees, each weighing 40–60 kg, were selected for study based on negative findings in tests for HBsAg, anti-HBsAg, anti-HBcAg, elevation in transaminase, liver histopathology and tuberculin reaction. The animals were separated into two groups, four test animals and four controls. Each of the four test animals was given three 40-μg doses of yeast-derived HBsAg vaccine in 1 ml volume i.m. at 4-week intervals. All eight animals were then challenged by intravenous injection of 1,000 chimpanzee infectious doses of subtype adr or ayw virus in 1 ml of human hepatitis B plasma. Antigen and antibody titres were measured by commercial (Abbott) RIA kits (AUSRIA, AUSAB and CORAB for HBsAg, anti-HBsAg and anti-HBcAg, respectively). SGOT and SGPT assays were performed by the Sigma-Frankel (no. 505) and by the UV absorption (Boehringer-Mannheim) procedures, respectively. SGOT titres >40 and SGPT titres >30 were considered elevated. The subtype adr and ayw human plasmas used for challenge were obtained from Drs R. Gerety and E. Tabor of the Office of Biologics, US Food and Drug Administration; they were of measured viral infectiousness for chimpanzees and were subtyped serologically. The animals were bled at weekly intervals during the 36-week period of observation, covering 12 weeks before virus challenge and 24 weeks after. Liver biopsies were taken at 4-week intervals using a Menghini 16T needle. The tissues were fixed in 10% buffered formalin solution and the haematoxylin/eosin-stained sections were prepared by Dr A. Phelps of these laboratories under blind code number. The tests were carried out in animals that were held in isolation in the facilities of Dr William E. Greer at the Gulf South Research Institute, New Iberia, Louisiana. –, All remained negative.

Human HBsAg is composed of a sequence of 226 amino acids of which the a antigen determinant is dominant. Small differences in amino acid sequence may occur at several positions in the polypeptide chain and are responsible for the subtype specificities[24]. In previous studies, chimpanzees that were cross-challenged with heterologous subtypes of hepatitis B virus after recovery from infection or vaccination with human plasma-derived antigens, were solidly protected due to the common group specificity of the dominant a antigen that is present in all HBsAg subtypes[25]. A protective efficacy trial in man of subtype ad vaccine of human plasma origin has shown strong protection against the homologous subtype[26,27] and, most recently, against the heterologous subtype ay[28] in studies carried out on the staffs of renal dialysis centres where subtype ay hepatitis is most common. The positive cross-protection afforded against heterologous subtype ayw virus challenge in chimpanzee immunized with type adw vaccine of yeast origin, indicates that the a antigen remains dominant in the recombinant-produced antigen obtained from human plasma.

We thank Dr C. E. Carty and F. X. Kovach for assistance in fermentation, B. J. Harder, N. Grason and J. Bailey for assistance in antigen purification, Dr B. Wolanski and R. Ziegler for electron microscopy, and H. E. Darmofal, J. T. Deviney, K. I. Guckert, R. R. Roehm and L. W. Stanton for assistance in the animal tests.

Received 11 July; accepted 16 November 1983.

1. Deinhardt, F. & Gust, I. D. *Bull. Wld Hlth Org.* **60,** 661–691 (1982).
2. Hilleman, M. R. *et al. Viral Hepatitis* (eds Vyas, G. N., Cohen, S. N. & Schmid, R.) 525–537 (The Franklin Institute Press, Philadelphia, 1978).
3. Hilleman, M. R. *et al.* in *Viral Hepatitis 1981 int. Symp.* (eds Szmuness, W., Alter, H. & Maynard, J.) 385–397 (The Franklin Institute Press, Philadelphia, 1982).
4. Buynak, E. B. *et al. J. Am. med. Ass.* **235,** 2832–2834 (1976).
5. Adamowicz, P. *et al.* in *INSERM Symp.* No. 18 (eds Maupas, P. & Guesry, P.) 37–49 (Elsevier, Amsterdam, 1981).
6. Coutinho, R. A. *et al. Br. med. J.* **286,** 1305–1308 (1983).
7. Valenzuela, P. *et al. Nature* **280,** 815–819 (1979).
8. Edman, J. C., Hallewell, R. A., Valenzuela, P., Goodman, H. M. & Rutter, W. J. *Nature* **291,** 503–506 (1981).
9. Valenzuela, P., Medina, A., Rutter, W. J., Ammerer, G. & Hall, B. D. *Nature* **298,** 347–350 (1982).
10. Alexander, J. J., Bey, E. M., Geddes, E. W. & Lecatsas, G. *S. Afr. med. J.* **50,** 2124–2128 (1976).
11. Barin, F., Maupas, P., Coursaget, P., Goudeau, A. & Chiron, J. P. in *INSERM Symp.* No. 18 (eds Maupas, P. & Guesry, P.) 263–266 (Elsevier, Amsterdam, 1981).
12. Moriarty, A. M., Hoyer, B. H., Shih, J. W-K., Gerin, J. L. & Hamer, D. H. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2606–2610 (1981).
13. Dubois, M. F., Pourcel, C., Rousset, S., Chany, C. & Tiollais, P. *Proc. natn. Acad. Sci. U.S.A.* **77,** 4549–4553 (1980).
14. Burrell, J. C., Mackay, P., Greenway, P. J., Hofschneider, P. H. & Murray, K. *Nature* **279,** 43–47 (1979).
15. Lerner, R. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 3403–3407 (1981).
16. Dreesman, G. R. *et al. Nature* **295,** 158–160 (1982).
17. Bhatnagar, P. K. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 4400–4404 (1982).
18. Prince, A. M., Ikram, H. & Hopp, T. P. *Proc. natn. Acad. Sci. U.S.A.* **79,** 579–582 (1982).
19. Gerin, J. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 2365–2369 (1983).
20. Smith, G. L., Mackett, M. & Moss, B. *Nature* **302,** 490–495 (1983).
21. Miyanohara, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 1–5 (1983).
22. Hitzeman, R. A. *et al. Nucleic Acids Res.* **11,** 2745–2763 (1983).
23. Deters, D., Muller, U. & Homberger, H. *Analyt. Biochem.* **70,** 263–267 (1976).
24. Ono, Y. *et al. Nucleic Acids Res.* **11,** 1747–1757 (1983).
25. Gerety, R. J., Tabor, E., Purcell, R. H. & Tyeryar, F. J. *J. infect. Dis.* **140,** 642–648 (1979).
26. Szmuness, W., Stevens, C. E., Zang, E. A., Harley, E. J. & Kellner, A. *Hepatology* **1,** 377–385 (1981).
27. Francis, D. P. *et al. Ann. intern. Med.* **97,** 362–366 (1982).
28. Szmuness, W. *et al. New Engl. J. Med.* **307,** 1481–1486 (1982).
29. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *biol. Chem.* **193,** 265–275 (1951).
30. Laemmli, U. K. *Nature* **227,** 680–685 (1970).
31. Morrissey, J. H. *Analyt. Biochem.* **117,** 307–310 (1981).

Antisera to a synthetic peptide of the *sis* viral oncogene product recognize human platelet-derived growth factor

Henry L. Niman

Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California 92037, USA

It has recently been reported that the sequences of the *sis* oncogene of simian sarcoma virus (SSV) and of human platelet-derived growth factor (PDGF) are very similar[1,2], establishing the most solid link yet between the mitogenic actions of growth factors and the transforming proteins of retroviruses. To investigate molecular mechanisms of transformation I have produced antisera against synthetic peptides corresponding to segments of the protein sequences predicted by the nucleotide sequences of viral oncogenes. Applying this approach to the case of *sis* and PDGF, I report here the results of probing outdated human platelets with an antiserum directed against a synthetic peptide representing residues 139–155 of the predicted sequence of the SSV transforming protein, p28^{sis} (ref. 3). I detected peptides of apparent molecular weights (MWs)

0028–0836/84/020180—04$01.00

© 1984 Macmillan Journals Ltd

30,000 to 31,000 (30–31K) and 16–18K, which correspond to the apparent molecular weights of nonreduced and reduced PDGF[4–8]. In addition, a peptide of MW 21,000 was detected in platelets and a protein of MW 56,000 was detected in SSV-infected marmoset cells.

The *sis* peptide used in this study represents the predicted amino acid residues 139–155 of p28^{sis} (Table 1). If the sequences of PDGF-1 and -2 are colinear with p28^{sis}, these residues would correspond to positions 67–83 and 73–89 of PDGF-1 and -2, respectively. The amino acid sequence of residues 73–80 of PDGF-2 has been determined[1] and all eight amino acids are identical to the first eight amino acids of the synthetic peptide. In addition, the sequence of PDGF corresponding to residues 146–155 of p28^{sis} has been determined[2] and of the nine amino acids which have been identified, all are identical to the corresponding residues in the synthetic peptide. Thus, of the 17 synthesized amino acids which represent the predicted sequence of residues 139–155 of p28^{sis}, 16 have been identified in PDGF and all are identical.

The activities of two sequence-specific antisera are shown in Fig. 1. These antisera were prepared by immunizing mice with synthetic peptides coupled to keyhole limpet haemocyanin (KLH). The anti-*sis* antiserum was made against the peptide shown in Table 1. The anti-*myb* serum was made against a peptide representing residues 2–18 of the predicted sequence of the transforming protein of avian myeloblastosis virus[9]. The anti-*sis* serum reacts with the *sis* synthetic peptide and KLH but not with the *myb* peptide. The anti-*myb* antiserum reacts with the *myb* peptide and KLH but not the *sis* peptide. To test these sera for activity with cognate structures in intact molecules, purified PDGF (given by R. Ross) and an extract of marmoset cells nonproductively infected with SSV (NPV-SSV) were probed with the *sis* and *myb* antisera. Probing the purified PDGF with the anti-*sis* serum readily detected a doublet of MW 16–18K (Fig. 2, lane 1) corresponding to the major reduced forms of PDGF-1 and -2 (refs 4–8). In addition, a major band of 56K was specifically detected by the anti-*sis* serum when probing the cell extract (Fig. 2, lane 2). These activities were blocked by the *sis* conjugate (data not shown). Overexposure of the film also revealed a minor band of 28K (data not shown). The 28K protein corresponds to the predicted size of the SSV-coded transforming protein[3,10] while the 56K protein is the predicted size of the dimer. Although the assay conditions readily reduced the purified PDGF, the major band detected by the anti-*sis* serum in the cell extract was resistant to reduction.

To analyse the size of PDGF in platelets, an extract made from outdated platelets was probed with the anti-*sis* and anti-*myb* antisera. The platelets were treated as described for the first two steps of PDGF purification[4]. This extract was electrophoresed into a polyacrylamide gel and then transferred to nitrocellulose[11]. The extract was then probed with the anti-*sis*

Table 1 Location of the *sis* synthetic peptide

p28^{sis}	1	MTLTWQGDPIPEELYKMLSGHSIRSFDDLQRLLQGDSGKE
p28^{sis}	41	DGAELDLNMTRSHSGGELESLARGKRSLGSLSVAEPAMIA
PDGF-2†	1	SLGSLTIAEPAMIA
PDGF-2†	1	SLGSLTIAEPAMIA
PDGF-1*	1	SIEEAVPA
PDGF-1†	1	SIEEAVPA
p28^{sis}	81	ECKTRTEVFEISRRLIDRTNANFLVWPPCVEVQRCSGCCN
PDGF-2*	15	ECKTREEVFCICRRL?DR?????????PPCVEVKRCTGCCN
PDGF-2†	15	ECKTRTEVFEISRRLID?TTANFLVWPPCVEVQRCSGCCN
PDGF-1*	9	VCKTRIVIYEISRRELD???ANFL
PDGF-1†	9	VCKTRTVIYEIPRSQVDPTSANFLVWPPCVEVQRCSGCCN
p28^{sis}	121	NRNVQCRPTQVQLRPVQVRKIEIVRKKPIFKKATVTLEDH
PDGF-2*	55	NRNVKCRPSQVQLRP?QVRKIEIVRK
PDGF-2†	55	NRNVQCRPTQVQLRPVQVRKIE???KKPIFKKA?V?LEDH
PDGF-1†	49	NRNVQCRPTQVQL?PVQ????????KKPIFKKA?V?LEDH
p28^{sis}	161	LACKCEIVAAARAVTRSPGTSQEQRAKTTQSRVTIRTVRV
PDGF-2†	95	LACKC?IVAAA
PDGF-1†	89	LACKC?IVAAA
p28^{sis}	201	RRPPKGKHRKCKHTHDKTALKETLGA

The sequences of p28^{sis}, PDGF-1 and PDGF-2 are from refs 3, 1 and 2, respectively. The presentation of the ref. 2 sequence assumes peptide III originates from PDGF-2, peptide IV originates from PDGF-1, and peptide V originates from both PDGF-1 and PDGF-2. Residues in p28^{sis} that are not identical in all of the sequences determined are underlined. The peptide used in this study is boxed.

* Ref. 1; † ref. 2.

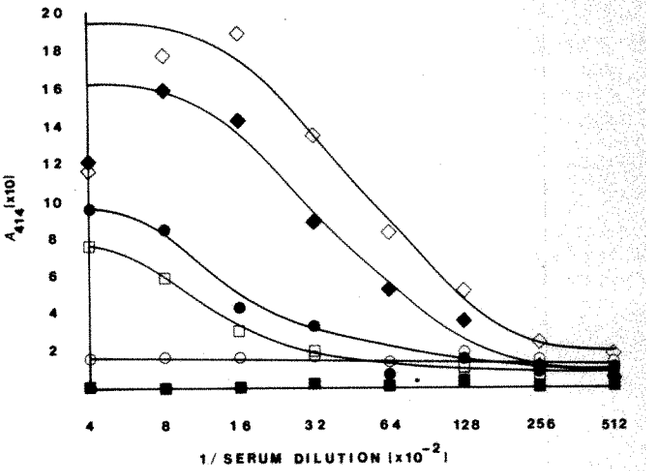


Fig. 1 Immunoreactivities of *sis* and *myb* sequence-specific antisera. The anti-*sis* and anti-*myb* antisera are represented by closed and open symbols, respectively. The antigens are the *sis* peptide (○, ●); the *myb* peptide (□, ■); and KLH (◇, ◆).
Methods: Peptides representing residues 139–155 of the predicted sequence of the SSV transforming protein p28^{sis} (ref. 3) or residues 2–18 of the predicted sequence of the avian myeloblastosis virus[9] were synthesized using solid state methods as described elsewhere[31]. A cysteine was added to the carboxyl end of each sequence for coupling purposes. The amino acid composition of each peptide was determined. For immunization, the peptides were coupled to KLH as described elsewhere[32]. The peptide conjugates were administered at ~50 μg per mouse per injection. On day 0, each conjugate was mixed with an equal volume of Freund's complete adjuvant and injected intraperitoneally (i.p.). On day 19 each conjugate was mixed with a final concentration of 5 mg ml^{-1} alum and injected i.p. For the *sis* conjugate, the final injection, in phosphate-buffered saline (PBS), was given intravenously (i.v.) on day 62 and plasma was taken from blood on day 67 by orbital puncture. For the *myb* conjugate a second alum injection was given on day 41; the final injection was given i.v. on day 143 and the plasma was collected on day 148. To test the specificity of the antisera, 250 ng of unconjugated peptide or 500 ng of KLH were dried onto the bottom of microtitre wells and fixed with methanol as described previously[33]. To prevent nonspecific adsorption, the wells were incubated at 37 °C for 4 h with 3% bovine serum albumin (BSA). 25 μl of serial twofold dilutions of mouse plasma, starting with a 1:400 dilution in tissue culture medium supplemented with 10% fetal calf serum, were incubated with peptide or KLH for 16 h at 25 °C. After washing 10 times with distilled H_2O, 25 μl of rabbit anti-mouse κ (Litton) diluted 1:500 with 1% BSA in PBS were added for 2 h at 37 °C. Then after washing 10 times with distilled H_2O, 25 μl of glucose oxidase-conjugated goat anti-rabbit IgG diluted 1:500 in 1% BSA in PBS were added for 1 h at 37 °C. The amount of glucose oxidase bound was determined by using 50 μl of 100 μg ml^{-1} ABTS dye (Boehringer-Mannheim) in the presence of 1.2% glucose, 10 μg ml^{-1} horseradish peroxidase in 0.1 M phosphate buffer, *p*H 6.0. The optical density at 414 nm was read in a Titertech microscanner.

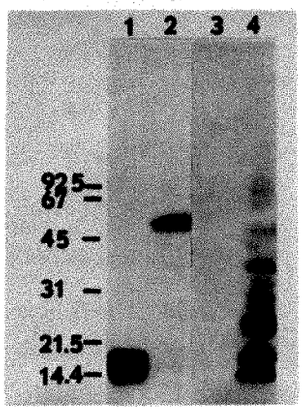


Fig. 2 Immunological detection of purified PDGF and SSV transforming protein on nitrocellulose filters. Approximately 700 ng of purified PDGF with 150 μg carrier BSA (lanes 1, 3) or a lysate of 10^6 SSV-infected cells prepared as described previously[12] (lanes 2, 4) were electrophoresed onto a 7.5–17% polyacrylamide gel and transferred to nitrocellulose as described[11]. After blocking nonspecific binding with 3% BSA and 0.1% Triton X-100 in PBS, 4 ml of mouse anti-*sis* or anti-*myb* sera diluted 1:100 with 3% BSA, 0.1% Triton X-100 were incubated for 16 h at 25 °C. After washing three times with 0.1% Triton X-100 in PBS, 10 ml of rabbit anti-mouse IgG1 (Litton) diluted 1:300 with 3% BSA, 0.1% Triton X-100 in PBS were incubated with the nitrocellulose for 1 h at 37 °C; after washing three times with 0.1% Triton X-100 in PBS, the strips were incubated with 10^7 c.p.m. of ^{125}I-protein A and washed with 0.1% Triton X-100 in PBS. The ^{125}I-labelled lanes were visualized with XRP-1 film (Kodak) and Cronex Hi-plus intensifying screen (Dupont) (−70 °C for 3.5 h). The anti-*sis* serum (lanes 1, 2) was obtained from a mouse immunized using the following protocol. On day 0 the *sis* peptide conjugate was given i.p. at ~50 μg in Freund's complete adjuvant. On days 15 and 31, 50 μg of the conjugate was mixed with 5 mg ml^{-1} alum and injected i.p. The plasma was isolated from blood taken on day 77 by orbital puncture. The anti-*myb* serum (lanes 3, 4) was obtained as described in Fig. 1 legend. The molecular weight standards were lysozyme, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, BSA and phosphorylase *b*. All of the bonds visualized in lane 4 with the anti-*myb* serum were also seen on longer exposures of lane 2 (probed with the anti-*sis* serum). Only a major band of 56K is seen specifically in lane 2. (Longer exposure revealed a specific 28K protein in lane 2; data not shown.)

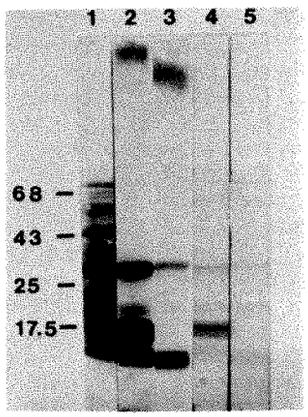


Fig. 3 Immunological detection of nonreduced and reduced PDGF on nitrocellulose filters. Lane 1 is total protein stained with amido-black. Lanes 2 and 3 were developed with ^{125}I; lanes 4 and 5 with dimethoxybenzidine. The platelet extract was probed with the anti-*sis* serum (lanes 2, 4) or the anti-*myb* serum (lanes 3, 5). The molecular weight standards were BSA, ovalbumin, chymotrypsinogen and β-lactoglobulin.

Methods: Using outdated human platelets (16 units; obtained from the San Diego Blood Bank), the first two steps of the PDGF purification were followed[4]. Platelets were collected by centrifugation at 28,000*g* for 20 min at 4 °C, then washed twice by resuspending them in 400 ml of 9-vol 17 mM Tris-HCl *p*H 7.4, 0.15 M NaCl, 0.1% glucose; 1 vol 0.8 g citric acid monohydrate, 2.2 g dextrose (anhydrous), 2.6 g sodium citrate (dihydrate) in 100 ml, and centrifuged at 28,000*g* for 10 min at 4 °C. The washed platelets were resuspended in 16 ml of 0.08 M NaCl, 0.01 M PO_4 *p*H 7.4 and boiled for 10 min. Phenylmethylsulphonyl fluoride and Trasylol were added to a final concentration of 1 mM and 3% respectively. The lysate was mixed with 8 ml CM Sephadex C-50 previously equilibrated with 0.08 M NaCl; 0.01 M PO_4 *p*H 7.4. The platelet pellet was extracted twice with 1 M NaCl for 24 h at 4 °C. After pelleting the insoluble material, the supernatant was dialysed against 0.08 M NaCl, 0.01 M PO_4 *p*H 7.4. PDGF extracted from the pellet was also mixed with 0.5 vol CM Sephadex C-50 equilibrated with 0.08 M NaCl, 0.01 M PO_4 *p*H 7.4. The beads were poured into a 15×1.5 cm column, and washed with 6 column vol of 0.08 M NaCl, 0.01 M PO_4. PDGF was eluted with two column vol of 1 M NaCl. Trasylol was added to a final concentration of 3%, then the eluate was dialysed against 0.08 M NaCl, 0.01 M PO_4 *p*H 7.4. All three extracts were pooled and concentrated with a 10K exclusion filter (Amicon). The extract from ~2.5 units of platelets was adjusted to 0.5% SDS, 5% 2-mercaptoethanol, boiled for 2 min and electrophoresed into a 5–17% polyacrylamide gel. The protein was electrophoretically transferred to nitrocellulose as described[11] and cut into strips. After blocking nonspecific binding with 3% BSA and 0.1% Triton X-100 in PBS, 4 ml of mouse plasma diluted 1:200 were incubated with the nitrocellulose strips. After washing three times with 0.1% Triton X-100 in PBS, the strips were incubated with either 10^7 c.p.m. ^{125}I-labelled protein A or a 1:1,000 dilution of peroxidase-conjugated goat anti-mouse serum (Tago), and washed with 0.1% Triton X-100 in PBS. The ^{125}I-labelled strips were visualized with XRP-1 film (Kodak) and Cronex Hi-plus intensifying screens (Dupont) (−70 °C for 48 h). The peroxidase conjugate was developed with 0.009% H_2O_2, 0.0025% 3,3′-dimethoxybenzidine dihydrochloride (Kodak), 10 mM Tris *p*H 7.4.

and anti-*myb* antisera (Fig. 3): lane 1 is a stain of the total protein bound to nitrocellulose. Although many proteins could be visualized with amido-black staining, PDGF was not present in sufficient quantities to be seen. Figure 3, lanes 2 and 3 show the results of probing with the anti-*sis* and anti-*myb* antisera. Bound antibody was detected with ^{125}I-labelled protein A. The specific bands detected with the anti-*sis* antisera migrated with an apparent MW of ~30K as well as 18–16K. In addition, a minor band of ~21K was detected. Visualization of antibody by benzidine staining[12] of peroxidase-conjugated rabbit anti-mouse IgG (Fig. 3, lanes 4, 5) produced similar results except that the higher molecular weight proteins were resolved as a major band of ~31K and a minor band of 30K. Similarly, the lower bands were resolved as major bands of ~18K and 16K, with protein of a more heterogeneous size migrating between these two bands. In addition, a minor band of ~21K was detected. The specificity of the anti-*sis* antiserum was further tested by preincubation with synthetic peptide conjugates. Incubation of anti-*sis* antiserum with the unrelated *myb* peptide conjugate (Fig. 3, lane 1) did not affect binding to the specific bands seen in Fig. 2, whereas incubation with the *sis* peptide conjugate eliminated detectable binding to all of the specific bands. Only a nonspecific binding (also detected by the anti-*myb* serum, lane 3) which co-migrated with the major protein in the extract was visualized. The mobilities of ~31K and 17K correspond to the unreduced and reduced forms of human PDGF[4–8]. The detection of the ~31 K and 21K bands indicates that the higher molecular weight forms of PDGF in platelets are resistant to reduction.

The data presented immunologically confirm the sequence homology between the SSV oncogene and human PDGF. The factor(s) responsible for the resistance of some of the PDGF and *sis* gene product to reduction is unknown. However, the mitogenic activity of PDGF is associated with the nonreduced forms[6,13,14]. Stimulation of tyrosine-specific kinase activity by PDGF represents the third example of phosphorylation associated with proteins of oncogenes. Members of the *ras* gene family bind GTP and autophosphorylate pp21 (ref. 15). Members of the oncogene family having sequence homology with the carboxyl portion of $pp60^{src}$ phosphorylate IgG *in vitro* and stimulate

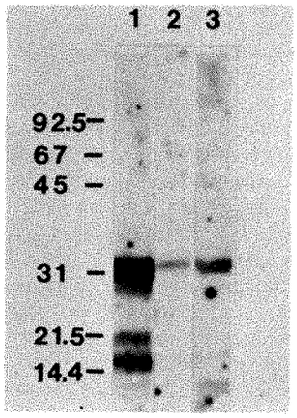


Fig. 4 Immunoreactivity with PDGF is blocked by the synthetic peptide. Platelets were extracted as described in Fig. 3 legend except that Trasylol was added to a final concentration of 1% after elution from the CM Sephadex C-50 column. The extract from ~2.5 units of platelets was adjusted to 0.5% SDS, 5% 2-mercaptoethonal, boiled for 5 min and electrophoresed into a 7.5–17% polyacrylamide gel. Transfer was as described in Fig. 3 legend. The plasma used in lanes 1 and 2 was taken from a mouse immunized as described in Fig. 2 legend except that there was no i.v. injection; instead, the third i.p. injection was in alum and given on day 215 and the plasma was collected on day 223 by orbital puncture. Lane 1, platelet extract probed with anti-*sis* antiserum preincubated with the *myb* conjugate (30 μg for 1 h at 37 °C). Lane 2, anti-*sis* antiserum preincubated with the *sis* conjugate (30 μg for 1 h at 37 °C). Lane 3, extract probed with the anti-*myb* serum. The molecular weight standards were lysozyme, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, BSA and phosphorylase *b*.

tyrosine phosphorylation in cell extracts[16–23]. Although p28^{sis} does not seem to autophosphorylate or label IgG *in vitro*[10], PDGF stimulation of tyrosine phosphorylation in cell extracts[24–27] suggests that phosphorylation reactions may be involved in numerous pathways important in cell proliferation and transformation.

These pathways can be further characterized using sequence-specific antibodies. This report describes a peptide that readily produces antibodies which recognize the synthetic peptide as well as the cognate structure in the intact molecule. Such peptides can be used for high-frequency isolation of hybridomas secreting useful monoclonal antibodies[12]; these antibodies can be used to map the active site of PDGF. Antibodies which do not block activity can be used to isolate PDGF receptors using immunoaffinity procedures. Monoclonal antibodies will be useful in the characterization of PDGF-related molecules and associated substrates (for example, tumour cells of mesenchymal origin have been shown to express RNA related to v-*sis*[28]). In addition, monoclonal antibodies against sequences unique to each of the PDGF peptides can facilitate their isolation and separation for additional sequencing. Apart from the use of sequence-specific monoclonal antibodies for the characterization of biochemical pathways used in cell proliferation and transformation, selected antibodies should have diagnostic and therapeutic value.

During the preparation of this manuscript, Robbins *et al.* reported[29] the detection of a 56K dimer of the *sis* transforming gene product and the immunological detection of this gene product by an antiserum directed against PDGF. Similarly, Deuel *et al.*[30] showed the reactivity of an anti-PDGF antiserum with a 20K protein in SSV-infected cells.

I thank R. Ross, D. Bowen-Pope and E. Raines for supplying PDGF, R. A. Lerner for discussions, R. A. Houghten for synthesizing and analysing peptides, the San Diego Blood Bank for supplying outdated platelets, R. Naso for supplying SSV-infected marmoset cells, and B. Heilman and D. Schloeder for technical assistance. This work was supported in part by NIH grant CA25803. This is publication no. 3162-MB from the Research Institute of Scripps Clinic.

Received 12 August; accepted 18 November 1983.

1. Doolittle, R. F. *et al. Science* **220,** 275–277 (1983).
2. Waterfield, M. D. *et al. Nature* **304,** 35–39 (1983).
3. Devare, S. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 731–735 (1983).
4. Antonides, H. N., Scher, C. D. & Stiles, C. D. *Proc. natn. Acad. Sci. U.S.A.* **76,** 1809–1813 (1979).
5. Heldin, C. H., Westermark, B. & Wasteson, A. *Biochem. J.* **193,** 907–913 (1981).
6. Antoniades, H. N. *Proc. natn. Acad. Sci. U.S.A.* **78,** 7314–7318 (1981).
7. Deuel, T. F. *et al. J. biol. Chem.* **256,** 8896–8899 (1981).
8. Johnson, A., Heldin, C. H., Westermark, B. & Watson, A. *Biochem. biophys. Res. Commun.* **104,** 66–74 (1982).
9. Rushlow, K. E. *et al. Science* **216,** 1421–1423 (1982).
10. Robbins, K. C., Devare, S. G., Reddy, E. P. & Aaronson, S. A. *Science* **218,** 1131–1133 (1982).
11. Niman, H. L. & Elder, J. H. *Virology* **123,** 187–205 (1982).
12. Niman, H. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 4949–4953 (1983).
13. Heldin, C.-H., Wasteson, A. & Westermark, B. *Expl Cell Res.* **109,** 429–437 (1977).
14. Raines, E. W. & Ross, R. *J. biol. Chem.* **257,** 5154–5160 (1982).
15. Shih, T. Y. *et al. Nature* **287,** 686–691 (1980).
16. Hunter, T. & Sefton, B. M. *Proc. natn. Acad. Sci. U.S.A.* **22,** 1311–1315 (1980).
17. Kawai, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **77,** 6199–6203 (1980).
18. Feldman, R. A., Hanafusa, T. & Hanafusa, H. *Cell* **22,** 757–765 (1980).
19. Witte, O. N., Dasgupta, A. & Baltimore, D. *Nature* **283,** 826–831 (1980).
20. Barbacid, M., Beemon, K. & Devare, S. G. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5158–5162 (1980).
21. Neel, J. C., Ghysdael, J. & Vogt, P. K. *Virology* **109,** 223–228 (1981).
22. Feldman, R. A., Wang, L. H., Hanafusa, H. & Balduzzi, P. C. *J. Virol.* **42,** 228–236 (1982).
23. Simon, M. A., Kornby, T. B. & Bishop, J. M. *Nature* **302,** 837–839 (1983).
24. Ek, B., Westermark, B., Wasteson, A. & Heldin, C. H. *Nature* **295,** 419–420 (1982).
25. Nishimura, J., Huang, J. S. & Deuel, T. F. *Proc. natn. Acad. Sci. U.S.A.* **79,** 4305–4307 (1982).
26. Ek, B. & Heldin, C. H. *J. biol. Chem.* **257,** 10486–10492 (1982).
27. Pike, L. K., Bowen-Pope, P. E., Ross, R. & Krebs, E. G. *J. biol. Chem.* **258,** 9383–9390 (1983).
28. Eva, A. *et al. Nature* **295,** 116–119 (1982).
29. Robbins, K. C., Antoniades, H. N., Devare, S. G., Hunkapiller, M. W. & Aaronson, S. A. *Nature* **305,** 605–608 (1983).
30. Deuel, T. F. *et al. Science* **221,** 1348–1350 (1983).
31. Bittle, J. L. *et al. Nature* **298,** 30–33 (1982).
32. Green, N. *et al. Cell* **28,** 477–487 (1982).
33. Niman, H. L. & Elder, J. H. in *Monoclonal Antibodies and T Cell Products* (ed. Katz, D. H.) 23–51 (CRC Press, Boca Raton, Florida, 1982).

Primary structure homology between the product of yeast cell division control gene *CDC28* and vertebrate oncogenes

Attila T. Lörincz & Steven I. Reed

Biochemistry and Molecular Biology Section, Department of Biological Sciences, University of California, Santa Barbara, California 93106, USA

In the budding yeast, *Saccharomyces cerevisiae*, division is controlled in response to nutrient limitation[1] and in preparation for conjugation[2]. Cells deprived of an essential nutrient or responding to mating pheromones cease division and become synchronous in the G_1 interval, apparently constrained from completing a critical event. This event has been given the operational designation of 'start'. We have isolated a large number of start mutations which confer on *S. cerevisiae* cells a conditional inability to complete start[3,4] (Fig. 1) presumably because they define genes which must be expressed for the start event to be successfully completed. We have described the isolation on plasmids of one of the start genes, *CDC28*, by genetic complementation[5] and initial characterization of its product[6,7]. We now describe the DNA sequence of the gene *CDC28*.

Comparison of the amino acid sequence encoded by *CDC28* with those of several members of the vertebrate protein kinase family, including a number of viral oncogenes, reveals a significant degree of sequence homology indicating that the latter may have arisen from genes that are essential for cell proliferation and more specifically, for the normal process of cell division control.

The dideoxy method of Sanger *et al.*[8] was used in conjunction with modified M13 phages[9] to sequence a 1.7-kilobase (kb) segment of DNA containing the *CDC28* gene (Fig. 2) and an open reading frame of 298 codons was revealed (Fig. 3). Preliminary nuclease protection experiments[10] indicate that the

© 1984 Macmillan Journals Ltd

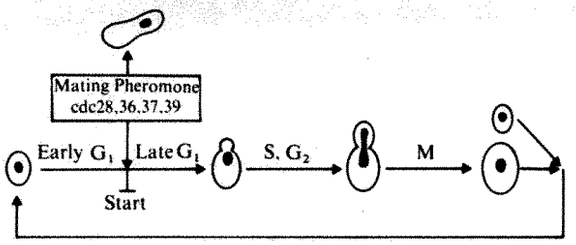


Fig. 1 The yeast cell cycle showing only the relevant details. Execution of start, the controlling event in the cell cycle, depends on both nutritional and pheromonal status. Four genes required for completion of start based on mutational analysis are *CDC28, CDC36, CDC37* and *CDC39* Mutations at these loci produce conditional arrest at start and morphological alterations which cause mutant cells to resemble those responding to mating pheromone. Execution of start signals commitment to completion of the cell division cycle. The cell cycle intervals G_1, S, G_2 and M are shown. The shaded body inside the cell represents the nucleus.

presumptive initiating codon (nucleotide position +1 in Fig. 3) was contained in the *CDC28* mRNA and that the gene contained no introns (data not shown).

The predicted primary structure of the *CDC28* product was subjected to a high resolution computer search which compared it with a large data base of known and predicted protein sequences[11]. As can be seen in Fig. 3, reasonably good homology is detected when the *CDC28* primary structure is compared to those of two distantly related members of the protein kinase family[12]. A 25% identity is obtained when the *CDC28* product is aligned with bovine cyclicAMP-dependent protein kinase[13] and a 22% identity is obtained with v-*mos*, the Moloney murine sarcoma virus oncogene[14]. Furthermore, when subjecting these alignments to the 'jumble test' described by Doolittle[11], the degree of homology was assessed as greater than 6.5 standard

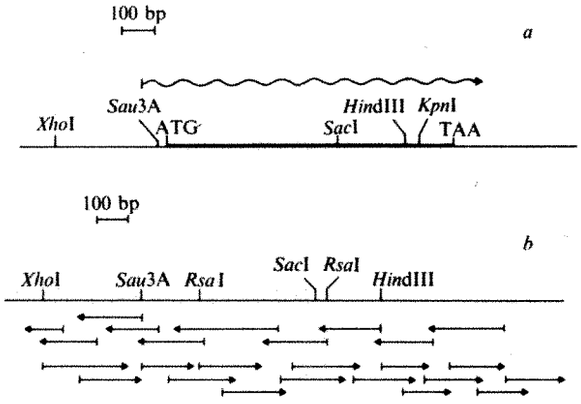


Fig. 2 Sequencing strategy for *CDC28. a*, A region of DNA which had previously been shown to contain the *CDC28* gene[6] with principal restriction sites. The *CDC28* mRNA (wavy line) was oriented[7] and end points determined by S_1 nuclease protection experiments[10]. The open reading frame found by sequence analysis begins approximately 75 nucleotides downstream from the 5′ terminus and ends 90 nucleotides upstream from the 3′ terminus (data not shown). *b*, The directions and limits of the sequencing runs below a map of the region which gives only the restriction sites used as sequencing end points. Part of the gene was sequenced by cloning restriction fragments and *Bal*31 deleted restriction fragments, mobilized using *Eco*RI linkers[7], into M13-derived sequencing vectors[8,9]. However, most of the gene was sequenced by a strategy where M13 clones were subjected to progressive *Bal*31 deletion and sequenced directly. Deletions were initiated at a centrally located unique restriction site and deletion end points were juxtaposed to the sequencing primer site by digestion with a proximal restriction site in the cloning linker segment[9] followed by religation. Deletion end points were aligned by means of restriction mapping so that specific phages could be chosen for sequencing. This approach has the advantages of being extremely rapid and efficient.

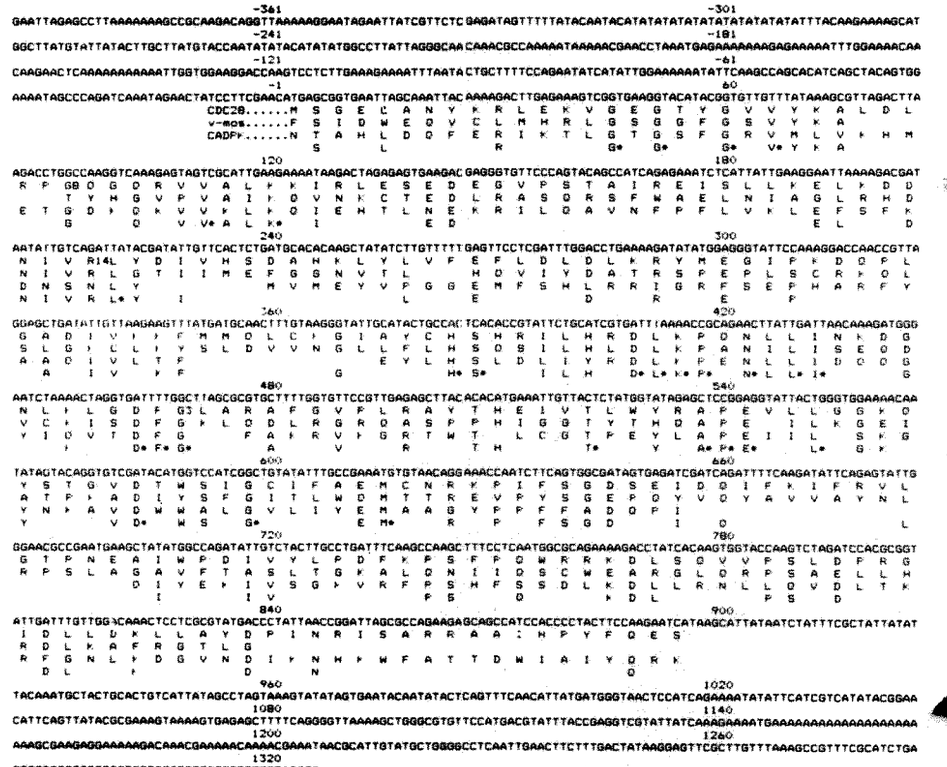


Fig. 3 The nucleotide sequence of the *CDC28* region of the yeast genome and the predicted primary structure of the *CDC28* product. The first nucleotide of the initiating codon (AUG) is given as position +1. Note that the consensus for nucleotides surrounding eukaryotic translational initiation codons is obeyed[18]. The predicted primary structure of the *CDC28* product has been aligned for maximal homology[11] with the predicted primary structure of the oncogene v-*mos* from Moloney murine sarcoma virus[14] (one line down) and the primary structure of cyclicAMP-dependent protein kinase (CADPK) from bovine cardiac muscle[13] (two lines down). This display does not necessarily constitute the maximal homology between the latter two proteins. Where gaps were necessary to obtain optimal alignment, they are indicated by leaving blank spaces (v-*mos* and CADPK sequences) or by inserting the symbol and the number of positions comprising the gap (*CDC28* product sequence). Amino acid homologies observed between the *CDC28* product and either one of the other proteins are indicated (three lines down). Where a homology exists for all three sequences, the amino acid is followed by an asterisk. Homologies observed between v-*mos* and CADPK are not indicated. In addition, both of these sequences have been truncated so that only the region of homology with *CDC28* is displayed. Other noteworthy features of the nucleotide sequence include a stretch of dAT 24 nucleotides long beginning at position −315 and a run of 22 consecutive adenines beginning at position 1146.

deviations above a mean derived by processing a large number of random sequences of identical composition and length via the same algorithm[11]. There is no doubt, then, of the relatedness of the *CDC28* product to these diverse members of the protein kinase family, and good reason to propose that the *CDC28* product is, in fact, a protein kinase itself.

Amino acids common to *CDC28* and either of the two aligned primary structures are indicated in Fig. 3. It is interesting that even though some positions are conserved among all three sequences, many of the relationships hold in one or other of the comparisons but not both. The simplest explanation is that all three sequences are derived from the same ancestral sequence with divergence having occurred early in eukaryotic evolution. Similar matches were obtained with a number of other related viral oncogene product sequences including: Rous sarcoma virus oncogene (*src*; 21% homology), feline sarcoma virus transforming gene (*fes*; 25.5% homology), avian Fujinami sarcoma virus transforming gene (*fps*; 24.5% homology), avian Y73 sarcoma virus transforming gene (*yes*; 25% homology), mouse virus 3611 oncogene (v-*raf*; 24% homology). Most of these have been demonstrated to encode active protein kinases. For a minority, of which v-*mos* is an example, membership in the protein kinase family has been inferred from sequence data, as it has not yet been possible to demonstrate enzymatic activity *in vitro*[15].

Does the sequence homology between the yeast cell cycle gene *CDC28* and viral oncogenes extend to any functional properties? Sequence comparisons between viral oncogenes and their cellular counterparts or proto-oncogenes suggest that the viral forms result from rare transduction events and that their improper expression in the infected host contributes to the transformed state[16] in which normal control of cell division has been lost. An analogous situation in the yeast system is a loss of division control in the fission yeast *Schizosaccharomyces pombe* when carrying the *S. cerevisiae CDC28* gene on a plasmid[17]. Moreover, the *CDC28* gene can complement conditional-lethal *cdc2* mutations in *S. pombe*[17] even though these two organisms are only distantly related and their mode of cell cycle control, at least superficially, dissimilar. Whether the explanation is that the *CDC28* product is unresponsive to normal *S. pombe* control signals or that the plasmid-associated abnormally high gene dosage overwhelms the regulatory capacity of the system, the behaviour of the *S. cerevisiae CDC28* gene in *S. pombe* strikes an interesting parallel with the expression of viral oncogenes in cells of higher eukaryotes and suggests that the gene products in the two yeast species have been highly conserved in the course of evolution. It is probable, then, that underlying division control mechanisms are conserved as well and that, in light of the protein kinase homology, phosphorylation of key target proteins may be involved.

We thank Dr Russell Doolittle for performing the sequence comparisons. This work was funded by NIH grant RO1 GM28005 and NSF grant PCM81-02308 to S.I.R. S.I.R. was supported in part by American Cancer Society Faculty Research Award FRA-248.

Received 24 August; accepted 24 October 1983.

1. Johnston, G. C., Pringle, J. R. & Hartwell, L. H. *Expl Cell Res.* **105,** 79–88 (1977).
2. Bucking-Throm, E., Duntze, W., Hartwell, L. H. & Manney, T. R. *Expl Cell Res.* **76,** 99–110 (1973).
3. Hartwell, L. H., Culotti, J., Pringle, J. & Reid, B. *Science* **183,** 46–51 (1974).
4. Reed, S. I. *Genetics* **95,** 561–577 (1980).
5. Nasmyth, K. A. & Reed. S. I. *Proc. natn. Acad. Sci. U.S.A.* **77,** 2119–2123 (1980).
6. Reed, S. I., Ferguson, J. & Groppe, J. C. *Molec. cell. Biol.* **2,** 412–425 (1982).
7. Reed, S. I. *Gene* **20,** 255–265 (1982).
8. Sanger, F., Nicklen, S. & Coulsen, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
9. Messing, J., Crea, R. & Seeburg, P. A. *Nucleic Acids Res.* **9,** 308–321 (1981).
10. Berk, A. J. & Sharp, P. A. *Cell* **12,** 721–732 (1977).
11. Doolittle, R. F. *Science* **214,** 149–159 (1981).
12. Barker, W. C. & Dayhoff, M. O. *Proc. natn. Acad. Sci. U.S.A.* **79,** 2836–2839 (1982).
13. Shoji, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 848–851 (1981).
14. Van Beveren, C. *et al. Nature* **289,** 258–262 (1981).
15. Papkoff, J., Nigg, E. A. & Hunter, T. *Cell* **33,** 161–172 (1983).
16. Duesberg, P. A. *Nature* **304,** 219–223 (1983).
17. Beach, D., Durkacz, B. & Nurse, P. *Nature* **300,** 706–709 (1982).
18. Kozak, M. *Microbiol. Rev.* **47,** 1–45 (1983).

Similarity of the *Cin1* repetitive family of *Zea mays* to eukaryotic transposable elements

Nancy S. Shepherd, Zsuzsanna Schwarz-Sommer, Jutta Blumberg vel Spalve, Manju Gupta, Udo Wienand & Heinz Saedler

Max-Planck-Institut für Züchtungsforschung, Egelspfad, D-5000 Köln 30, FRG

It has been suggested that the middle repetitive class of sequences that make up a large proportion of the eukaryotic genome have been amplified and dispersed by DNA transposition[1,2]. Transposition is a phenomenon first postulated by Barbara McClintock on the basis of her genetic analysis of mutants in *Zea mays*[3]. Since then, DNA transposition has been studied genetically in various plant systems[4–6] and is well documented on the molecular level in both prokaryotes and eukaryotes[7]. This has included the isolation of DNA inserts at various loci in several plants[8–15]; however, the prevalence of transposition in plants is not established. We report here DNA nucleotide sequence data which show that some members of the *Cin1* middle repetitive family of maize[16,17] have features characteristic of known transposable elements. One cloned *Cin1* repeat has a 6-base pair (bp) perfect inverted repeat sequence at its ends. The terminal five base pairs (5′ TGTTG ... CAACA 3′) are identical to the termini of *Drosophila copia* transposable elements[18]. Two other *Cin1* alleles are flanked by 5-bp direct repeats. A comparison is made with the long terminal repeat (LTR) of the *copia*–Ty*1*–retrovirus families of moveable genetic elements.

To define the extent of the *Cin1* element we compared the sequence of a given DNA fragment containing the insert with that of the homologous DNA fragment from which the insert is absent, using the NF1 and LC1 recombinant clones isolated from *Z. mays*. NF1 is a 5.7-kilobase (kb) clone from a Northern Flint line of maize which was found to differ from the 5.0-kb LC1 clone of a midwestern maize, line C, by the *Cin1*-001 insert (Fig. 1)[19]. Figure 2*a* shows DNA sequence analysis of the two clones in the region surrounding the *Cin1* insert. The two sequences are quite homologous (90%) except for the *Cin1*-001 insert, the left end of which is shown to be 5′ TGTTGG 3′ while the right end is 5′ CCAACA 3′, thus forming a short, 6-bp perfect inverted repeat at the ends of the element.

Further evidence that the ends of the element are assigned correctly comes from a *Teosinte guerrero* recombinant clone, TG2 (ref. 8). Annual teosintes are thought to be the progenitor of *Z. mays*; however, *T. guerrero* is not one of its closest relatives[20,21]. The TG2 clone is homologous (but not identical) to the LC1 maize clone[8]. There is a strong correlation between the *Teosinte* sequence and that of LC1 (96%) and both show a complete absence of any portion of the sequence which we have assigned to the *Cin1* repeat (Fig. 2*a*).

The final approach used to define the ends of the *Cin1* element was to compare the DNA sequence surrounding the repeat with that found on other recombinant clones containing the *Cin1* repeat. Figure 2*b, c* shows this sequence for two such recombinant clones, LC102 and LC103; the sequences of the *Cin1* elements were clearly similar (87 and 82%, respectively; N.S.S., unpublished data), while the border regions flanking the element were completely different. The ends of the *Cin1* repeat on clone LC102 showed the identical 6-bp inverted repeat as seen for the *Cin1*-001 allelle, but the LC103 clone has one base change, C → T, in this inverted repeat.

From the above analysis we conclude that the *Cin1* element terminates in a 6-bp perfect inverted repeat (5′ TGTTGG ... CCAACA 3′). The presence of short, terminal inverted repeats is a characteristic of most known transposable elements; however, this particular DNA sequence is different from the

© 1984 Macmillan Journals Ltd

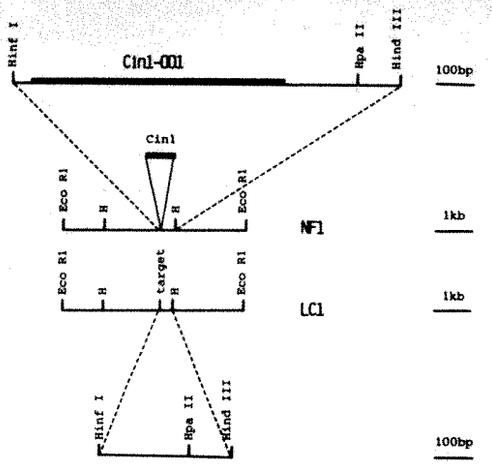


Fig. 1 The *Eco*RI and *Hin*dIII (H) restriction maps of the NF1 and LC1 maize recombinant clones[19]. The two clones were aligned to show their size similarity, except for the *Cin1*-001 sequence (thick bar) which is present on the NF1 clone.

inverted repeat sequence of other known plant DNA inserts[8,12–14]. The terminal 5 base pairs are identical to the termini of the *copia* transposable element of *Drosophila melanogaster*[18], and *Cin1* is the first plant DNA insert examined that contains the TG-CA dinucleotides present at the termini of many eukaryotic transposable elements[7,22].

Another characteristic of transposable elements is the duplication of target site sequence on integration[7]. This criterion is also difficult to analyse for *Cin1* because of the lack of a true target site or an immediate progenitor allele. There is a 5-bp direct repeat flanking the *Cin1* element on both the LC102 and LC103 clones (Fig. 2). The flanking sequence is not identical for the two clones (ATAAT for LC102 and GTTAG for LC103), and their presence is consistent with the notion of the *Cin1* element producing a target site duplication on integration.

Unlike the previously mentioned alleles, the *Cin1*-001 allele on the NF1 clone is not flanked by a 5-bp direct repeat. A direct repeat of 4 bp (CTCG) is present, but displaced from the *Cin1*-001 terminus on one side by two Ts. The significance of this is unknown; however, the presence of an extra base separating a direct repeat from the terminus of an insertion has been observed recently in another system[23].

The fact that the DNA sequence of the *Teosinte* clone shows greater homology with the LC1 sequence than with the NF1 sequence, does not necessarily mean that the LC1 sequence is an older form than that of NF1. *Z. mays* and annual teosintes easily interbreed and are often present in the same field[20,21]. Therefore, it is uncertain whether or not this particular DNA fragment in the *T. guerrero* line has been exchanged with maize during growth of *Teosinte* in the wild. It is just as reasonable to assume that NF1 is the precursor of the others, and that *Cin1* had been excised from the sequence. In this case, the continued presence of the CTCG repeat plus the extra G is reminiscent of recently observed nucleotide alterations of the direct repeat on excision of the *Ds* transposable element of maize[13]. Thus, the DNA sequence of the LC102 and LC103 clones as well as that of the NF1/LC1 set of clones is consistent with the idea of *Cin1* transposition.

It has been mentioned that the TG-CA dinucleotides at the termini of *Cin1* are similar to those of other eukaryotic transposable elements (see above), including several *copia*-like elements of *Drosophila*, the Ty*1* element of yeast, mouse intracisternal A-particle (IAP) genes[24], and the majority of retrovirus proviruses (for reviews see ref. 7). These elements contain a left and a right LTR which form a large direct repeat at the ends of the element and which also end in the dinucleotides TG-CA[7,22,24]. Although the LTR is defined as the terminal direct repeat of a larger element, solo structures which resemble LTRs in size, hybridization or internal sequence organization, have also been found[25–29].

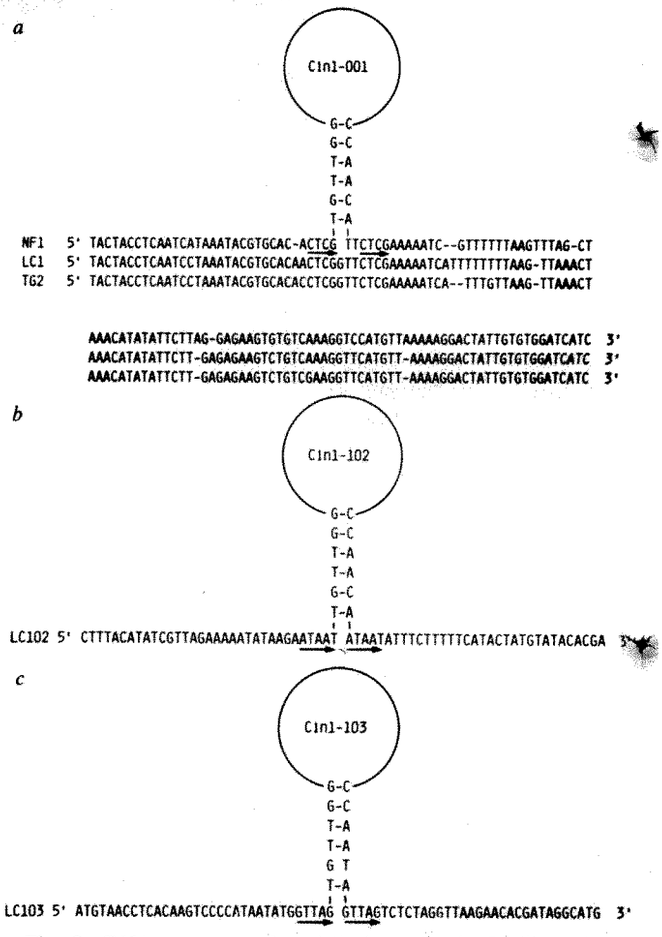


Fig. 2 DNA sequence of regions immediately flanking *Cin1* alleles present on the maize recombinant clones NF1 (*a*), LC102 (*b*), and LC103 (*c*)[16,19]. *Cin1*, as defined in the text, is shown as a stem–loop structure, and direct repeats which flank the element are indicated by arrows. The corresponding homologous DNA sequences of the LC1 and TG2 recombinant clones[8], which do not contain the *Cin1*-001 allele, are also given. The DNA sequences of NF1 (including the *Cin1*-001 sequence of Fig. 3), LC1 and LC1002 were obtained for both DNA strands using standard Maxam and Gilbert sequencing[39] of overlapping restriction fragments. For TG2 and the left side of LC103, only one DNA strand was sequenced, but in both cases the sequence was easily read from a 16 or 8% sequencing gel.

By analogy, the *Cin1* element is similar in size to an LTR, contains the terminal TG-CA dinucleotides, and is found dispersed in the maize genome. The internal structure of the *Cin1*-001 allele is also analogous to that of an LTR[22] (Fig. 3) in that it contains signals for the initiation of RNA transcription[30,31] followed by a poly(A) addition signal[32], and signals thought to be important for the termination of RNA synthesis[33,34]. However, it is unknown whether *Cin1* is transcribed or if it is ever found as the terminal direct repeat of a larger moveable element.

The similarities of *Cin1*-001 to the LTR of retroviruses are striking, but care should be exercised when extending this structural similarity to a mechanistic one. Retroviruses integrate into the animal genome via reverse transcription of the viral RNA genome into DNA[22]. The possibility of reverse transcription in plants has been the subject of many recent articles[35–38] but definitive proof is yet to be provided. Whether or not these sequences are conserved in all *Cin1* alleles is unlikely, for *Cin1* has been shown to be a heterogeneous family[16] and any transposition event may have occurred many years ago, thus allowing mutational drift to occur.

The findings presented here concerning the *Cin1* element of *Z. mays* suggest that it has characteristics typical of a transposable element: it is found in many copies, it has short inverted



Fig. 3 DNA sequence of the *Cin1*-001 allele present on the NF1 recombinant clone. The 6-bp perfect inverted repeat at the ends of the element is marked by a thick arrow. The *Cin1* sequences that are similar to the organization of a retrovirus LTR[22] are as follows. The sequence ACATT (overlined with dashes) is identical to the 'CAT' box[30] found in the LTR of spleen necrosis virus[22]. It is followed 34 bases later by a Goldberg–Hogness 'TATA' box at bases 413–419 (consensus sequence: $TATA^{\hat{A}}_{T}A^{T}_{A}$)[31], a perfect poly(A) addition signal (5′ AATAAA 3′)[32] at 467–472 (overlined), and a CA dinucleotide at position 485 (preferred site for polyadenylation, normally found 10–30 bp downstream from the poly(A) addition signal of eukaryotic genes)[32]. The 15-bp perfect inverted repeat (thin arrows) and the T-rich region (72% T) of the upper strand between bases 516 and 540 (brackets) are both features which may influence termination of RNA transcription[33,34]. The longest open reading frame with an AUG near the 5′ end is located on the lower strand. It begins at position 438 (ATG:*), terminates with the stop codon at position 229 (TAA:*), and possibly encodes a protein of 69 amino acids. A Goldberg–Hogness 'TATA' box is found 85 bp upstream from this open reading frame on the lower strand at bases 572–578. Other 'TATA' boxes and poly(A) addition signals are also present at various, unmarked positions. The total A + T content of *Cin1*-001 is 65%.

repeats at the end which are identical to a known transposable element, and in two cases it was found to be flanked by a 5-bp direct repeat. We conclude, therefore, that DNA transposition has played a part in the dispersion of short repetitive sequences in *Z. mays*.

We thank L. Glahn and R. Berntgen for technical assistance, and Dr Peter Czernilofsky for critical reading of the manuscript.

Received 9 August; accepted 21 November 1983.

1. Britten, R. J. & Davidson, E. H. *Fedn Proc.* **35,** 2151–2157 (1976).
2. d'Eustachio, P. & Ruddle, F. H. *Science* **220,** 919–924 (1983).
3. McClintock, B. *Carnegie Instn Wash. Yb.* **45,** 176 (1946).
4. Peterson, P. A. *Theor. appl. Genet.* **40,** 367–377 (1970).
5. Fincham, J. R. S. & Sastry, G. R. K. *A. Rev. Genet.* **8,** 15–50 (1974).
6. Fincham, J. R. S. *Genetics Suppl.* **73,** 195–205 (1973).
7. Shapiro, J. A. (ed.) *Mobile Genetic Elements* (Academic, New York, 1983).
8. Saedler, H. *et al.* in *Genetic Rearrangement, 5th John Innes Symp.* (eds Chater, K. F. *et al.*) 107–115 (Croom Helm, London, 1983).
9. Geiser, M. *et al. EMBO J.* **1,** 1455–1460 (1982).
10. Burr, B. & Burr, F. A. *Cell* **29,** 977–986 (1982).
11. Strommer, J. N. *et al. Nature* **300,** 542–544 (1982).
12. Goldberg, R. B., Hoschek, G. & Vodkin, L. O. *Cell* **33,** 465–475 (1983).
13. Peacock, W. J. *et al.* in *15th Miami Winter Symp.* (1983).
14. Döring, H. P., Tillman, E. & Starlinger, P. *Nature* **307,** 127–130 (1984).
15. Fedoroff, N., Wessler, S. & Shure, M. *Cell* **35,** 235–242 (1983).
16. Gupta, M., Bertram, I., Shepherd, N. S. & Saedler, H. *Molec. gen. Genet.* **192,** 373–377 (1983).
17. Gupta, M., Shepherd, N. S., Bertram, I. & Saedler, H. *EMBO J.* (in the press).
18. Levis, R., Dunsmuir, P. & Rubin, G. M. *Cell* **21,** 581–588 (1980).
19. Shepherd, N. S. *et al. Molec. gen. Genet.* **188,** 266–271 (1982).
20. Iltis, H. H. & Doebley, J. F. *Am. J. Bot.* **67,** 994–1004 (1980).
21. Wilkes, H. G. *Science* **177,** 1071–1077 (1972).
22. Weiss, R., Teich, N., Varmus, H. & Coffin, J. *RNA Tumor Viruses* (Cold Spring Harbor Laboratory, New York, 1982).
23. Rechavi, G., Givol, D. & Canaani, E. *Nature* **300,** 607–611 (1982).
24. Kuff, E. L. *et al. Nature* **302,** 547–548 (1983).
25. Cole, M. D., Ono, M. & Huang, R. C. C. *J. Virol.* **38,** 680–687 (1981).
26. Cameron, J. R., Lok, E. Y. & Davis, R. W. *Cell* **16,** 739–751 (1979).
27. Hughes, S. H., Toyoshima, K., Bishop, J. M. & Varmus, H. E. *Virology* **108,** 189–207 (1981).
28. Brown, S. D. M. *Gene* **23,** 95–97 (1983).
29. Streeck, R. E. *Nature* **298,** 767–769 (1982).
30. Efstratiadis, A. *et al. Cell* **21,** 653–668 (1980).
31. Corden, J. *et al. Science* **209,** 1406–1414 (1980).
32. Proudfoot, N. J. & Brownlee, G. G. *Nature* **252,** 359–362 (1974).
33. Rosenberg, M. & Court, D. *A. Rev. Genet.* **13,** 319–353 (1979).
34. Lai, E. C. *et al. Cell* **18,** 829–842 (1979).
35. Varmus, H. *Nature* **304,** 116–117 (1983).
36. Hull, R. & Covey, S. N. *Trends biol. Sci.* **8,** 119–121 (1983).
37. Pfeiffer, P. & Hohn, T. *Cell* **33,** 781–789 (1983).
38. Toh, H., Hayashida, H. & Miyata, T. *Nature* **305,** 827–829 (1983).
39. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499–560 (1980).

A large increase in enzyme–substrate affinity by protein engineering

Anthony J. Wilkinson*, Alan R. Fersht*, D. M. Blow†, Paul Carter‡ & Greg Winter‡

* Department of Chemistry and † Department of Physics, Imperial College of Science and Technology, London SW7 2AY, UK
‡ MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

A single point mutation has been engineered in the tyrosyl-tRNA synthetase that improves its affinity (K_M) for its substrate ATP by a factor of 100. In the crystal structure of the tyrosyl tRNA synthetase (of *Bacillus stearothermophilus*), the side-chain hydroxyl of Thr 51 appears to make a weak hydrogen bond with the AMP moiety of the substrate intermediate, tyrosyl adenylate. In the absence of substrate, however, the hydroxyl group should make a strong hydrogen bond with water which would favour dissociation of the enzyme–substrate complex. We have used oligodeoxynucleotide-directed mutagenesis to construct two point mutants at this site: one to remove the hydroxyl group (Thr 51 → Ala 51) and the other, in addition, to distort the local polypeptide backbone (Thr 51 → Pro 51). We report here that both mutants have increased activity (k_{cat}/K_M for ATP) but one mutant (Pro 51) shows a massive 25-fold increase due mainly to a lowered K_M for ATP. This demonstrates dramatically the potential of *in vitro* mutagenesis for improving the affinity of an enzyme for its substrate.

The activities of such bacterial enzymes as β-lactamase[1], 'evolved β-galactosidase'[2,3], amidases[4,5] and ribitol dehydrogenase[6] have been successfully directed towards novel substrates *in vivo* by selecting spontaneous mutations of the enzyme. We are exploring an alternative approach in which we inspect a crystallographic model of the enzyme and attempt to predict the effect of changing a side chain in the active site. We then construct the required mutant by site-directed mutagenesis of the gene, express the enzyme in *Escherichia coli* and measure catalytic activity[7,8].

The tyrosyl-tRNA synthetase (TyrTS) of *B. stearothermophilus* catalyses the aminoacylation of $tRNA^{Tyr}$ in a two-step mechanism in which tyrosine is activated to form enzyme-bound tyrosyl adenylate and then transferred to $tRNA^{Tyr}$. The location of tyrosyl adenylate in the crystal structure indicates that hydrogen bonds are made to the ribose moiety from the side chains of Cys 35, Thr 51 and His 48 (refs 9–11). The Cys 35 interaction has been confirmed by constructing Gly 35 and Ser 35 mutants: these have lowered affinities for ATP resulting either from the complete removal of the hydrogen bond in TyrTS(Gly 35) or from placing the −OH group in TyrTS(Ser 35) slightly too far from the hydrogen bond acceptor on the ATP[7,8]. Interestingly, TyrTS(Ser 35) has a lower affinity for ATP (calculated from k_{cat}/K_M for aminoacylation) than does TyrTS(Gly 35), despite the possibility of a weak hydrogen bond. This probably stems from Ser 35 exchanging a good hydrogen bond with water in the free enzyme for a poor one with ATP on forming the enzyme–substrate complex[8]. As the hydroxyl group of Thr 51 makes a long hydrogen-bonding contact to the ring oxygen (O-1) of the ribose, we predicted that removal of the weak hydrogen bond by constructing an Ala 51 mutant might improve the affinity of the enzyme for ATP.

The Ala 51 mutant was constructed as described previously[7,12] (using a synthetic oligodeoxynucleotide, 5′ AAATGGCGGCCAAGTG 3′) and characterized by dideoxy sequencing[13]. Not only was the Ala 51 lesion confirmed, but also the entire TyrTS gene was sequenced to prove that no other mutations had been introduced inadvertently. For this purpose, a family of five synthetic oligodeoxynucleotide primers staged throughout the TyrTS gene was used (Fig. 1*A*), allowing the complete sequence to be determined on a single salt gradient

0028-0836/84/020187—02$01.00

© 1984 Macmillan Journals Ltd

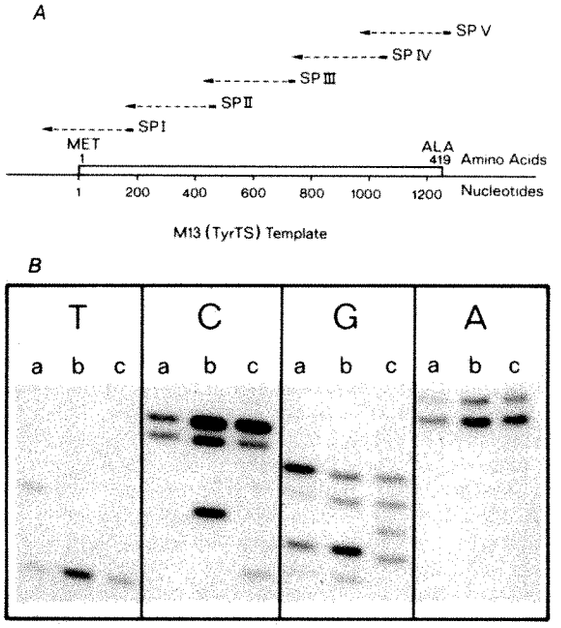


Fig. 1 Sequencing of mutant TyrTS gene. *A*, Oligodeoxynucleotide primers: SPI, 5′ CCCGCCTGCTGGAAGCG 3′; SPII, 5′ TGAACCGACTCTTT 3′; SPIII, 5′ CTCGTACGGCGACGTTT 3′; SPIV, 5′ AAGCGGAACGTCGCCTC 3′; SPV, 5′ TTACTATGCCCAGCGC 3′, complementary to the sequence of the TyrTS gene[15] cloned in M13, were synthesized using phosphotriester chemistry on a polydimethylacrylamide kieselguhr support[16] and purified by ion-exchange HPLC. Templates from the mutants M13TyrTS(Ala 51) and M13TyrTS(Pro 51) were prepared[17] and the genes sequenced by primer extension and the dideoxy chain termination method. As >300 nucleotides from the primer can be read from gradient gels using [α-^{35}S]thio-dATP[14], the complete sequence of each gene may be determined in a single gel. *B*, Sequences of M13TyrTS(Thr 51) (tracks a), M13TyrTS(Ala 51) (tracks b) and M13TyrTS(Pro 51) (tracks c) as determined using the primer SPI. Detection of sequence changes was facilitated by running the T, C, G or A tracks of the three clones in adjacent slots.

gel[14]. By running together the corresponding T, C, G and A tracks of mutant and wild-type clones, sequence changes can be readily detected (Fig. 1*B*).

Mutation of Thr 51 to Ala 51 causes a small change in k_{cat} for both exchange (Table 1) and aminoacylation (Table 2), but decreases K_M for ATP by a factor of two for both reactions. The specificity constant, k_{cat}/K_M, is accordingly increased two-fold, indicating that the enzyme–substrate interaction energy is increased by 0.38 kcal mol^{-1} in the Ala 51 mutant. This is consistent with our prediction that a small increase in enzyme affinity might be engineered by deleting a poor hydrogen bond with the substrate.

Residues 47–61 all have ϕ, ψ angles in the range characteristic of an α-helix (−57°, −48°)[11], but the helix is slightly irregular at the amino-terminus, and the hydrogen bond between Gly 47 (CO) and Thr 51 (NH) may not form. In the homologous *E. coli* enzyme where residue 51 is proline[15], this hydrogen bond could not exist as the nitrogen is locked into the pyrrolidine ring. This must cause a small distortion in the polypeptide backbone around residues 47–51. To examine the effect on the affinity of ATP, we constructed a Pro 51 mutant of the *B. stearothermophilus* enzyme (as above, using the primer 5′ CAAAATCGGGGCCAAGT 3′). The mutation leads to large kinetic changes. In the activation reaction, there is a 15-fold lowering of K_M for ATP and a near doubling of the value of k_{cat}. These combine to give a 25-fold increase in the specificity constant (≡1.9 kcal mol^{-1} of activation energy). In the charging reaction, K_M for ATP is reduced 130-fold for TyrTS(Pro 51), but k_{cat} is also reduced by a factor of 2.6 so that the specificity constant is 50 times higher than that for the wild type (≡2.4 kcal mol^{-1} of activation energy). The detailed stereochemical explanation of this mutation must await further work: for example, it is conceivable that the distortion of the polypeptide backbone introduced by Pro 51 may alter the position of His 48, and increase its interaction with the substrate. However, one factor in the improved affinity must be the removal of the poor hydrogen bond of Thr 51 with ATP.

Table 1 Pyrophosphate exchange activity of tyrosyl-tRNA synthetases

Enzyme	k_{cat}(s^{-1})	K_M (ATP) (mM)	k_{cat}/K_M (s^{-1} M^{-1})
TyrTS	7.6	0.9	8,400
TyrTS(Ala 51)	8.6	0.54	15,900
TyrTS(Pro 51)	12.0	0.058	208,000

Synthetase exchange activity was measured at 25 °C, *p*H 7.8, in 144 mM Tris-HCl, 10 mM $MgCl_2$, 0.1 mM phenylmethane sulphonyl chloride, 10 mM 2-mercaptoethanol, 2 mM pyrophosphate, 50 μM tyrosine, and 100–250 nM enzyme (assayed by active site titration).

Table 2 Aminoacylation activity of tyrosyl-tRNA synthetases

Enzyme	k_{cat} (s^{-1})	K_M (ATP) (mM)	k_{cat}/K_M (s^{-1} M^{-1})
TyrTS	4.7	2.5	1,860
TyrTS(Ala 51)	4.0	1.2	3,200
TyrTS(Pro 51)	1.8	0.019	95,800

The same buffer was used as for Table 1, plus 100 μM ^{14}C-Tyr, 20 μM $tRNA^{Tyr}$ and 25–50 nM enzyme.

Would the mutation to TyrTS(Pro 51) confer a selective advantage on the cell, given its improved K_M with ATP? In addition to any change in other properties of the protein that have as yet not been investigated, such as thermostability, there are clear kinetic disadvantages of TyrTS(Pro 51) *in vivo* because of the high cellular ATP concentration. The intracellular concentration of ATP (2–3 mM) is so high that the wild-type and mutant enzymes are nearly saturated with ATP, thus the reaction rate is controlled by k_{cat} and not k_{cat}/K_M (ref. 8). This confers a slight disadvantage on TyrTS(Ala 51) and a greater disadvantage on TyrTS(Pro 51) in the overall charging reaction (Table 2). However, *in vitro*, TyrTS(Pro 51) is far more active at lower ATP concentration (<2 mM). Also, in the tyrosine activation reaction, TyrTS(Pro 51) is far more active at all concentrations of ATP. (Selective pressure is exerted on the overall aminoacylation reaction rather than on the partial reaction of activation.)

Irrespective of the consequences *in vivo*, the dramatic improvement of K_M for ATP *in vitro* has significant implications for industrial enzymology by demonstrating that it is possible to engineer large improvements in enzyme activity by site-directed mutagenesis.

Received 16 September; accepted 4 November 1983.

1. Hall, A. & Knowles, J. R. *Nature* **264,** 803–804 (1976).
2. Hall, B. G. *Genetics* **89,** 453–465 (1978).
3. Hall, B. G. & Zuzel, T. *Proc. natn. Acad. Sci. U.S.A.* **77,** 3529–3533 (1980).
4. Paterson, A. & Clarke, P. H. *J. gen. Microbiol.* **114,** 75–85 (1979).
5. Turberville, C. & Clarke, P. H. *FEMS Microbiol. Lett.* **10,** 87–90 (1981).
6. Wu, T. T., Lin, E. C. C. & Tanaka, S. *J. Bact.* **96,** 447–456 (1968).
7. Winter, G., Fersht, A. R., Wilkinson, A. J., Zoller, M. & Smith, M. *Nature* **299,** 756–758 (1982).
8. Wilkinson, A. J., Fersht, A. R., Blow, D. M. & Winter, G. *Biochemistry* **22,** 3581–3586 (1983).
9. Monteilhet, C. & Blow, D. M. *J. molec. Biol.* **122,** 407–417 (1978).
10. Rubin, J. & Blow, D. M. *J. molec. Biol.* **145,** 489–500 (1981).
11. Bhat, T. N., Blow, D. M., Brick, P. & Nyborg, J. *J. molec. Biol.* **158,** 699–709 (1982).
12. Zoller, M. & Smith, M. *Nucleic Acids Res.* **10,** 6487–6500 (1982).
13. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
14. Biggin, M. D., Gibson, T. J. & Hong, G. F. *Proc. natn. Acad. Sci. U.S.A.* **80,** 3963–3965 (1983).
15. Winter, G., Koch, G. L. E., Hartley, B. S. & Barker, D. G. *Eur. J. Biochem.* **132,** 383–387 (1983).
16. Gait, M. J., Matthes, H. W. D., Singh, M., Sproat, B. S. & Titmas, R. C. *Nucleic Acids Res.* **10,** 6243–6254 (1982).
17. Winter, G. & Fields, S. *Nucleic Acids Res.* **8,** 1965–1974 (1980).

DON'T TAKE THIS TEST . . .

unless you would like to learn more about publications from the world's largest biological and biomedical information service.

QUESTIONS

1. Where would you look for journal article abstracts in *any* life science field?
   - A. **Biological Abstracts**
   - B. Your local Bookstore
   - C. Library Card Catalogue
2. Which publication provides over 1500 synopses of books in the life sciences each year?
   - A. Reader's Guide to Periodical Literature
   - B. **Biological Abstracts/RRM** (Reports, Reviews, Meetings)
   - C. Scientific American
3. Who publishes **BA** and **BA/RRM**?

ANSWERS

1. **Biological Abstracts (BA),** produced biweekly, provides informative abstracts of current published research from the biological and biomedical journal literature.
2. Rely on **Biological Abstracts/RRM** (also biweekly) to keep up with and select materials for your life science collection. In addition, **BA/RRM** indexes notes, symposia papers, research reports, reviews and meetings.
3. We do. BioSciences Information Service, also known as BIOSIS.

For a FREE brochure about our products and services, call (215) 587-4800 or toll free (800) 523-4806 (continental USA, outside PA), or write: BIOSIS User Services, 2100 Arch Street, Philadelphia, PA 19103-1399, USA

Circle No.17 on Reader Service Card.

Announcing the new current-awareness journal from Cambridge

New for 1984

BIO/ESSAYS

Editor-in-chief: **Dr W.J. Whelan** *University of Miami*

Cambridge University Press is pleased to announce this important new monthly which will be launched in July 1984 and published for the ICSU Press, the publishing house of the International Council of Scientific Unions.

BIO/ESSAYS meets the demand for a current-awareness journal devoted to reviews, comment, debate and progress in the frontier areas of molecular, cellular, and developmental biology and their impact on science and society.

Whilst BIO/ESSAYS will concentrate primarily on advances in knowledge from basic research, it will also pay significant attention to actual or potential applications in medicine, industry and agriculture and is of particular interest to those working, or planning a career, in this field. It will debate speculative or controversial aspects of biological research, and disseminate authoritative views and exchanges of views.

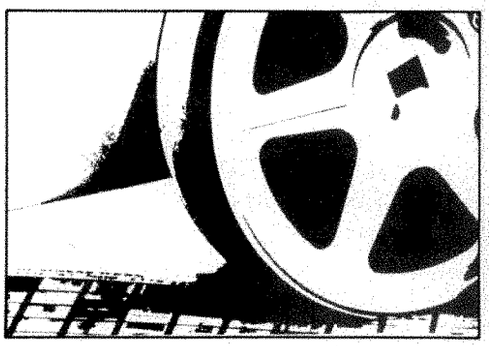
Please contact Aileen Askwith, Journals Publicity Controller, for further information.

CAMBRIDGE UNIVERSITY PRESS

The Edinburgh Building, Shaftesbury Road, Cambridge CB2 2RU, England

Circle No.22 on Reader Service Card.

nature
is available in Microform.



University Microfilms International

Please send additional information

for _____ (name of publication)

Name_____

Institution_____

Street_____

City_____

State_____ Zip_____

300 North Zeeb Road, Dept. P.R., Ann Arbor, Mi. 48106

Circle No.61 on Reader Service Card.

Leucocyte Typing

Human Leucocyte Differentiation Antigens Detected by Monoclonal Antibodies

Specification - Classification - Nomenclature

Editors: **A. Bernard, L. Boumsell, J. Dausset, C. Milstein, S.F. Schlossman**

1984. 158 figures. 249 tables. Approx. 820 pages.
Cloth DM 124,–; approx. US$48.00
ISBN 3-540-12056-4

Leucocyte Typing comprises the proceedings of the first international collaborative effort to define human leucocyte antigens by monoclonal antibodies. Involving practically all research groups active in the field, this unusually large study was sponsored by INSERM, IUIS and WHO, who will be basing their recommendations with regard to standardization and nomenclature on the workshop results.
The book deals with technical details, surveys the large number of antileucocyte reagents that have been produced, discusses current problems, and draws conclusions concerning the future development of leucocyte typing; as such it is indispensable to the research immunologist. However, it also reviews the progress that has been made and provides a clear guide for the nonspecialist who wishes an up-to-date overview of this new field. The definition of antigens by monoclonal antigens now concerns almost all medical specialties and a study of this kind is essential for present clarity and future progress.

2461/4/2h

Springer-Verlag
Berlin
Heidelberg
New York
Tokyo

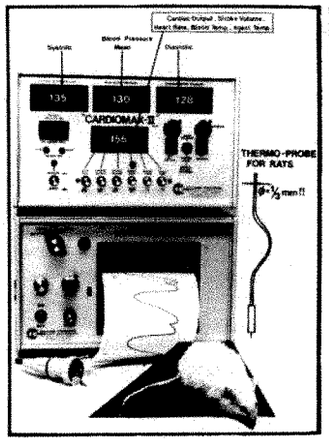
Tiergartenstr. 17, D-6900 Heidelberg 1 or 175 Fifth Ave., New York, NY 10010, USA or 37-3, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan

Circle No.24 on Reader Service Card.

RATS!!!
CARDIAC OUTPUT BY THERMODILUTION

"CARDIOMAX-II-R" is the only computer which can measure cardiac output of rats (also guinea pigs, rabbits, dogs, chickens, horses and humans). For rats, it uses unique Columbus Instruments thermodilution microprobes **1/3 mm in size!** It also measures systolic, diastolic and mean blood pressures and heart rate up to 1000 beats per minute. Stroke volume is also automatically computed.

Why Measure Cardiac Output of Rats?



Development of salt sensitive and salt resistive strains of rats provided inexpensive models for studying hypertension. Dynamics of rat circulatory system is similar to dogs or humans. Studies on rats cost only a fraction of studies using dogs.

Automatic Measurements Without Supervision

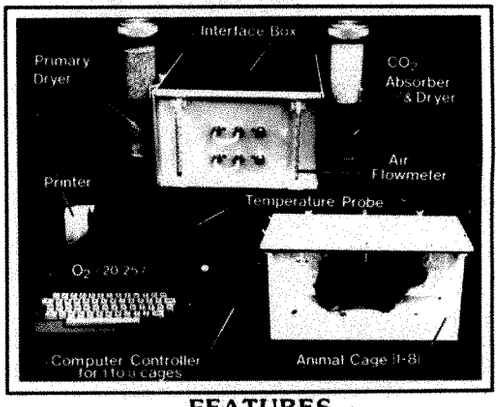
All parameters (cardiac output, stroke volume, three blood pressures, blood temperature, heart rate, time of day) can automatically be measured periodically and printed when using **"Cardiomax-II-R"** with Columbus Instruments Automatic Injector and Printer.

For more information, call or write to:
COLUMBUS INSTRUMENTS INTERNATIONAL CORPORATION
Supplier of individual instruments and total measuring systems
950 N. HAGUE AVE., COLUMBUS, OHIO 43204 U.S.A.
PHONE: (614) 488-6176 TELEX: 246514

Circle No.03 on Reader Service Card.

O_2-consumption
CO_2-production
Respiration Rate

Simultaneously measured in 1 to 8 animal cages



FEATURES

• One controller for 1 to 8 animal cages • Continuous measurement of O_2, CO_2 and Respiration Rate over a 24 hour period in ml/kg of body weight/hour in STP units. • Printer prints results every four (4) minutes for each of 1 to 8 animals as well as oxygen percent level in each animal cage • System adaptable to small or large animals • Continuous airflow through system with oxygen level measurements at the output and input • High accuracy, >98% of full measuring range e.g. typical error ±30 ml oxygen/kg/hour in 3000 ml oxygen/kg/hour range • User adjustable alarm warns user of possible hypoxia • Reuseable absorbants • Auto reference assures high accuracy, system periodically measures ambient air and corrects itself • Optional RS-232 interface for communication with other computers • Insensitive to temperature variations in the animal cage.

Columbus Instruments also supplies monitors for: Animal Activity, Cardiac Output, Drinking and Feeding, Respiration Rate and Startle Response.

For more information, call or write to:
COLUMBUS INSTRUMENTS INTERNATIONAL CORPORATION
Supplier of individual instruments and total measuring systems
950 N. HAGUE AVE., COLUMBUS, OHIO 43204 U.S.A.
PHONE: (614) 488-6176 TELEX: 246514

Circle No.04 on Reader Service Card.

Mathematical pragmatism

Stephen Toulmin

The Nature of Mathematical Knowledge. By Philip Kitcher. *Oxford University Press: 1983. Pp.287. £15, $25.*

IT WAS more than time for a shift of focus in the philosophy of mathematics. With very few exceptions (Henri Poincaré or Hugo Dingler, for example) twentieth-century writers in this field have worked under the shadow of Gottlob Frege; and Frege's classic work, *The Foundations of Arithmetic* (1884), apparently demolished for good the attempt to give mathematics a basis in our empirical experience. In particular, Frege's denunciations of "the genetic fallacy", and of "psychologism", were part of a larger, Platonizing attack on the whole subject, and they have been understood to rule out any hope of introducing historical and mental factors into the analysis of mathematical concepts and propositions.

Since Frege, there have been few truly radical changes in the subject. For all of his originality, Ludwig Wittgenstein's posthumous *Remarks on the Foundations of Mathematics* (1967) never wholly emerged from under Frege's shadow. And although Imre Lakatos's early *Proofs and Refutations* (1963–1964) placed some emphasis on the historical mutability of basic mathematical notions like "validity", "rigour" and "truth", his alliance with Karl Popper led him, later on, to downplay those insights. Instead, he moved toward a more timeless view of the subject matter of mathematics, as part of a distinct "Third World", set apart from all changing physical and mental things.

Although Frege was a contemporary of Ernst Mach, who showed how far the basic concepts of physical theory are the products of human effort over time — so that, as many philosophers of science today would agree, philosophical accounts of scientific concepts need to be *historisch-kritisch dargestellt* — the corresponding insight in the philosophy of mathematics has hitherto made little headway. Nowadays, philosophers rarely focus on the empirical roots of mathematical concepts and procedures, because they have set themselves an oversimplified choice: either, they assume, mathematical truths must be based on empirical experience in the present — the view attributed to John Stuart Mill, which Frege demolished so devastatingly — or, in reaction, they deny to mathematics any empirical basis beyond the self evidence of its axioms and, like Frege himself, treat its claims as transhistorical.

Yet a powerful argument can be presented for thinking that these are not the only options. Must our understanding of the procedures and concepts of mathematics, and even their "meaning", rest solely on observations and experiences that can be pointed to *in the present*? Or do they not depend, equally, on those *past* experiences that led earlier mathematicians to adopt the systems and formalisms they actually did, rather than other systems or formalisms, which were equally consistent and conceivable? This alternative philosophical approach need not, of course, be taken to imply that mathematical statements function as simple reports of past experience. Rather, it asks whether we can fully grasp the intellectual force and scope of mathematical theories and theorems, if we look only at their formal features, in isolation both from the historical contexts in which they took the forms they did, and from the psychological contexts in which children can master them in the present. This richer alternative is one that presses itself on us the more urgently, at a time when philosophers of science have moved so far towards an alliance with historians of scientific thought, and when the developmental studies of L.S. Vygotsky and Jean Piaget have redirected our attention towards the learning of mathematical concepts and procedures.

Philip Kitcher's book, *The Nature of Mathematical Knowledge*, is a pioneer attempt to give a thorough philosophical account of mathematics from this third, and novel, point of view. As is the case with many such enterprises, the book opens up more issues than it can cover to general satisfaction. Still, it has many striking features. It opens with a dozen pages of introduction, which provide a clear and courageous statement of the central programme of the new approach, and this deserves a critical reading by all philosophers interested in the subject, quite aside from the rest of the text. (For instance, the introduction hints at many connections that the author does not pursue further: e.g. at parallels between his view of mathematics and J.J. Gibson's "ecological realism" as a view of sense perception.) Among other good things, subsequent chapters explore helpfully the relations between the "historical–critical" account of change in natural science that has developed in recent years, and the novel picture of historical change in mathematical concepts and procedures that will have to be built up, if the new approach establishes itself as a new research programme for the philosophy of mathematics.

Professor Kitcher's argument falls into three parts. Four chapters clear away philosophical undergrowth, and prepare the ground for the constructive work which occupies the author in Chapters 5 to 9, while a long final chapter is given over to a careful study of the development of "Analysis" as a formal branch of mathematics. (I first wrote "a branch of *pure* mathematics", but struck this out, for Professor Kitcher's approach undermines the traditional contrast between pure and applied mathematics. From his point of view, the only distinctions are ones of degree: based on the comparative distances of different mathematical concepts and theorems from the practical contexts and experiences out of which they initially crystallized.)

Recently, debate within the philosophy of mathematics has become rather specialized and inbred: as a result, those not up in the subject may find the opening part of the book obscure. But such readers are urged to turn to Chapter 5, where much in the constructive exposition of the new position is presented directly and clearly, without reference to current professional controversies. In particular, Professor Kitcher's discussion of the historical character of conceptual change in natural science and mathematics should be read and discussed by many people — working mathematicians and general philosophers, as well as others — who have no particular commitment to one or another of the current rival positions in the philosophy of mathematics.

If this book is in the end somewhat tantalizing, it is for the best of reasons: viz., that the author throws out many more novel ideas and suggestions than he has the space to follow up. Still, at a few points, this gap between promise and achievements affects his argument. To give just one example, his reading of psychology is limited to certain "individualist" traditions: apart from his passing reference to J.J. Gibson, the other main references are (for example) to J.A. Fodor and Noam Chomsky. Hence, for instance, he treats the *warranting* of a belief as a "mental process" in the head of an individual thinker, and ignores the alternative view that treats *warrants* rather as elements within collective procedures of argument that are developed and applied collectively, or "in the public domain".

Minor shortcomings apart, however, this is a valuable and important book. After the careful and thorough criticism of "creationism" in his earlier book, *Abusing Science*, it is a pleasure to see Philip Kitcher making such an original contribution to the philosophy of mathematics, and to general philosophy also. All told, it marks him as having one of the freshest and most versatile minds at work in Anglo-American philosophy today. □

Stephen Toulmin is a member of the Committee on Social Thought at the University of Chicago. His most recent book is The Return to Cosmology: Postmodern Science and the Theology of Nature *(University of California Press, 1983).*

All the way through mammalian skin

Sam Shuster

Mammal Skin.
By V.E. Sokolov.
University of California Press: 1983. Pp.695. £61.50, $72.50.

AFTER many dark years, the academic wilderness of the outer cover has been blown and biomedical scientists are coming out of the warmth of internal prying to the rewarding uncertainties of the cold surface. The spate of cutaneous publishing is part of the celebration and in that sense it may be churlish to ask for sober assessment rather than whoopee.

As might have been expected from a field in which the drive has mostly been pragmatic (disease, fur and pelt production), some topics have been dealt with rather more than others. As might also be expected of an organ whose great complexity is the unique issue of a commune rather than a marriage of other tissues, expertise can easily be maintained in one aspect side by side with the deepest oblivion of neighbourhood experts working on a different topic not a μ or two away. From this it follows that most large skin texts are multi-author jobs and they, and we, all suffer from it. The thrust of this over-long welcome to this singular book must by now be apparent: we have for the first time in the English language a notably unique and personal account of the comparative structure of the mammalian skin.

There is a short general introduction on structure followed by a systematic description covering the entire immense range of mammalian skin, the book ending with a section on adaptive changes. The whole is so exhaustive that you can follow your pet themes right through their evolutionary vicissitudes, and, if you've the stamina left, crack your equally pet theories against reality: you will not travel far without finding some extraordinary and interesting new facts which, at very least, will give you a clutch of new experiments for students. This then is an old-fashioned work of scholarship the like of which is all too rarely seen.

Nonetheless there are some serious defects. The thinking, information and methodology are essentially of the 1960s and earlier, although the original 1974 Russian edition is said to have been updated (in compensation it is salutary to be reminded how well simple methods work — for instance the evidence for the thermo-regulatory function of the fins of certain aquatic mammals); some of the data is thin; and there are some surprising omissions — coat colour and pattern is an opportunity lost. Yet despite the many flaws, including a rudimentary index and indescribably bad photomicrographs, this is an outstanding contribution to the literature. But I do hope that next time round the book gets a thorough revision to bring it into the 1980s; it certainly deserves it. □

Sam Shuster is Professor of Dermatology at the University of Newcastle upon Tyne.

AMERICAN NUCLEAR SOCIETY

AMERICAN NUCLEAR SOCIETY'S PUBLICATIONS

Complete Nuclear Publications and Services Worldwide.

Inquire about specific interests.

For Free Publications and Services Catalog, contact:

American Nuclear Society
555 N. Kensington Avenue
La Grange Park, IL 60525
U.S.A.

Circle No.11 on Reader Service Card.

Superficial science

J.E. Inglesfield

Surface Physics, 2nd Edn.
By M. Prutton.
Clarendon: 1983. Pp.138. Hbk £12.50; pbk £6.95.

EVEN before starting to study the chemisorption of carbon monoxide on nickel, a fledgling surface scientist (unless he is a theoretician) will have to be familiar with vacuum techniques, Auger spectroscopy and the theory of low-energy electron diffraction. Professor Prutton's *Surface Physics* is an ideal guide for such a student in his first few terms, introducing him to the techniques used for surface analysis and structure determination, and the main concepts in surface electronic structure, thermal motion of surface atoms and adsorption. Detail is eschewed, but the account is commendably clear and there is an up-to-date list of references.

One of the remarkable features of surface science is the variety of techniques which are routinely used to unravel surface structure. This, quite justifiably, is the topic of the longest chapter in the book, and brief but lucid accounts are given of low-energy electron diffraction (LEED), reflection high-energy electron diffraction (RHEED), field ion microscopy (FIM), ion scattering, photoelectron diffraction and surface-extended X-ray absorption fine structure (SEXAFS). These techniques are based on a whole range of physics, from solving the electronic Schrödinger equation in LEED and SEXAFS to following the classical trajectories of Ar^+ ions with kilovolt energies in ion scattering — the physics of the techniques is briefly but clearly explained. We can expect more techniques (and more confounded acronyms) in the third edition — surely on the way are grazing incidence X-ray diffraction, low-energy atom diffraction and most impressive of all, the scanning tunnelling microscope.

The drawback of a book such as this is that it is inevitably bitty. This makes me less sure about its use by undergraduates. An excellent final-year undergraduate course can certainly be based on surface science, as it provides so many examples of physical principles — particularly quantum mechanics — in action. For example, the electron beam in a LEED experiment will be completely reflected by the surface when its energy coincides with a forbidden energy gap in the bulk band structure, because at such an energy electrons cannot propagate inside the solid. The concept of energy gaps leads to surface states — wavefunctions localized at the surface because their energies lie in a bulk gap below the vacuum zero of energy — of which the dangling bonds on a semiconductor surface provide one example. In the space available Professor Prutton can only touch on topics such as surface states, and the unifying role of surface electronic structure has to be neglected. Undergraduates *will* find the book useful when they want to find out how surface states have been found experimentally.

Anyone beginning a career in a surface science lab will find this little book invaluable. Theoreticians can survive a bit longer than experimentalists without knowing what an ultra-high vacuum is, or what a LEED apparatus looks like, but they cannot get by indefinitely and they too ought to read this book when they begin research. One of the real delights of surface science is that it is still possible to understand most of what's going on, and this book provides an excellent piece of elementary education in the subject. □

J.E. Inglesfield is Head of the Theory and Computational Science Division at the Science and Engineering Research Council's Daresbury Laboratory, Warrington.

Meeting of lizards and ecologists

Mark Williamson

Lizard Ecology: Studies of a Model Organism.
Edited by Raymond B. Huey, Eric R. Pianka and Thomas W. Schoener.
Harvard University Press: 1983. Pp.501. $35, £29.75.

FRASER Darling used to advise that the way to start writing is to put a semi-colon in the middle of a blank page. A similar technique has been used in this survey. Most of the chapters started as papers given at a symposium in December 1980, and these have been topped and tailed with introductions to the three main sections and to the book as a whole, and a final overview. The intention is to cover not all but several aspects of lizard ecology, evaluating progress in the past fifteen years.

Much of modern ecological theory stems from the study of birds, particularly from the work of David Lack and Robert MacArthur. The title, and some of the comments, suggests that lizards could be equivalent to birds as model organisms for understanding ecology. They are certainly different, less diverse but far from uniform. The major difference is that lizards are low-energy animals, with the advantages of being easy to observe and capture, hardy in captivity, and many species, particularly in the American west, are abundant. But although the low-energy theme recurs through the papers it is a muted refrain; most authors expound their subject for its own sake, and only by inference compare their subject with the high-energy birds and mammals.

The three sections cover the main aspects of modern ecology. The first quarter of the book is on physiological ecology, an overview and four weakly co-ordinated chapters in different styles. Of the three chapters concerned with energy, one is a long and experimentally sophisticated field study of one species (*Uta stansburiana*), one a short survey of locomotion and metabolism in the laboratory, the third a not very successful attempt to understand distributional limits from the effect of temperature on activity. The other chapter in this section is on the physiological consequences of lizard malaria; this disease causes roughly a 20% decrease in physiological performance, but it may not affect population density. The rest of the book is similar in style, a mixture of detailed studies, broad surveys and loose ends.

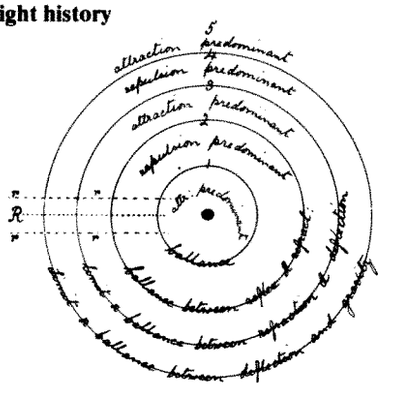
The second section, another quarter of the book, is on behavioural ecology and is much concerned with the slippery topic of home range, in *Uta* again, and in relation to sex dimorphism. A short chapter by Regal on adaptive zone and behaviour does address the question of how lizards differ from birds and mammals, the physiological and anatomical limitations of ecological performance, and the diversity of life styles in lizards. Other chapters are less successful.

That leaves half the book for population and community ecology, which is certainly not unreasonable in relation to the excitement of work in those fields in the past couple of decades. The section starts with a sound and helpful overview by Thomas Schoener, and ends with a personal, and more amusing, conclusion by the ornithologist Peter Grant. In between, half the space is taken by *Anolis* in the West Indies. On the Greater Antilles this genus has had a most interesting adaptive radiation, and Ernest Williams extends his previous study on Puerto Rico to encompass Hispaniola and Jamaica, around fifty species in all. In contrast, on the Lesser Antilles there are never more than two species on any one island, and Roughgarden, Heckel and Fuentes consider whether there has been co-evolution of competing populations. Their empirical evidence is reasonably convincing, their mathematical modelling less so. But certainly studies on *Anolis* are important for all ecologists, evolutionists and biogeographers. Chapters by Dunham, by Huey and Pianka, and by Case are also concerned with the measurement of competition, and whether niche overlap is a quantifiable concept. This is an active and controversial field, and the papers here make a useful contribution.

Overall this volume is as patchy as most reports on symposia; the attempts at cohesion are rather flimsy. For instance, the references are grouped at the end, but arranged by chapter and not unified. Lizards have much to tell ecologists, but for the moment those outside the field will have to make their own assessment of the extent to which knowledge of lizard ecology bears on their interests. □

Mark Williamson is Professor of Biology at the University of York.

Light history



William Herschel's diagram of 1780, showing his conception of the force field surrounding a particle of matter. The illustration is taken from *Optics after Newton*, by Geoffrey Cantor, recently published by Manchester University Press. Price is £20.

More or less belief in reproduction

John Habgood

The Biology of Religion.
By V. Reynolds and R.E.S. Tanner.
Longman: 1983. Pp.332. £14.95, $30.

THOSE who enjoy out-of-the-way information will find plenty to entertain them in this fascinating book. Where else could one lay one's hand on comparative figures for obesity among Trappist and Benedictine monks? Or on the Tibetan custom of drinking tea out of a cauldron which has just held a corpse? Or find a discussion of the relationship between wet-nursing, infant mortality and Christian beliefs about sexuality in seventeenth-century France? There are paragraphs here on everything from Islamic divorce to religious massacres. The hugely varied religious beliefs and practices of mankind have been combed for anything which might be thought to have a bearing on the health, survival and reproductive potential of those who profess them. The result is a book which is a delight to read, or to browse in, and which assembles an uncommonly comprehensive range of scientific studies on religious practice across the world.

The bulk of the material is arranged in terms of the human life-cycle, and the religious rituals and moral attitudes associated with each stage in human development in different religious cultures are assessed in terms of their reproductive consequences. There is a somewhat shorter section which tackles specifically the relationship between religion and disease. The results are not always what the protagonists of different religions might wish to believe. There is an uncomfortable suggestion, for instance, that the Flagellants, whose response to the Black Death in the fourteenth century was to go around the countryside flogging themselves to demonstrate their penitence, were actually instrumental in spreading infection.

The layout of the book, with its wide margins and numerous line-drawings, might give the impression that it is designed more for the coffee table than as a serious scientific study. But although the authors hope that general readers will be interested, as surely they will, their main purpose is to expound and test a hypothesis about cultural evolution, using religion as a convenient cultural indicator. It is on this level, therefore, and not as an entertaining compendium, that the work must ultimately be judged.

In the authors' own words, the hypothesis is

that the more unpredictable the total environment is perceived to be, the more people will

want to create and support ideas favouring high levels of reproduction. They will accept the loss of some of their children as inevitable. Conversely, the less unpredictable the environment is perceived to be, the more people will develop ideas favouring low levels of reproduction.

The hypothesis is tested, in the particular case of religion, by classifying different religious strategies as reproductively negative or positive, and then looking for correlation between broad religious traditions and such indicators of environmental stability as energy consumption and gross national product. Not surprisingly, Protestant Christians turn out to be wealthy energy-consumers with inhibited attitudes towards sex, whereas Hindus are just the opposite.

Such a bald summary does not do justice to the considerable subtlety often exercised in the interpretation of religious ideas — the perceptive discussion of celibacy is a case in point — nor to the sensitive exploration of ways in which cultural and physical evolution might in practice interrelate. I welcomed also the frank recognition that there is vastly more to religion than the biologically adaptive dimension on which the authors concentrate. But even within its own terms the main hypothesis seems to me to be thin and unconvincing. I was surprised, for example, to find no mention of the widely canvassed view that Protestant Christianity, far from being a response to an environment perceived as stable, has been a major factor in helping to bring that environment under control. In other words, it is not only the relationships between causes and effects which are uncertain in this immensely complicated field; there is also the question of which is which. □

John Habgood is Archbishop of York. Earlier in his career he was a physiologist and pharmacologist at the University of Cambridge.

Hoyle: comparative excellence

P.N.R. Usherwood

Muscles and their Neural Control.
By Graham Hoyle.
Wiley: 1983. Pp.688.
£61.30, $85.85.

GRAHAM Hoyle's monograph, *Comparative Physiology of the Nervous Control of Muscular Contraction,* was required reading in the late 1950s and early 1960s when comparative neurobiology was still a fledgling undergraduate subject. At that time "according to Hoyle", took on an entirely new meaning. After almost 30 years, the revised version, *Muscles and their Neural Control,* has appeared. It is well written, beautifully illustrated and conveys a most comprehensive, if at times birds-eye view of muscle structure and function across the animal kingdom. It is a remarkable achievement, but hardly a surprising one as the author stands out amongst neurobiologists for the breadth of his research and the length of his bench career. Representing as it does a distillation of his life's work, it commemorates an era which is now sadly slipping away from us.

The book is intended for undergraduates and is at its best when describing those muscle preparations which formed the stepping stones of the author's career — there are numerous references to published and unpublished work of Hoyle and his students — but the style falters when less familiar territory is encountered. His description of muscle fine structure is closely linked to cogent functional correlates and, together with a detailed phylogenetic survey of muscle innervation, this provides a successful approach for those seeking a comparative perspective of muscle.

This account of contemporary knowledge and ideas in nerve-muscle physiology is laced with words of caution from a scientist who clearly resents the oft pejorative interpretation of the term comparative. He is also unhappy about current views on the molecular mechanisms of contraction which have emanated mainly from studies of vertebrate muscle. Past challenges to the cross-bridge hypothesis fashioned from his discovery, or rediscovery, of ultrathin filaments in some muscles have met "a very sceptical, unreceptive audience bent solely upon tracking down the nature of X-bridges". Unfortunately, whilst demanding recognition for our own discoveries we often pay scant regard to other "still small voices" crying in the wilderness of contemporary science. Admittedly authors of undergraduate texts have no choice but to clarify their messages by reducing the surrounding noise of science debate and controversy. Hoyle is no exception in this respect, but I doubt whether dissenters from the vesicle hypothesis for synaptic transmission will appreciate his unquestioning acceptance of this proposal.

In the 1950s it was still possible to pursue a successful career as a comparative physiologist; although it was a struggle to keep abreast of the literature and relevant technological development. It is no longer possible to be a "Jack of all Phyla", as a comparison between Hoyle's original monograph and this new book will clearly show. Despite this, I feel *Muscles and their Neural Control* will be as successful as its predecessor and will, in time, become a classical work. □

P.N.R. Usherwood is Professor and Head of the Department of Zoology, University of Nottingham.

Towards a new phase in physics?

Louis Lyons

Quark Matter Formation and Heavy Ion Collisions.
Edited by M. Jacob and H. Satz.
World Scientific (distributed by Wiley): 1983. Pp.586. £49, $70.

PROTONS and neutrons, the constituents of nuclei, are themselves composed of quarks, bound together by gluons. Richard Feynman is quoted as once saying that the chance of discovering anything fundamental in proton–proton collisions is about the same as that of learning about how balance wheels and mainsprings operate by hurling one watch at another. If that is so, what use can there be in attempting to study the collisions of heavy nuclei with each other?

The hope is that, with a large enough number of protons and neutrons (or equivalently, quarks and gluons), a thermodynamic approach may be possible. Furthermore, it is even conceivable that, at high enough energies, the resulting nuclear matter may become so compressed that new phenomena, not accessible in collisions of just a pair of protons, may occur. In particular, increasing attention has been given to the idea that at high densities the quarks and gluons, rather than being confined inside a single proton or neutron, could wander around freely in a quark–gluon plasma extending over the volume of the two nuclei involved in the collision. Such a phase may have existed during the first millisecond of the Universe.

Several crucial questions in this field immediately come to mind. Does such a phase transition in fact exist, and if so under what conditions? Would we expect the new phase to be attainable in heavy ion collisions (and preferably with accelerators already existing)? What would be the best experimental signatures for deducing whether such a quark–gluon plasma had been created?

Such questions were all addressed at length during a workshop held in Bielefeld in May 1982. These published proceedings give a comprehensive view of both experimental and theoretical facets of the subject at the time. They are clearly required reading for nuclear or elementary particle physicists interested in working in this field (although they would have to update their information from material presented at the subsequent conference at Brookhaven in September 1983). The rest of the scientific community, however, can probably safely wait for a few years until some of the basic questions can be answered by the experiments which are now being planned. □

Louis Lyons is a Lecturer in the Department of Nuclear Physics, University of Oxford.

Automatic DNA sequencing

Akiyoshi Wada describes a system that takes the grind out of DNA sequencing

DNA SEQUENCING analysis is of fundamental importance today in pure and applied molecular biology. But the current procedure for analysing DNA sequences is both tedious and time-consuming. In the case of the Maxam-Gilbert scheme, more than thirty steps of microchemical manipulation are involved and these can only be carried out manually by a skilled chemist.

However, even using manual procedures the world-wide rate of analysis may exceed 10^6 bases per year soon, judging by the current rate of additions to the DNA database. But this seemingly high rate of input is far below what is needed to meet the expected demands of sequence analysis in the future. There are many genomes still to be sequenced and even a single human genome has 3×10^9 bases.

An automated DNA sequencer recently developed by Seiko Instruments and Electronics of Tokyo, Japan, should take some of the drudgery out of DNA sequencing and speed up data production. The instrument is a microchemical robot that can carry out the fragmentations of 16 DNA solutions — that is it can test for the four bases (A,G,C and T) in each of four different DNA solutions. It performs the fragmentation in four to five hours, following the Maxam-Gilbert protocol. In addition, it can carry out two sets of operations sequentially so that 10 hours after the start button is pushed, 32 solutions are prepared for electrophoresis. The analysis may be started in the evening as the laboratory closes and then processed material can be collected the next morning.

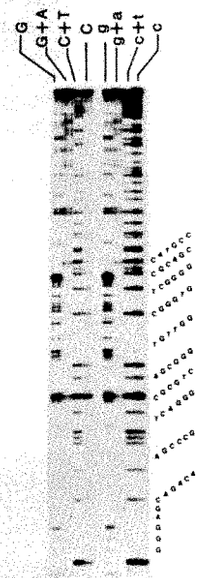
The fully automatic chemical operations performed by this Seiko robotic instrument include the quantitative addition of reagents, mixing by vortexing, centrifugal separation, draining, vacuum drying, and the heating and cooling of solutions under treatment. The volume of solutions to be handled can be varied from 1 μl to 1 ml. The time schedule and conditions of reaction steps in a series of operations are controlled by a microcomputer command which is input by the operator before the start of the sequencing process. Programmable functions extend this microchemical robot's range of performance to include any of a series of complex chemical reactions of microliter order[1].

The instrument automates just one aspect of the DNA sequencing procedure and will earn a niche in the market by virtue of its labour-saving capacity rather than by any change in the speed or economy of sequencing. The time it takes to determine a sequence is only a little less than that needed to complete a sequence manually, the sequencer runs the same number of solutions as would a skilled technician and it does not save on reagents. But its designers believe that because it can run for 10 hours when the laboratory is effectively closed and because it frees the chemist to carry out other aspects of the sequencing procedure, it will be commercially viable. Tests have shown that the instrument can perform chemical reactions at the same level of efficiency and reliability as a skilled technician. What is more, the automatic sequencer requires no training and works steadily for 24 hours a day without complaining — something which should make it very attractive to the potential buyer!

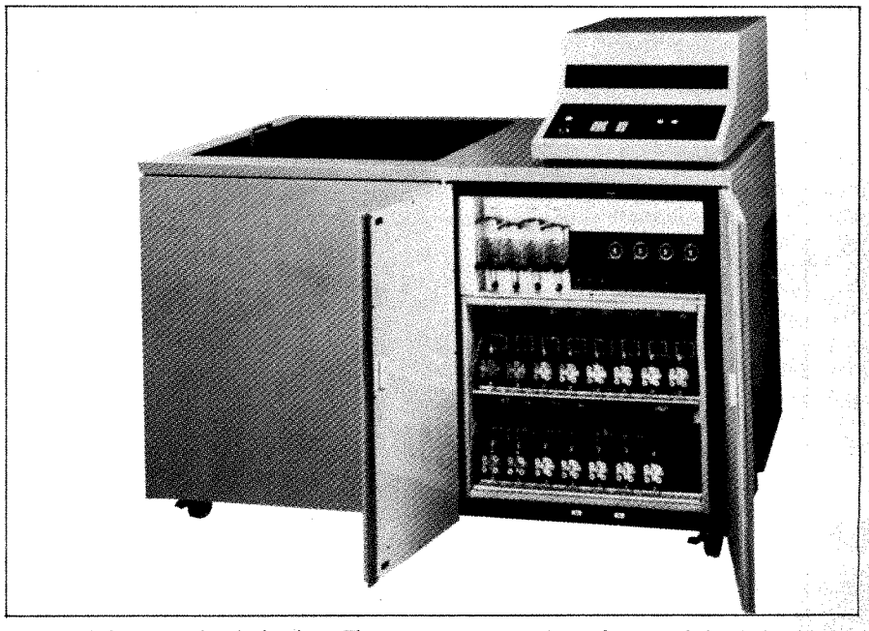
The machine should be commercially available later this year, although details of the instrument's price and its exact date of launch have not yet been released.

Akiyoshi Wada is professor of biophysics in the Department of Physics, University of Tokyo and the chairman of the project 'DNA extraction, analysis and synthesis'. The DNA sequencer was developed as part of this project under the auspices of the Science and Technology Council of Japan.

1. Wada, A., Yamamoto, M. and Soeda, E. *Rev. Sci. Instr.* (in the press).



Comparison of sequencing patterns prepared manually (left) and prepared with the sequencer (right).



View of the microchemical robot. The computer system is at the top of the desk and the temperature control dial, stock solutions and pump systems, which are normally under cover, can be seen inside.

General laboratory equipment

● A range of products for use in the DNA laboratory is available from Pharmacia. The products include a centrifugation medium which can be used to separate nucleic acids and proteins. This medium, called CSTFA, allows all types of RNA to be separated by isopycnic centrifugation. Cedium chloride is also available for the preparation of density gradients to purify DNA, and dextran sulphate can be provided to increase the rate of hybridization of nucleic acid molecules. Pharmacia also produces Sephacryl S-1000 which may be used for the removal of low molecular weight material from DNA preparations and in the purification of bacterial plasmid DNA.
Circle No. 100 on Reader Service Card.

● The Schleicher & Schuell Selectasol system is a desk-top chamber for thin layer chromatography (TLC) solvent selection. The Selectasol system is based on the circular development technique. It can be used with TLC ready plates and foils as well as with chromatography papers of between 5 × 10 cm and 20 × 20 cm. Up to 16 different solvent systems can be tested on a standard 20 × 20 cm TLC plate. All the chromatograms develop under the same temperature and relative humidity conditions.
Circle No. 101 on Reader Service Card.

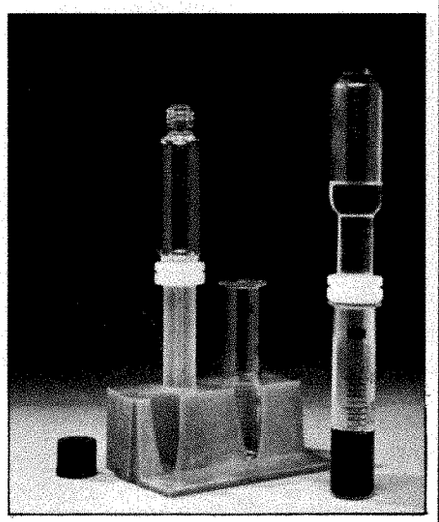
● Two new automatic sample changers are now available from Bioscan. These instruments, the Auto Changer 1000 and 3000, are both compatible with Bioscan's line of radioisotope imaging scanners. Designed to hold from one to three 20-cm thin layer chromatography plates, both instruments provide a system of optical tabs permitting the user to mark up to 60 chromatographic lanes for automated analysis.
Circle No. 102 on Reader Service Card.

● Wheaton Instruments has introduced a new automatic diluter/dispenser which allows samples to be dispensed either manually, semi-automatically or automatically. To accommodate liquids of varying viscosities, the Wheaton diluter/dispenser has an adjustable syringe plunger speed control and an adjustable delay control. Each model is equipped with an interchangeable syringe kit consisting of a sample with a plunger, a reagent syringe with a plunger and a valve assembly. This kit may be removed and autoclaved.
Circle No. 103 on Reader Service Card.

To mark the DNA Winter Symposium taking place in Miami, Florida from 16 to 20 January 1984, this week's selection features products for the molecular biology laboratory. These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● A new solvent extraction system, designed to replace the separating funnel, is now available from Associated Biolabs. This new Mixxor stirs up the sample and the extraction solvent and then allows them to settle spontaneously into an upper and lower phase. The upper phase can then be decanted without the risk of contaminating it with the lower phase. The Mixxor consists of a glass mixer-reservoir and a glass mixer-separator with a ground glass piston. When fitted together, these can be used in a pumping action. On the 'down' stroke the sample and solvent are forced through a narrow axial channel in the piston into the separator. This produces intimate mixing and leads to very efficient mass transfer. Five strokes of the pumping action produce the same mixing as shaking a separating funnel 40 times. After mixing, the phases are allowed to separate spontaneously. The piston is then adjusted, with the help of a holder-spacer, so that the lower phase is at the very top of the axial channel. The upper phase can then be decanted while the lower phase remains in place. Mixxors are available in four sizes (2, 5, 10 and 20 ml) and are supplied with their own special interlocking racks. These allow an unobscured view of the phase separation.
Circle No. 104 on Reader Service Card.

● An illustrated brochure now available from Beckman Instruments describes the company's system 1 DNA synthesizer. System 1 uses the phosphoramadite chemistry, which means that its cycle times are short and side reactions are minimal. The brochure also describes the system 1+, which includes the DNA synthesizer and a matched HPLC purification system available as a single package of instruments. Both system 1 and system 1+ can be obtained through Beckman's Base-Pak plan.
Circle No. 105 on Reader Service Card.

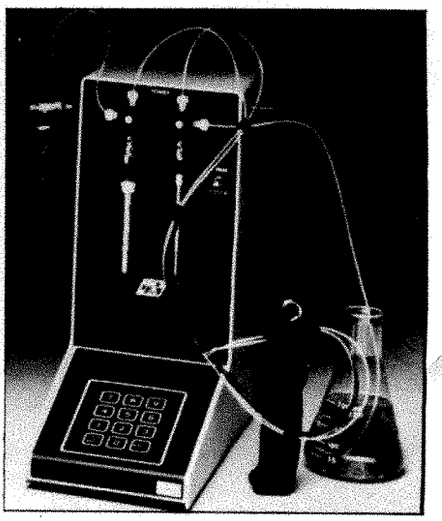


The Mixxor from Associated Biolabs

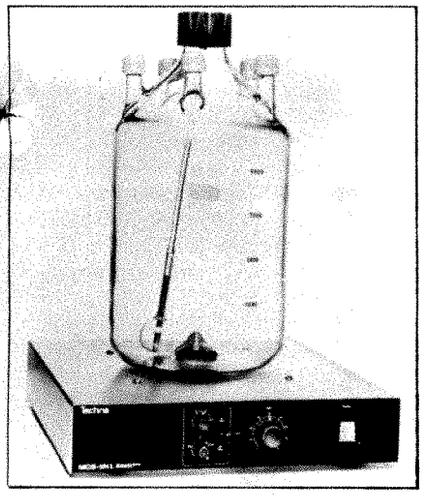
● A new digital syringe diluter/dispenser has been introduced by Dynatech Laboratories. The model 110 is a pipettor/diluter that has a separate dispenser. It has a microprocessor-controlled stepping motor drive and a separate synchronous motor to power the valves. The model 110 can achieve an accuracy of ± 1% at total syringe volume. Volumes to be dispensed are programmed in microlitres into the diluter's memory through a 12-key membrane-type keypad which has a four-digit LED display and eight back-lit legends. Stroke rates can also be programmed. Up to 14 programs can be stored in the diluter's memory and are retained even when power is cut off.
Circle No. 106 on Reader Service Card.

● A line of test tube shakers and rotators has been introduced by Labindustries. The Labquake rotator models provide a constant rotation at 8 r.p.m. Two models are offered which have different capacities and can handle tubes from 10–19 mm diameter to tubes of 27–37 cm diameter, and up to 180 cm long. The Labquake shaker models feature two removable trays. They hold tubes of any diameter up to 162 cm long, and up to 800 g in weight. Speeds may vary from 22 reversals per minute to 70 reversals per minute. Tubes are added and removed while the motor is running. All the shakers and rotators operate on standard 110 V a.c., 50–60 Hz, or 220–240 V a.c., 50–60 Hz. The shakers and rotators are also available in combination models which are supplied with clip bars and interchangeable dual trays.
Circle No. 107 on Reader Service Card.

● A line of sample-handling vials and accessories is available from Kontes. These Microflex vials have a conical cavity which makes it easier to retrieve contents with a syringe needle. Five sizes are available — 0,1, 0.3, 1.0, 2.0, 3.0 and 5.0 ml. Optional closures and finishes are available.
Circle No. 108 on Reader Service Card.



Dynatech Laboratories' diluter/dispenser



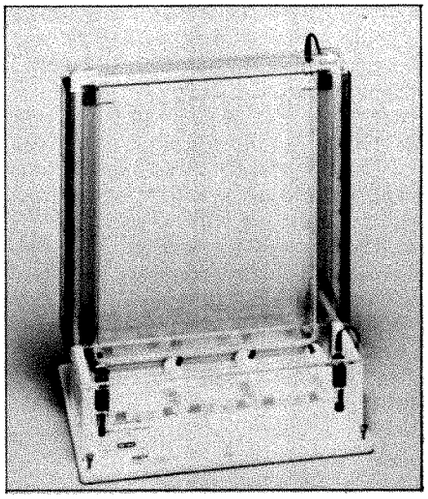
One of Techne's microcarrier stirrers

● A range of Techne's microcarrier stirrers is now available from Gallenkamp. These stirrers have been designed to increase cell yields by up to 60%. The stirring system is made from borosilicate glass, siliconized in order to inhibit cell growth on the glass surface. A special 'softstart' prevents turbulence on start-up and the subsequent uniform motion of the stirrer rod aids cell growth. Continuous stirring from 0 to 80 r.p.m. or interval stirring from 6 seconds to 5 minutes 'on' and 2 minutes to 2 hours 'off' is possible. The culture vessels are sealed to prevent contamination. The stirrers can be used with an incubator. Four models are included in the range.
Circle No. 109 on Reader Service Card.

● A series of 5cm × 5mm internal diameter mini-columns has been released by HPLC Technology. The columns are packed with the Techsphere, Spherisorb and Hypersil phases and are guaranteed to produce efficiences of 5,000 theoretical plates per 5 cm column. The 3 μm Techsphere, Spherisorb and Hypersil ranges include the Silica, C 1, C8, C18 (ODS), C18 (ion pair), CN (cyano), NH_2 (amino), phenyl and diol phases).
Circle No. 110 on Reader Service Card.

● The AE100 balance has been added to Mettler's AE line of electronic analytical balances. The AE100 has a weighing range of 0 to 109 g. The weighing chamber is accessible from both sides and from the top. A data output option makes it possible to transfer weights to a printer, calculator or computer.
Circle No. 111 on Reader Service Card.

● A range of regulated power supplies from 500 to 4,000 V has been produced by Fotodyne for electrophoresis, DNA sequencing and electrofocusing. The Fotodyne 4,000 V power supply offers constant power, constant current and constant voltage regulation with automatic crossover capability between all modes.
Circle No. 112 on Reader Service Card.



The model 1800 from Bio-Rad

● The new model 1800 DNA sequencing cell from Bio-Rad is designed to reduce the 'smile' effect found when gels are run. The design maintains an even temperature over the gel during electrophoresis. The upper buffer is in contact both with the top of the gel sandwich and with the entire area across one side, so that heat dissipates evenly. Both the upper and lower buffer chambers are incorporated into a single stand that also serves to support the glass sandwich. The sandwich itself is clamped against leakproof seals so that it forms one wall of the extended upper buffer chamber and is in direct contact with the buffer.
Circle No. 113 on Reader Service Card.

● The Drummond Scientific Company has recently added several new dispensing manifold styles to accommodate both 96-well and 24-well microtest plates. For 96-well plates, four-position, eight-position and 12-position manifolds are available. For 24-well microtest plates, 2-position and 6-position manifolds have been added. When attached to the Drummond model 590 digital micro-dispenser, these manifolds can deliver precise aliquots of sample to several positions simultaneously. The manifolds are made of stainless steel.
Circle No. 114 on Reader Service Card.

● A new catalogue detailing BDH calibration kits for gel filtration and gel electrophoresis, SDS polyacrylamide gel electrophoresis and RNA electrophoresis is now available from BDH Chemicals.
Circle No. 115 on Reader Service Card.

● A fluorescence accessory is now available from Beckman Instruments that converts a UV/visible spectrophotometer into a fluorimeter. The accessory can be used with the DU-6 or DU-7 or DU-8B UV/visible spectrophotometer. In using the accessory, the exciting wavelength is first determined by the monochromator setting. The emitted wavelength is then selected with optional filters.
Circle No. 116 on Reader Service Card.

● Tri-Continent Scientific has added an ultra-micropipettor to its line of positive displacement micropipettors. The new pipettor has flexible Teflon tips to facilitate delivery to fragile surfaces, such as those used in electrophoresis, radial immunodiffusion and thin layer chromatography. The pipettor, the Ultra-Micro Absoluter, comes in two models — the 0.5–5μl version and the 2–10 μl version. The capillary tip is reusable.
Circle No. 117 on Reader Service Card.

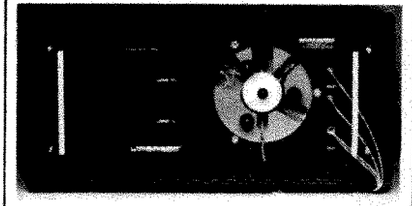
● Linear Instruments has developed the model 1210 recorder designed for use in liquid or gas chromatography. The model 1210 features a recessed zero adjustment and front-mounted, push button control of power, chart drive and full scale spans. It has an advance/rewind knob for positioning the chart. Chart drive speed is 1 cm min^{-1}, but speeds of 0.25, 0.5, 2 or 4 cm min^{-1} can be achieved through gear changes. The 1210 is a flatbed model which uses 200-mm paper, but it can be wall mounted.
Circle No. 118 on Reader Service Card.

● Heraeus has extended its range of sterile tissue culture vessels by the introduction of Flexiperm. The Flexiperm silicone vessels have an adhesive lower side that sticks to all smooth surfaces to form chambers suitable for culturing cells. The Flexiperm-Slide is used in combination with a standard microscope slide of 75 × 26 mm. The Flexiperm-Disc is intended for use with a Petriperm tissue culture dish, but it may also be placed in a petri dish of polystyrene or glass.
Circle No. 119 on Reader Service Card.

● Wheaton Instruments has introduced the new Step-Pette repetitive syringe dispenser. This delivers pre-set volumes of aliquots from a single draft of fluid. Autoclavable plastic syringes, supplied with the Step-Pette, permit volumes of 10 to 1,000 μl to be pipetted each time the thumb knob is depressed. Once the syringe is primed, 10 to 58 dispensings can be performed depending on the volume of the syringe and adjustment setting selected.
Circle No. 120 on Reader Service Card.

ADVERTISEMENT

Liposomes



LIPOPREP® the equipment for the **reproducible preparation of stable unilamellar liposomes of defined size** in the **range** of **30-300 nm Ø** with extremely narrow particle size distribution for e.g. the efficient incorporation of (therapeutic) agents incl. antibodies.
DIANORM-Geräte, P.O. Box 126, D-8 München 65, FRG

Circle No.25 on Reader Service Card.

Biologicals

● *Sau* 1 and *Stu* 1 are the latest introductions to Boehringer Mannheim's programme of restriction enzymes. *Sau* 1 recognizes and cleaves the sequence CC/TAGG producing 5′ overlapping ends. It is an isoschizomer of *Cyn* 1 and *Mst* II, and therefore useful in the diagnosis of sickle cell anaemia. *Stu* 1 recognizes and cleaves the sequence AGG/CCT, producing blunt ends. It is an isoschizomer of *Aat* I and *Gdi* I.
Circle No. 121 on Reader Service Card.

● 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside for the visual detection of β galactosidase positive *Escherichia coli* colonies or phage plaques is available from BCL. It comes in pack sizes ranging from 25 mg to 2.5 g. Solutions of X gal at 40 mg ml^{-1} in *N, N*′-dimethylformamide are stable for at least 2 weeks when stored at 4°C.
Circle No. 122 on Reader Service Card.

● New England Nuclear has introduced a new reverse transcriptase system (^{32}P). This should increase yields of double-stranded cDNA (ds-cDNA) from mRNA templates and eliminate unnecessary steps. The system includes all enzymes necessary to make ds-cDNA as a final product. The enzymes, avian myeloblastosis virus (AMV) reverse transcriptase, S_1 nuclease, and DNA polymerase I, are purified and their activities are optimized for the reverse transcriptase system. The system also includes deoxycytidine 5′-triphosphate, (α-^{32}P)- for labelling reaction products, ribonuclease A inhibitor, standardized buffer solutions and distilled water. All components are supplied ready-for-use.
Circle No. 123 on Reader Service Card.

● Reverse transcriptase that has been purified from avian myeloblastosis virus is now available in the UK from Anglian Biotechnology in conjunction with Dr G.E. Houts. Dr Houts has been providing the product for the National Cancer Institute of America. The transcriptase is allegedly free from contaminating RNase activity.
Circle No. 124 on Reader Service Card.

ADVERTISEMENT

MAP

Analytical Instrument Manufacturers, Custom built spectrometers for geological applications.

For further details see reader reply service.

Circle No.26 on Reader Service Card.

● New England Nuclear has introduced a new nick translation system (^{35}S) that complements its tritium- and ^{35}P-labelled systems. This pre-tested system optimizes preparation of DNA probes labelled with dATPαS, (^{35}S) which are suitable for *in situ* hybridization and Southern transfers. For *in situ* hybridizations, autoradiography exposure times are reduced using the ^{35}S label in place of the tritiated analogue, while the resolution necessary for silver grain counting at the light microscope level is maintained. The system is sufficient for 20 reactions and includes deoxyadenosine 5′ (α-thio)-triphosphate (^{35}S)-, DNA polymerase I, deoxynucleoside triphosphate mixture, DNase 1 and control plasmid DNA.
Circle No. 127 on Reader Service Card.

● Kor Biochemicals has listed details of its 30 growth factors, cell attachment factors, hormones, transport factors and other related biochemicals.
Circle No. 128 on Reader Service Card.

● Promega Biotec is now manufacturing an *in vitro* translation system. The system is composed principally of New Zealand white rabbit reticulocyte lysate preparation. Other components that are added to the lysate include an enzymatic energy generating system, a mixture of tRNAs and haemin. This means that the two translation-qualified amino acid mixtures, one of which lacks leucine and the other of which lacks methionine, and a control sample of brome mosaic virus mRNA must be added to the solution before use. These items are provided in separate vials. The treated rabbit reticulocyte lysate is provided in 200-μl aliquots. This eliminates the need for thawing and refreezing. Promega also manufactures and sells RNasin ribonuclease inhibitor, Packagene single extract lambda DNA packing system, GammaPrep labelled nucleotide synthesis systems and restriction endonucleases, including *Sin*I, *Sca*I, and *Dpn*I, as well as DNA and RNA modifying enzymes.
Circle No. 129 on Reader Service Card.

● Bethesda Research Laboratories (BRL) has introduced Nacs Prepac, a prepacked system for nucleic acid purification. Nacs is BRL's family of ion-exchange resins for use in nucleic acid applications and column chromatography. Filled with Nacs 52, a gravity flow resin, Nacs Prepac can be used in plasmid purification and clone screening, removing unincorporated nucleotides from labelling reactions, and recovering nucleic acids from electrophoresis gels. It is available in two forms — the convertible, for use as a mini-column or pipette tip and the cartridge, for use with a syringe. Both the cartridge and convertible are compatible with a variety of common loading and elution accessories. A Prepac contains enough resin to bind up to 40 μg of A_{260} material.
Circle No. 130 on Reader Service Card.

● Schleicher & Schuell has introduced the S&S Quick-Blot kit. This is a quantitative method for purifying and immobilizing mRNA directly from whole cells or viruses onto nitrocellulose. The new Quick-Blot kit permits selective immobilization of multiple samples of mRNA using dot-blotting techniques in conjunction with the S&S Minifold, a 96-well microsample filtration manifold. The kit can be used to immobilize mRNA from polyribosomes, chromatin, mitochondria, bacteria, yeasts or viruses. Because no baking is required, the mRNA remains biologically active on the nitrocellulose and can be easily translated into polypeptide, reverse-transcribed into cDNA, or released for further manipulation. Each Quick-Blot kit provides sufficient materials to test 120 samples and consists of four basic ingredients: detergents that help rupture the plasma membrane; sodium iodide solutions to promote interaction between mRNA and mRNC and solubilize whole cells at room temperature; NaI-Plus reagent, which furthers the removal of contaminating DNA and protein; and mRNC membrane, an RNA grade nitrocellulose.
Circle No. 131 on Reader Service Card.

● Three new products have been produced by New England BioLabs. The first is a restriction endonuclease, *Hin*P_1I which is derived from *Haemophilus influenzae* P_1. The second is a range of pBR322 *Pst* clockwise site primers for dideoxynucleotide sequencing of DNA. These are specific for the sequence 5′ . .GCTAGAGTAAGTAGTT. .3′. The third new product is a series of dephosphorylated pBR322 cloning vectors which includes *Bam*H1 cleaved and dephosphorylated pBR322, *Hin*d III cleaved and dephosphorylated pBR322, *Eco*R I cleaved and dephosphorylated pBR322 and *Pst* I cleaved and dephosphorylated pBR322.
Circle No. 132 on Reader Service Card.

● Enzo Biochem has added viral specific Patho-Gene kits to the Enzo Bio-Probe system. These kits detect the presence of a virus in fixed cells and tissue preparations. The Patho-Gene kits available contain biotinylated probe DNAs specific for herpes simplex virus I and II, cytomegalovirus, hepatitis B and Epstein Barr virus. The DNA is hybridized and then visualized by a colorimetric enzyme assay for biotin. The Patho-Gene kits are provided with Detek I-hrp, a streptavidin horseradish peroxidase complex.
Circle No. 133 on Reader Service Card.

● Hydroxylapatite, which is used to characterize DNA hybrids and to study reassociation kinetics, is now available from Bio-Rad. It comes in bottles that show the conditions for optimal separation of single and double-stranded DNA on the label.
Circle No. 134 on Reader Service Card.

NATURE CLASSIFIED

LONDON OFFICE Keith Walesby 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024)
AMERICAN OFFICES NEW YORK Miss Gene Fein 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900
SAN FRANCISCO JJHS (415) 392 6794 ●**PASADENA** JJHS (213) 796 9200
CHICAGO Didier & Broderick (312) 498 4520 ● **BOSTON** CEL Assoc (617) 848 9306
CANADIAN OFFICE TORONTO Peter Drake 32 Front Street West, Suite 200 Toronto, Ontario M5J1C5 Telephone (416) 364 1623

RATES UK — Rest of world — £11.00 per centimetre. Minimum 3 cm. £2 is charged for the re-direction of replies to advertisements with a box number.

CONDITIONS. The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.

MOLECULAR BIOLOGY

The Department of Biological Sciences at Dartmouth College seeks candidates for a senior faculty position in the area of molecular biology. The person selected will be expected to pursue an active research program, participate in departmental courses in his or her area of biological interest, and on behalf of the department take the lead in developing an interdepartmental, institution-wide program in molecular genetics. A tenure appointment will be made at the level of Professor, to begin 1 July 1984. We urge all who wish to be considered for the position to submit a current résumé, copies of their most important publications and names of four references.

The search committee will begin reviewing the applicant pool on 15 February 1984. Applications and nominations should be sent to: Secretary, Search Committee, Department of Biological Sciences, Dartmouth College, Hanover, NH 03755. Affirmative Action/Equal Opportunity Employer.

(NW184)A

BIRKBECK COLLEGE (University of London) POSTDOCTORAL RESEARCH ASSISTANT Analysis and Prediction of Protein Structure

Applications are invited for a SERC funded post to work with Dr M Sternberg on the analysis and prediction of protein structure with the aim of obtaining rules to guide protein engineering. This is a theoretical project in the Department of Crystallography which has a colour Evans & Sutherland graphics system and access to the Cray 1-S at ULCC. Experience of computing and macromolecular structure is desirable. The appointment is for three years starting between 1st April and 1st October 1984. Initial salary, according to age and experience, will be between £8,376 and £9,266 pa including London Allowance.

Applications including curriculum vitae and names of two referees should be sent to the Assistant Secretary (Personnel), Birkbeck College, Malet Street, London WC1E 7HX, England. (986)A

UNIVERSITY OF EDINBURGH FACULTY OF MEDICINE

SENIOR LECTURER IN PHYSIOLOGY

Applications are invited from graduates with considerable experience of teaching physiology, a higher degree in medicine or science, experience of supervising post-graduate research assistants, and a capacity to maintain an independent research programme. The successful candidate will be required to contribute to teaching in the department's undergraduate courses and to participate in their co-ordination. The salary is in the range £13,515 to £16,925.

Further particulars are available from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications (8 copies), including names and addresses of three referees, should be lodged not later than 10th February, 1984. Please Quote Reference No. 525. (957)A

UNIVERSITY OF PENNSYLVANIA SCHOOL OF VETERINARY MEDICINE

DEPARTMENT OF PATHOBIOLOGY

PARASITOLOGY FACULTY POSITION

is inviting applications for a tenure probationary faculty position of Assistant or Associate Professor in the Laboratory of Parasitology, with a starting date of 1 July 1984. Applicants are expected to have a DVM and/or a PhD degree. A demonstrated ability and interest in research and teaching in parasitology is essential.

Candidates are requested to submit curriculum vitae and the names of at least three references to: Dr Mark Haskins, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104. Application deadline is 1 March, 1984, or thereafter until the position is filled.

The University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer. (NW194)A

Head of Drug Metabolism

Bombay

The Boots Company (India) Ltd., is seeking for its recently established Research Centre in Bombay, a Biochemist who will be in charge of the Drug Metabolism laboratory.

The successful candidate will be involved in the establishment of the unit and will be responsible for designing and conducting drug metabolism and pharmacokinetic studies.

Candidates must have a PhD in Biochemistry with at least five years of postdoctoral/industrial experience in drug metabolism, pharmacokinetics and bioavailability studies. Preference will be given to candidates with experience in:

* instrumental methods of analysis including HPLC
* handling radiolabelled compounds
* dealing with regulatory issues related to all aspects of human studies of drug development and pharmacokinetics.

The position offers an attractive remuneration, support for relocation and good future prospects.

A curriculum vitae along with the names of three referees should be sent to: T. W. Flower, Employment Manager (Technical), The Boots Company PLC, Station Street, Nottingham, NG2 3AA.

The Boots Company PLC

(950)A

Advanced Drug Delivery Research

Our client is recognised as one of the largest and most respected pharmaceutical companies in the world. In line with its policy to lead the field in technological innovation, significant investment has been allocated to set up a new research unit to specialise in advanced drug delivery research. The unit will have approximately 45 staff, and aims to be pre-eminent in its field by the late 1980's.

Opportunities now exist for

SENIOR SCIENTISTS

to help initiate research programmes and to develop multi-disciplinary teams within the unit.
You will combine an impressive academic or industrial background with a proven record of resourceful and, ideally, creative research experience in the fields of cell biology, immunology, virology, bio-chemistry or pharmacy.
The unit is based in Southern England, and the benefits and relocation package is geared to attract the best.
In the first instance write with full CV regarding project number L3102 to:
The Caldwell Partners, 24 Buckingham Gate, London SW1E 6LB.

The Caldwell Partners International
Toronto / Montreal / Calgary / Vancouver / Houston / London

(954)A

CSIRO

Microbiologist

$A24,344 – $A35,806
Division of Plant Industry
Canberra ACT Australia

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Forest Research is a member of the Institute of Biological Resources.

The CSIRO Division of Plant Industry conducts research on problems fundamental to plant biology and agricultural production. As a contribution to improved utilization of renewable energy resources, the Division has a program of research into possible uses of cellulosic crop residues as energy sources for the biological fixation of atmospheric nitrogen.

Duties: To conduct laboratory and field experiments into biological nitrogen fixation associated with microbial populations growing on decomposing wheat stubble, and possibly other crop residues. The research will concentrate in the first instance on definition of ecological aspects and quantification of nitrogen fixation, to be followed by participation in laboratory studies to improve natural systems, and the development of improved practical applications.

Qualifications: Applicants should hold a PhD in relevant aspects of microbiology, or equivalent qualifications, followed by experience in field measurements of biological nitrogen fixation and proven ability to conduct independent research. They should also be prepared to participate actively in collaborative research and in the development of practical applications.

Tenure: Indefinite with Australian Government superannuation benefits available.

Applications: Stating full personal and professional details, names of at least two referees and quoting reference No. A4633, should be directed to:

The Chief,
CSIRO Division of Plant Industry,
GPO Box 1600,
CANBERRA ACT 2601.
AUSTRALIA.
By February 10, 1984.

(W645)A

Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Public Health Service
Department of Health and Human Services

ASSOCIATE DIRECTOR FOR CHEMICAL AND PHYSICAL CARCINOGENESIS

The National Cancer Institute (NCI) develops the overall national strategy against cancer, coordinates Federal Government and non-Federal Government cancer programs, and supports international programs of cancer research. The Division of Cancer Cause and Prevention directs the NCI's major research effort related to determining the cause of cancer as well as basic research on possible means of cancer prevention. The Associate Director for Chemical and Physical Carcinogenesis plans, directs, and conducts a basic and applied research program in which agents known or suspected to have carcinogenic and/or tumor promoting activity are evaluated from the standpoint of mechanism of action, metabolism, interactions with biologically important macromolecules, and related areas. The incumbent's responsibilities will also include basic research involving the development of effective agents to prevent or reverse the process of carcinogenesis. The Associate Director will be responsible for the management of both intramural and extramural research programs in chemical and physical carcinogenesis. The chemical and physical carcinogenesis program activities represent a budget of approximately $70 million, eight in-house laboratories, approximately 189 full-time equivalent positions, as well as an extramural component which administers a $49 million grant and contract program.

This is a Civil Service position in the Senior Executive Service (SES) with a salary range from $56,945 to $63,800 per annum. In addition, M.D. candidates may be eligible for a Physician's Comparability Allowance up to $10,000 per year. (Alternately, candidates may be eligible for appointment in the Commissioned Corps of the U.S. Public Health Service.) The individual selected, if not presently in SES, must serve a one year probationary period.

Candidates must meet established mandatory professional/technical and managerial/executive requirements. Candidates will be further evaluated on the extent they exceed the minimum requirements and on additional factors including scientific reputation/peer recognition, training and education and honors and awards. A copy of the requirements may be obtained by contacting Mr. Sherman Hatchett, Personnel Management Branch NCI (301) 496-6864.

Applications (Personal Qualifications Statement SF-171) are to be sent to the above individual at: The National Cancer Institute, Building 31, Room 3A23, Bethesda, Maryland 20205, postmarked no later than February 29, 1984. A current curriculum vitae and bibliography must accompany all applications. Applications from women and/or minority group members are especially solicited.

The National Institutes of Health is an EQUAL OPPORTUNITY EMPLOYER.

(NW188)A

ICRISAT

INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS (ICRISAT) near Hyderabad, India needs the following personnel

PULSE AGRONOMIST

(To be based at ICRISAT Center, Hyderabad, India)

To lead programs of research involving both agronomy and microbiology in relation to N fixation, and to work on chickpea and pigeonpea in a multi-disciplinary program also involving plant breeding, entomology, pathology, and biochemistry. The agronomic work will include studies on varietal response to chemical inputs, response to environmental factors controlling yield such as photoperiod, light, and temperature, and studies on limiting factors including drought, high salt concentration, and high soil pH. The microbiology program will emphasize the development of suitable strains of rhizobia for inoculants and selection criteria for legume host genotypes for breeding programs. Research on legume inoculant production will also be an important activity. *Qualifications:* PhD in Agronomy, Physiology or Microbiology. Significant research experience in crop production and in relation to N uptake or fixation by legumes. Experience in organizing, guiding and coordinating research and in working with a team of research scientists. A minimum of 10 years experience is desirable.

AGRONOMIST — FARMING SYSTEMS RESEARCH PROGRAM

(To be based at ICRISAT Center, Hyderabad, India)

To conduct research both at the research center and at selected on-farm locations. His specific responsibility will be to lead a research team in the development and evaluation of cropping system components of farming systems technologies, working in close collaboration with principal scientists in the farming systems and crop improvement programs. The objectives of ICRISAT's Farming Systems Research Program are to identify more productive farming systems for defined agroclimatic regions. The technologies developed must recognize the resource base of farmers in the rainfed seasonally dry semi-arid tropics, and meet the special needs of small farm holders of limited means. *Qualifications:* PhD in Agronomy (crop production, soil management) or Crop Physiology. Several years of relevant experience and a solid record of scientific achievement with evidence of a systematic approach to the solution of agronomic problems are essential. Should be able to work with scientists from other disciplines to identify and solve crop production problems and with scientists from a wide range of cultures and professional levels in an international team of scientists. Research and/or teaching/extension experience in a developing country is desirable.

CROPPING SYSTEMS AGRONOMIST

(At ICRISAT Sahelian Center, Niamey, Niger)

To work in close coordination with other farming systems research staff in West Africa and at the ICRISAT headquarters. He will work in a team whose work is focused on evolving, evaluating, and testing more productive millet and groundnut based cropping systems for sub-sahelian agroclimatic region. The technologies developed must recognize the resource base of the small farmers and be acceptable to small farm holders of limited means. Research conducted by the agronomist will involve both on-station and on-farm research. For the latter, farmers participate in testing new soil management and cropping systems. *Qualifications:* PhD in Agronomy (crop production, soil management) or Crop Physiology with relevant field experience in crop production. Several years of post-doctoral experience working with scientists of other disciplines on research to identify and solve crop production problems is required; applicant also must be able to work with scientists from a range of cultures and professional levels in an international team of scientists. Agronomic research and/or teaching/extension experience in a developing country and a working knowledge of French desirable.

Salary is at principal staff level, according to qualifications and experience, with attractive fringe benefits.

Send résumé and names of three referees by 15 March 1984 to Director General, ICRISAT Patancheru PO 502 324, A.P., India.

(W658)A

In keeping with our historic interest in reproductive biology and our strong commitment to cell biology, the Worcester Foundation is seeking faculty candidates at all levels with expertise in

Cellular and Molecular Aspects of Reproduction

The Worcester Foundation offers excellent facilities and a highly interdisciplinary and interactive environment for independent researchers. The Foundation's campus is located in the pleasant New England town of Shrewsbury, Massachusetts, 38 miles west of Boston. Interested candidates should send a curriculum vitae with statement of research interests and names of three references to: **David E. Wolf, PH.D., C/O PERSONNEL OFFICE, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, Massachusetts 01545, USA**

AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER M/F/H

WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY

(NW107)A

UNIVERSITY OF SYDNEY LECTURERSHIP/SENIOR LECTURESHIP IN PATHOLOGY

Applications are invited from graduates with registrable medical qualifications and suitable postgraduate training to teach and conduct research in Pathology. Preference will be given to those with higher degrees in a field related to pathology. The Department conducts courses for students in the Faculties of Medicine, Dentistry and Science.

Salary range: Lecturer $A23,394 — $A30,734 pa Senior Lecturer $A31,390 — $36,585 pa Paraclinical loading $A5,848 pa.

Appointments to Lectureships/ Senior Lecturerships are usually probationary for three years and the University reserves the right not to proceed with any appointment for financial or other reasons.

Applications including curriculum vitae, list of publications and names of three referees by 3 February 1984 to the Registrar, University of Sydney, NSW 2006, Australia, from whom further information is available. Enquiries about teaching and research should be directed to Professor D A Cameron, Head of the Department of Pathology.

Equal employment opportunity is University policy.

Further general information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W659)A

HAMPSTEAD HEALTH AUTHORITY

HEAD OF MEDICAL PHYSICS

Salary: £16,090 — £19,117 plus £1,042 London Weighting Allowance

Applications are invited from appropriately qualified and experienced physicists for the above post following the retirement of Mr. H. S. Williams.

The person appointed will be required to advise the District Management Team of developments in Medical Physics.

The department has a staff of 20 and is engaged upon routine clinical services and research and development. The main areas of activity are in radiotherapy physics, nuclear medicine and radiation protection. There is also a substantial commitment to scientific computing and the department has its own electronics work shop.

Informal preliminary interview visits are encouraged and may be arranged by contacting Mr. J. Agnew, Acting Head of Medical Physics on 01-794 0500 ext 3208. Application and job description may be obtained from the Personnel Department, The Royal Free Hospital, Pond Street, Hampstead, NW3 2QG. Telephone 01-794 0500 ext 4286.

Closing date for completed applications 31 January 1984.

(969)A

HAMPSTEAD HEALTH AUTHORITY

SENIOR TOXICOLOGIST

The Rohm and Haas Company, a leading multinational chemical company, has an opening for a Senior Toxicologist at our corporate research headquarters located in Spring House, PA, an attractive suburb of Philadelphia.

The position is for an experienced toxicologist to provide liaison between toxicology and the industrial chemical business of the Rohm and Haas Company worldwide. The individual will be responsible for planning toxicology programs for existing and new industrial chemicals as well as for U.S. premanufacturing notices and EEC premarketing notices. Liaison with Regulatory Agencies in the U.S. and in Europe will be required.

A successful candidate will need a strong background in chemistry and a minimum of 3-5 years industrial toxicology experience, with an M.D., D.V.M. or Ph.D. degree or equivalent in a health-related discipline. The individual must be multilingual, e.g. English, French, and German, and must be willing to travel. Relocation in 2-3 years to Europe may be required.

We offer an excellent salary and benefits program, including relocation assistance, and an environment conducive to professional advancement. Please send a detailed resume, in confidence, to:

Recruiting and Placement #484

ROHM AND HAAS
PHILADELPHIA, PA. 19105

(NW189)A

An equal opportunity employer M/F.

National Institutes of Health Public Health Service

SENIOR INVESTIGATOR

The Laboratory of Biochemistry, Division of Cancer Biology and Diagnosis, National Cancer Institute, seeks a senior investigator who will conduct a strong independent research program. Applicant's interests should be in the molecular biology/genetics of prokaryotes or lower eukaryotes; they should have an outstanding record of original and independent research accomplishments. The position will be classified as GS 14-15 with a salary range of $42,722 to $65,327 per year depending on qualifications.

Send CV, bibliography, and a short statement of current research interests to Maxine Singer, Chief, Laboratory of Biochemistry, Building 37, Room 4E-28, National Institutes of Health, Bethesda, MD 20205 by February 1, 1984. (Telephone Number (301) 496-5957) U.S. citizenship and an M.D. or Ph.D. or equivalent in experience and training required. NIH is an Equal Opportunity Employer.

(NW217)A

Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Public Health Service
Department of Health and Human Services

ASSOCIATE DIRECTOR FOR BIOLOGICAL CARCINOGENESIS

The National Cancer Institute (NCI) develops the overall national strategy against cancer, coordinates Federal Government and non-Federal Government cancer programs, and supports international programs of cancer research. The Division of Cancer Cause and Prevention directs the NCI's major research effort related to determining the cause of cancer as well as basic research on possible means of cancer prevention. The Associate Director for Biological Carcinogenesis plans, directs, and conducts a basic research program in which the role of biological agents, genetic sequences, viral genes, and combinations of viral and cellular genes in carcinogenesis is investigated. This research, which emphasizes molecular biology, includes isolation and analysis of proteins responsible for transformation and identification of the genetic sequences that code the proteins, in addition to the role of RNA- and DNA-containing viruses in certain forms of cancer. The incumbent will be responsible for management of five intramural laboratories and an extramural component which administers a $51 million a year grant and contract program comprising the biological carcinogenesis activities of the DCCP. These activities are supported by a budget of approximately $70 million and approximately 181 full-time equivalent positions.

This is a Civil Service position in the Senior Executive Service (SES) with a salary range from $56,945 to $63,800 per annum. In addition, M.D. candidates may be eligible for a Physicians' Comparability Allowance up to $10,000 per year. (Alternately, candidates may be eligible for appointment in the Commissioned Corps of the U.S. Public Health Service.) The individual selected, if not presently in SES, must serve a one year probationary period.

Candidates must meet established mandatory professional/technical and managerial/executive requirements. Candidates will be further evaluated on the extent they exceed the minimum requirements and on additional factors including scientific reputation/peer recognition, training and education and honors and awards. A copy of the requirements may be obtained by contacting Ms. Patricia Rados, in the Personnel Management Branch, NCI on (301) 496-6864.

Applications (Personal Qualifications Statement SF-171) are to be sent to the above individual at: The National Cancer Institute, Building 31, Room 3A23, Bethesda, Maryland 20205, postmarked no later than February 29, 1984. A current curriculum vitae and bibliography must accompany all applications. Applications from women and/or minority group members are especially solicited.

The National Institutes of Health is an EQUAL OPPORTUNITY EMPLOYER.

(NW191)A

CSIRO

Plant Physiologist

$A24,344 – $A35,806
Division of Forest Research
Mount Gambier SA
Australia

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Forest Research is a member of the Institute of biological Resources.

General: The Division of Forest Research has important programmes in Mount Gambier, South Australia concerned with root growth and functions, nutrient supply to and uptake by trees, nutrient transiocation within trees, and water use by trees. Since these processes are strongly affected by soil water content and the water status of the trees, it is important to initiate work on whole plant water relations, the effects of tissue water status on growth and the interactions between water and nutrition.

The Mount Gambier Station has a total staff of fourteen. The Officer-in-Charge is responsible for integration of the regional programmes and for administration. The appointee will report to him on a day to day basis. General scientific direction comes from the Chief and the Program Leaders in Canberra although most operational aspects of the research will be the responsibility of the appointee. He/she will be in the Soils and Nutrition Program (Leader Dr. T. Talsma) and will be expected to work closely with the Officer-in-Charge at Mt Gambier, Dr. E. K. S. Nambiar. Collaboration with scientists in the ecophysiology program (Leader Dr. S. Linder) will be encouraged.

Duties: The appointee will undertake research into plant water use and the effects of soil-plant water relations on nutrient uptake and movement, and on short and long-term growth patterns.

Qualifications: Applicants should have a PhD degree or equivalent qualifications, supported by proven research activity in a relevant field. Some knowledge and background in environmental physics, as well as plant physiology, is essential.

Tenure: Appointment is for a three year term, with the possibility of a further term not exceeding two years. Australian Government superannuation benefits are available.

Applications: Stating full personal and professional details with the names of at least two referees and quoting reference No. A2872, should be directed to:

The Chief,
CSIRO Division of Forest Research,
PO Box 4008,
QUEEN VICTORIA TERRACE ACT 2600.
AUSTRALIA.
By February 10, 1984.

For further details about the Regional Station contact: Dr E. K. S. Nambiar (087) 25 5555. (NW646)A

DIRECTOR, CENTER FOR ENVIRONMENTAL MANAGEMENT

Tufts University is developing a National Center for Environmental Technology, Health & Policy Research, focusing on issues of hazardous waste. The Director will be responsible for all aspects of the planning and management of the Center. Initial tasks include articulation of the scientific program with Tufts faculty, the sponsoring agency and other public and private sector groups; recruitment, with Tufts faculty, of key scientific personnel; facility planning; and development of the scientific and administrative procedures of the Center. The successful candidate will be an accomplished researcher and administrator, with an advanced degree (e.g., Ph.D., J.D., M.D.) in an appropriate discipline; skill in management of a complex organization; and significant experience in hazardous waste management/research/regulation. The Director will be eligible for professorial appointment in the appropriate Tufts department. Inquiries or resumes (deadline April 1, 1984) should be directed to: Dr. Sol Gittleman, Provost, Chairman, Search Committee, Tufts University, Medford, MA 02155. An Affirmative Action/Equal Opportunity Employer.

(NW205)A

Biotechnology Careers

Challenging career opportunities currently exist in the expanding Biotechnology Section of the Celanese Research Company. The following research positions are available:

BIOCHEMICAL ENGINEER

The successful candidate will join our professional team of dedicated scientists engaged in developing biotechnological processes for the production of a variety of chemicals. The primary responsibility of this R&D position will be to provide effective interfacing between teams of research biologists and chemical engineers towards the successful commercialization of current industrial research programs. In addition to a Ph.D. in Biochemical Engineering or Microbiology applicants must possess a minimum of 2 years in-depth experience in fermentation with an emphasis on bioprocess control and development. Good communication skills and the ability to relate to people on all levels is essential.

MOLECULAR GENETICIST

The primary responsibility of this R&D position will be the application of state-of-the-art genetic and recombinant DNA technology to the development of microorganisms and processes for the production of a variety of chemicals. In addition to a Ph.D. in Bacterial Genetics or Molecular Biology the ideal candidate should have proven expertise in the manipulation of bacteria (preferably gram-negatives) using classic genetic technology in addition to expertise in the manipulation of microorganisms using recombinant DNA technology.

BIOCHEMIST/ PHYSIOLOGIST

This position requires a Ph.D. in Biochemistry or Microbial Physiology, preferably with at least 2 years postdoctoral experience in the area of microbial metabolism and/or the genetic manipulation of microorganisms. The successful candidate will be required to participate in on-going research programs in addition to initiating his/her own programs.

Located at our corporate research center in Summit, New Jersey, these positions offer salary commensurate with credentials and a comprehensive benefits package in addition to a professionally stimulating and rewarding environment.

To be considered for one of these exceptional opportunities please forward a resume with salary history in confidence to **Employee Relations, Dept. SB4, Celanese Research Company, 86 Morris Ave., Summit, New Jersey 07901.**

(NW207)A

CELANESE SUMMIT TECHNICAL CENTER

An equal opportunity employer M/F/H/V

SCOTTISH HEALTH SERVICE COMMON SERVICES AGENCY SCOTTISH NATIONAL BLOOD TRANSFUSION SERVICE

DIRECTOR: PROTEIN FRACTIONATION CENTRE

(Re-advertisement)

Salary £22,856 — £26,639

Applications are invited for the above post which is a senior management position within the Scottish National Blood Transfusion Service. The successful applicant, who will be expected to take up the appointment on 1 April 1984, will be responsible to the Management Committee of the Common Services Agency for the processing of all plasma received by the Protein Fractionation Centre and its conversion to therapeutic blood products.

Previous experience in the manufacture of biologicals in the industrial setting and in plasma fractionation would be an advantage.

Whitley Council Terms and Conditions of Service apply. Appropriate information and application forms are available from the Appointments Section, Scottish Health Service, Common Services Agency, Trinity Park House, South Trinity Road, Edinburgh to whom applications should be returned by 2 February 1984. Further particulars and any clarification may be obtained by telephoning 031 664 2317 (ext 259). Please quote reference number U564/N. (947)A

UNIVERSITY OF ALBERTA

DEPARTMENT OF ZOOLOGY

The Department will make one tenurable appointment at the rank of

ASSISTANT PROFESSOR

(minimum $29,720 per annum, 1983-84 scale) on July 1, 1984

This appointment is subject to the availability of funds. Applications are requested from candidates with a PhD degree or equivalent, preferably with postdoctoral experience. The Department is interested in an individual with training and research experience in the field of regulation and/or modelling of freshwater fish populations. The successful candidate will be expected to establish a vigorous research program in this field, in addition to sharing in the teaching program in the areas of vertebrate biology, ecology and fisheries management.

Interested candidates should send a curriculum vitae, a brief statement of research objectives, a list of publications and reprints, and the names and addresses of three referees to: Dr R E Peter, Acting Chairman, Department of Zoology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada. The deadline for receiving applications is March 7, 1984.

The University of Alberta is an equal opportunity employer but, in accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. (NW202)A

UNIVERSITY OF ALBERTA

DEPARTMENT OF ZOOLOGY

The Department of Zoology, University of Alberta invites applications from individuals who would qualify for support as a Research Scholar at the rank of non-tenured Assistant Professor (1983-84 salary $29,720 to $41,820/annum) or non-tenured Associate Professor (1983-84 salary $37,420 to $53,658). The Department is interested in an individual with training and research experience in the field of vertebrate developmental neurobiology, at the cell or molecular level. Applicants should minimally have a PhD degree or equivalent and postdoctoral experience, and must be highly qualified investigators capable of a vigorous independent biomedical research program. This position will be long-term and will be funded by an external agency such as the Alberta Heritage Foundation for Medical Research but the incumbent will enjoy the privileges of the University academic rank at which the appointment is made. Scholars normally receive extensive support to initiate a research program.

Applicants should forward their curriculum vitae with names of three referees and selected reprints to: Dr R E Peter, Acting Chairman, Department of Zoology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada by March 7, 1984.

The University is an equal opportunity employer, but in accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. (NW204)A

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF CHILD HEALTH

A POST-DOCTORAL RESEARCH FELLOW

is required for a project investigating the underlying causes of Respiratory Distress in premature infants. The work will include the use of cultured type II alveolar cells from fetal lung as a model system for the development of the capacity for surfactant production. It is intended to study the hormonal regulation and induction of the enzymes of lecithin biosynthesis and of surfactant-associated proteins in these cultures. Experience of protein purification, immunological or tissue culture techniques would be an asset.

The appointment will be for three years starting as soon as possible. The starting salary will be between £7,190 — £8,530 per annum on the Grade IA scale for Research and Analogous Staff.

Further information may be obtained from Dr A D Postle, Department of Child Health, Southampton General Hospital, Southampton (0703) 777222 ext 4336.

Applications (2 copies) giving date of birth, full curriculum vitae, together with the names and addresses of two referees should be sent to Mr A J Small, Staffing Department, The University, Southampton, Hampshire SO9 5NH, as soon as possible. Please quote ref: 1271/R/N. (976)A

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
DEPARTMENT OF PHYSICS
IMAGE ANALYSIS GROUP

APPOINTMENT OF TWO POSTDOCTORAL RESEARCH ASSISTANTS

Two posts for Research Assistants are available, to be filled as soon as possible, for work on the interpretation of images taken using synthetic aperture radar (SAR). The appointments are funded by Ministry of Defence and are tenable each for two years, with possible extension to a third and final year depending on progress.

Both Research Assistants should have doctorate degrees or a similar level of research experience. One post will be primarily theoretical and the other primarily computational/numerical analysis of large image data sets. The theoretical work concerns eg scattering from rough surfaces and image resolution enhancement incorporating the use of external constraints.

Candidates for the theoretical post should have experience in the general area of scattering theory (eg as in optical physics or particle physics), computational image analysis or applications of "fuzzy knowledge". Candidates for the computational post should be competent Fortran computer programmers and might have experience of the use of unitary transforms in physics — ideally, experience of the algorithms and transforms used in image processing. A DEC VAX 11/780 computer system is available in this Department.

The starting salary of one post will be £8,080. The starting salary for the second post is £8,975 pa. London Allowance of £1,186 pa is paid in addition to each salary. Further details can be obtained from Professor R E Burge, Physics Department, Queen Elizabeth College.

Applications with the referees should be sent by 1st February 1984 to Mrs J Staight, Assistant College Secretary, Queen Elizabeth College, Campden Hill Road, Kensington, London W8 7AH. (994)A

UNIVERSITY OF OXFORD
UNIVERSITY LECTURESHIP IN BIOCHEMISTRY AND TUTORIAL FELLOWSHIP AT ST ANNE'S COLLEGE

Applications are invited for a lectureship to be held in the Department of Biochemistry in the field of Enzymology. Stipend according to age on the scale of £7,190 — £15,085. The successful candidate may be offered a stipendiary Tutorial Fellowship at St Anne's College. Details of the post from Professor J Mandelstam, Department of Biochemistry, South Parks Road, Oxford OX1 3QU, to whom completed applications (8 typed copies, two from overseas candidates) and the names of two referees should be sent by 25 February 1984. (973)A

RESEARCH SCIENTIST/PRINCIPAL INVESTIGATOR

Biomedical Application of NMR and Stable Isotopes
Los Alamos National Laboratory

Applications are being accepted for an established research scientist to lead an active biochemistry/NMR program in the Isotope and Nuclear Chemistry Division. This position requires serving as the Principal Investigator for two component programs: "A National Stable Isotope Resource at Los Alamos," supported by the Division of Research Resources of the National Institutes of Health (NIH), and a related Department of Energy (DOE) sponsored program, "Carbon-13 NMR and Metabolism." Direction of the Stable Isotopes Resource will include the supervision of a basic research program to develop new biosynthetic approaches for the synthesis of compounds selectively labeled with stable isotopes of carbon, nitrogen, and oxygen. The DOE program activities include the design and execution of **in vivo** NMR studies of the metabolism of isotopically labeled compounds. The latter program will be carried out in collaboration with the University of New Mexico/Los Alamos Center for Non-Invasive Diagnosis by NMR spectroscopy.

A demonstrated record of accomplishment in the area of high resolution NMR spectroscopy is required. Additional experience in the areas of microbiology, biosynthesis, and analysis of metabolism by NMR techniques is also highly desirable. U.S. citizenship is required. This position provides a unique opportunity for the development of biomedical applications of the stable carbon, nitrogen, and oxygen isotopes produced at Los Alamos.

The Laboratory is operated by the University of California for the Department of Energy. We offer competitive salaries and a full range of employee benefits. Our beautiful mountain location in northern New Mexico has exceptional recreational activities. The school system is excellent and the community provides a casual, relaxed lifestyle.

For further information call Basil I. Swanson, Chairman, Search Committee, Isotope and Structural Chemistry Group, at 505-667-6045.

To apply, please submit resume and letters of recommendation in confidence to:
Madeline Lucas, DIV-84-Q
Personnel Services
Los Alamos National Laboratory
Los Alamos, NM 87545

University of California
Los Alamos
Los Alamos National Laboratory
Los Alamos, New Mexico 87545

An Affirmative Action/Equal Opportunity Employer (NW215)A

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
DEPARTMENT OF NEUROLOGY
RESEARCH ASSISTANT 1A

Required a postdoctoral research assistant to work on a project involving the electron microscopy of epileptic brain damage. Applicants must have experience in the electron microscopy of the CNS and have a minimum of three years experience of working in a university or hospital in the UK. The project is funded for two years from the Wellcome Trust.

Salary on the Research Staff 1A scale within the range £7,190 — £8,975 pa plus £1,186 pa London Allowance.

For further information contact Dr B Meldrum (Tel: 01-703 5411). For application form send stamped addressed envelope to the Assistant Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF (quoting Ref BM/N). Closing date for applications: 2 February 1984. (984)A

THE UNIVERSITY OF CALGARY
Research in Biochemistry

The University Biochemistry Group which comprises members of the Faculties of Medicine and Science offers an interdisciplinary environment for graduate and post-doctoral research in Biochemistry, Molecular Biology and Biotechnology. Highly qualified students with training in biochemistry, chemistry, biology or related disciplines may be admitted to programs leading to the MSc and PhD degrees.

Research/teaching assistantships ($8,775.00 to $11,700.00/yr) are available from the University. Studentships ($11,400.00/yr plus $2,500.00 research allowance) and post-doctoral fellowships ($20,760.00/yr plus $5,000.00 research allowance) are available from the Alberta Heritage Foundation for Medical Research and may be applied for via The University of Calgary. Interested individuals should send a curriculum vitae which includes courses taken and grades obtained and a statement of research interests to:

Dr Barry Lesser, Chairman
Graduate Studies Committee
University Biochemistry Group
The University of Calgary
2500 University Drive N.W.
Calgary, Alberta, Canada T2N 1N4.

(NW29)A

Neuropharmacologist

From £8650 p.a. gross *Ware, Herts*

Glaxo Group Research is Britain's leading pharmaceutical research organisation, with a distinguished reputation for developing safe and effective medicines.

Our Neuropharmacology Department is primarily concerned with the identification of novel and improved medicines for the treatment of diseases of the central nervous system, pain and diseases of the gastrointestinal tract.

Applications are invited from neuropharmacologists, who have recently obtained a PhD degree, to join one of our CNS project teams, This position offers considerable scope for innovative basic research and interaction with other neuroscientists in a multidisciplinary environment.

The starting salary will be related to experience. Our generous benefits package includes non-contributory pension and life assurance, participation in Glaxo Group profitability and, where appropriate, generous assistance with relocation to this attractive area of Hertfordshire.

Please apply to: Dr. D. J. Humphreys, Senior Personnel Officer, Glaxo Group Research Limited, Ware, Herts. SG12 0DJ. Tel: Ware (0920) 3993.

Glaxo Group Research Ltd.

(952)A

Recombinant

DNA Research

As part of its continuing commitment to innovatory research, Beecham Pharmaceuticals is currently engaged in the use of recombinant DNA technology as a means of obtaining human proteins of clinical utility.

We now have a permanent position for a post-doctoral molecular biologist within a group dedicated to cloning, characterisation and expression of human genes. Candidates should have at least two to three years relevant experience in recombinant DNA techniques. Experience of gene expression, though not essential, would be advantageous.

We offer a competitive salary and an extremely pleasant working environment on a 26 acre research site near Epsom Downs. Other benefits include free life assurance, a non-contributory pension scheme, a subsidised staff restaurant and flexible working hours. Furthermore, you will be working for a highly successful British company of international standing. Relocation costs may be paid where appropriate.

For an application form please write to or telephone the Site Personnel Manager, Beecham Pharmaceuticals Research Division, Biosciences Research Centre, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey. Telephone: Burgh Heath 53344 (ext 2207). Closing date: 9th February 1984. (981)A

Beecham Pharmaceuticals

DIAGNON CORPORATION

IMMUNOBIOCHEMIST

Diagnon Corporation, a developer and marketer of diagnostic reagents for clinical laboratories, has an immediate need for an energetic, versatile and creative PhD to serve as Group Leader of our Immunobiochemistry R&D program. Directing an initial staff of 5 technicians, the primary responsibility of the Group Leader is to develop state-of-the-art non-isotopic immunodiagnostic reagent systems utilizing monoclonal antibodies. In addition, the Group Leader will supervise, in the near term, technicians responsible for production of Diagnon's current RIA-based anemia diagnostic products.

The ideal candidate will have a PhD in protein biochemistry with strong training in immunology and a minimum of 5 years R&D experience, including but not limited to, ELISA, IRMA and/or FIA immunoassay development. Significant hybridoma expertise a definite plus.

Diagnon is prepared to offer a very attractive compensation package to the successful candidate for this challenging position with considerable growth potential that reports to the Vice President R&D. If you have an outstanding record of growth and accomplishment in immunobiochemistry R&D, we strongly encourage you to submit your résumé in complete confidence to

Diagnon Corporation
PO Box 960 Dept. 200
Laurel, MD 20707. (NW196)A

CSIRO

Research Fellow

$A24,344 – $A35,806

Division of Entomology
Darwin NT Australia

Field: Biological control of Northern Territory weeds.

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Entomology is a member of the Institute of Biological Resources.

General: The Division is the main centre in Australia for entomological research, which includes programs concerned with pest management. There is an ongoing program in biological and chemical control of Northern Territory weeds in collaboration with NT Department of Primary Production and the Australian Centre for International Agriculture Research. With the financial support of the NT Department of Primary Production, this program is being extended to include studies of week biology and population dynamics.

Duties: To undertake research on the biology and population dynamics of *Mimosa pigra, Sida spp.* and *Hyptis suaveolens* in the Northern Territory. The appointee will be a member of a research team which includes CSIRO and Department of Primary Production staff and will participate in developing strategies for control of these weeds. The research will be integrated with that of other staff working primarily on biological and chemical control. A significant involvement in field studies is anticipated.

Qualifications: A PhD degree in science with emphasis on ecology and population dynamics, or equivalent qualification, together with experience in the field of population dynamics.

Tenure: A term of two years and six months. Australian Government superannuation benefits are available.

Applications: Stating full personal and professional details, the names of at least two referees and quoting reference No M8009 should be directed to:

The Officer-in-Charge,
CSIRO Division of Entomology,
Private Bag No 3,
INDOOROOPILLY QLD 4068.
AUSTRALIA.
By February 10, 1984. (W643)A

Agriculture Research

The Northern Regional Research Center, US Department of Agriculture (USDA) is seeking skilled and imaginative scientists for the programs listed below.

1) Genetic engineering of rumen microorganisms. This research will focus on understanding and using the fermentative and biosynthetic properties of genetically modified obligate anaerobes for improved energy and protein metabolism and fermentation technology. Positions are open for a geneticist, a biochemist, and a microbiologist.

2) Control of Fusaria growth and mycotoxin production. The main emphasis will be on Fusaria and their trichothecene toxins employing genetic and biochemical techniques to define the primary metabolic events that trigger toxin production. Positions are open for biochemists, a plant pathologist and a geneticist.

3) Biochemistry of seed development and maturation in corn. The research will focus on the dynamics and mechanism of partitioning carbon and nitrogen into the biosynthetic pathways that result in protein, lipid, and polysaccharide. Positions are open for plant physiologists and plant biochemists.

This Center employs about 150 scientists engaged in diverse programs of basic and applied research. Benefits include a stimulating professional environment and relocation expense assistance. Salary is based on qualifications and experience, GS-11/12, $25,366 to $30,402. US citizenship is required.

Send curriculum vitaes for position in 1) to Dr. R. J. Bothast; 2) to Dr. R. W. Detroy and 3) to Dr. C. G. Crawford, Northern Regional Research Center, USDA, ARS, 1815 North University Street, Peoria, IL 61604 (309) 685-4011. The USDA is an Equal Opportunity Affirmative Action Employer. (NW197)A

University of Groningen, The Netherlands

junior-level tenured position in the area of biological structures

(vac.nr. 840101)

at the **Department of Chemistry**

Applicants are solicited for a position in the Laboratory of Chemical Physics. Candidates must be capable of developing a research programme employing X-ray crystallographic approaches to address fundamental biological problems.

The succesfull candidate will also be responsible for the computer program package as it is developed and used by staff and students for the determination and the study of large biological structures. Duties will further include some teaching in chemistry.

The Chemistry Department offers a stimulating environment in the area of molecular biology. The Laboratory of Chemical Physics is excellently equipped with rotating anode tubes, diffractometers and camera's, an Evans & Sutherland Picture System 2 with an Enraf Nonius TV-detector/diffractometer to be added soon. A Cyber 170/760 is available at the University Computing Centre.

Preference will be given to candidates under 35 years of age who have completed a PhD.

The salary offered will be between Dutch fl. 3300 - 6500 monthly, before deductions. The position will be available immediately.

Applications:
Applications with curriculum vitae and list of publications should be sent within 6 weeks of the publications of this issue of Nature to:the Chairman of the Personal Department of the University, P.B. 72, 9700 AB Groningen, the Netherlands. (W648)A

elf aquitaine

Research center ELF BIO RECHERCHES Toulouse Labège (south France)

To reinforce one of our young and enthusiastic research teams in biotechnology, we wish to recruit:

1) a **BACTERIAL GENETIST** *réf. N 106*

2) a **MOLECULAR BIOLOGIST** *réf. N 107*

with extensive experience in gene cloning technology,

3) a **BIOCHEMICAL ENGINEER** *réf. N 108*

with industrial experience in animal cell fermentation.

Positions 1) and 2) are senior appointments for which several years post doctoral research experience are required.
Successful candidates will assume full responsibility for their contribution to our multidisciplinary research projects and will be expected to maintain their high scientific level.
Knowledge of french would be an advantage, but is not essential.
Our new, well equipped research laboratories and pilot plants are situated in TOULOUSE near university and government research laboratories. Toulouse has an international airport and is within easy reach of the Mediterranean Coast.and the Pyrenees.

Full curriculum vitae together with a hand-written letter of application should be sent quoting selected reference to:

J. Palmier Elf Bio Recherches BP62 F. 31320 Castanet Tolosan France. (W657)A

MLM Conseil

SENIOR ORGANIC GEOCHEMIST

Division Head Required for Interdisciplinary Exploration Team

Woodward-Clyde Oceaneering, an expanding International Geological consulting firm based in Aberdeen, invites applications from individuals with a PhD Degree and a minimum of five years experience in Petroleum Geochemistry. Responsibilities will include developing and managing geochemical hydrocarbon exploration programmes in frontier basins, both terrestrial and marine, utilising current theories in organic geochemistry. Knowledge of a variety of instrumental methods such as GC, GC/MS, Fluorescence, Pyrolysis and Isotope Ratio/MS is desirable.

This position provides the opportunity of working with a multi-disciplinary exploration team consisting of Geologists, Geophysicists and Engineers. Résumés will be treated confidentially. Please state availability. The successful candidate would be expected to assume duties in March/April 1984.

Salary will be negotiated within the range of £15,000 to £22,500, to be commensurate with experience. Interviews will be scheduled in London and Aberdeen during the week of 30 February 1984. Reply to Box No 925 c/o Nature, 4 Little Essex Street, London WC2R 3LF. (925)A

UNIVERSITY OF GUELPH
ONTARIO VETERINARY COLLEGE
DEPARTMENT OF VETERINARY MICROBIOLOGY AND IMMUNOLOGY

CHAIRMAN

Applications and nominations are invited for the above position which will become vacant on July 1, 1984.

The Department embraces the disciplines of Veterinary Microbiology, Immunology, Epidemiology, and Public Health.

Candidates should possess the D.V.M. degree or equivalent and advanced training to the PhD level in one of the above disciplines.

The ability to administer and lead a dynamic department is essential.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

Applicants should submit a curriculum vitae, a list of their publications, and the names of three referees.

Applications will be accepted until March 15, 1984 or until the position is filled, and should be submitted to: Dean, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Position subject to final budgetary approval. Applications accepted until March 15, 1984. (NW200)A

RHODES UNIVERSITY INSTITUTE FOR FRESHWATER STUDIES

LIMNOLOGIST

Required for a three year contract project on eutrophication and limnology of turbid reservoirs, with the likelihood of extension by two years. The project entails the characterization of nutrient loading rates and the development of limnological management options for the reservoirs.

Appointment from 1 June 1984 or as soon as possible thereafter will be as a Research Officer (R12,657 × 780 — R16,557 × 936 — R22,173 per annum) or Senior Research Officer (R16,557 × 936 — R24,045 per annum) depending upon qualifications and experience, with additional service benefits and assistance with relocation. In addition, a 12 per cent pensionable allowance is payable. Applicants will preferably possess a PhD, and a proven independent research capability. The project will entail regular travel.

Applications with curriculum vitae, representative reprints of publications and names of three referees should be sent to Professor R. C. Hart, Institute for Freshwater Studies, Rhodes University, Grahamstown, South Africa, by 24 February 1984. (W651)A

CSIRO

Senior Oceanographers

$A31,092 — $A42,210
(2 positions)
Division of Oceanography
Marine Laboratories
Hobart TAS Australia

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Oceanography is a member of the Institute of Physical Sciences.

General: The CSIRO Marine Laboratories, which include the Division of Oceanography and the Division of Fisheries Research is Australia's principal marine research institution, employing over 200 scientists and support staff investigating the physical, chemical and biological features, including fisheries, of the oceans around Australia.

Two chartered vessels (53m and 43m) are used for research and a modern oceanographic vessel is being built to replace the 43m vessel. The Marine Laboratories have a VAX 11/750 computer, and on-line access to a Cyber 76 computer is available.

New Laboratories are nearing completion in Hobart.

Duties: The appointees will initiate and conduct research into the dynamics of the ocean, and will be expected to develop interest in processes and phenomena relevant or applicable to the Australian regional oceans, seas, straits, or continental shelves. Depending upon suitability, appointees may be invited to lead groups and develop new research initiatives within Divisional programmes. One appointee may be expected to direct particular interest to the Australian Northwest Shelf region.

Qualifications: A PhD degree or equivalent qualifications with extensive experience and substantial evidence of original research achievement in the field of physical oceanography, or a closely related geophysical descipline. Applicants should desirably have had substantial experience and success in the design and conduct of offshore oceanographic experiments, and the interpretation of their results in relation to available theory and applications; and/or substantial success in the development and verification of theoretical models of ocean or atmosphere dynamics, and a combination of analytical and numberical expertise.

Tenure: Indefinite with Australian Government superannuation benefits available.

Applications: Stating full personal and professional details, the names of at least two referees, and quoting reference Nos A6814 and A6815, should be directed to:

The Chief,
CSIRO Division of Oceanography,
GPO Box 1538,
HOBART TAS 7001.
AUSTRALIA.
By February 10, 1984. (W644)A

ACADEMIC APPOINTMENT IN IMMUNOLOGY

The position is within the Immunology Research Center. The successful candidate will be expected to conduct an independent research program in fundamental or applied Immunology. Opportunities exist as well to participate in research programs already underway in the Center or at the Institut, to supervise and train graduate students, to become involved in production activities and/or to assume administrative duties.

QUALIFICATIONS: A candidate must have obtained a PhD or the equivalent from an accredited university in Canada or elsewere and must have a training in Immunology. Preference will be given to candidates with post-doctoral or other experience indicating that the candidate can pursue his or her research autonomously. Priority will be given to Canadian citizens and permanent residents of Canada. The candidate is expected to possess or to be willing to acquire a working knowledge of French.

LEVEL OF APPOINTMENT: Rank will be determined on the basis of the qualifications and experience and the salary will be according to the scale presently in effect at Institute Armand-Frappier (University of Quebec).

TO APPLY: Send your *curriculum vitae* including a list of publications, 3 letters of recommendation and any other pertinent information before February 29, 1984, to: Service des ressources humaines, Institut Armand-Frappier, 531 boulevard des Prairies, Case postale 100, Laval-des-Rapides, Laval, Québec H7N 4Z3. (NW208)A

THE MACAULAY INSTITUTE FOR SOIL RESEARCH
DEPARTMENT OF MICROBIOLOGY

HIGHER SCIENTIFIC OFFICER/ SENIOR SCIENTIFIC OFFICER

Applications are invited for the post of Higher Scientific Officer/ Senior Scientific Officer to study the microbial transformations of sulphur and trace elements in soil and the availability of these nutrients to plant roots. Candidates should possess a First or Upper Second Class Honours Degree in Microbiology or Plant Science and have a minimum of two years relevant post-graduate experience for appointment at HSO, and a minimum of 4 years at SSO or a post-graduate degree in Microbiology.

Experience in either soil microbiology, plant nutrition, mathematical modelling or with radio isotopes would be advantageous.

Salary £7,149 — £9,561 (Higher Scientific Officer), £8,970 — £11,476 (Senior Scientific Officer), per annum, according to qualifications and experience. Non-contributory superannuation scheme operates.

Forms of application and further particulars of the post may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ, to whom they should be returned by 3 February 1984. Quote Ref. 83/ 17. (966)A

UNIVERSITY OF STOCKHOLM
DEPARTMENT OF ZOOLOGY
Ethologist

Applications are invited for a position as "eo docent" (roughly equivalent to US assistant professor). The position is primarily intended for a person with several years of post-doctoral research experience.

The position is advertised for a period of three years, but is usually renewed for another three year period. Starting salary L 18 (Swedish crowns 113.556 per annum = roughly 9.798 £ or 14.070 US$).

The position is a research and teaching position, and requires a minimum of 75 hours of lecturing per annum and supervision of graduate student research, some administrative duties, and a vigorous research programme. Applicants are selected primarily on the basis of their scientific publications, but teaching experience is also required.

Current research at the division of ethology comprises projects on: mate selection, communication, social and reproductive behaviour in birds, fishes and mammals, control and decision making in animal fighting behaviour.

For further information contact: Professor T Radesäter, Department of Zoology, University of Stockholm, S-106 91 Stockholm, Sweden (Telephone 00946-8340860 ext 322).

Applications including curriculum vitae plus one copy of published, accepted papers, a short summary of the applicants research and teaching experience, names and addresses of three referees and any other papers the applicant wishes to submit should be addressed to The Board of Stockholm University, Fredrik Bloms Hus, University of Stockholm, S-106 91 Stockholm, Sweden, and received no later than March 31, 1984. Quote reference number 79/84. (W661)A

CENTRE INTERNATIONAL DE RECHERCHES MEDICALES DE FRANCEVILLE — GABON

RECHERCHE

VETERINAIRE CLINICIEN ET CHIRURGIEN EXPERIMENTE

Adjoint au Responsable de son Centre de Primatologie

FONCTIONS

* Surveillance sanitaire et soins aux primates et autres animaux de Laboratoire.
* Participation aux différents protocoles de recherche en cours, basés principalement sur la Biologie de la Reproduction.
* Participation à la Gestion du Département.

Excellentes conditions de travail.
Contrat début avril 1984 pour 2 ans renouvelables.
Conditions habituelles d'expatriation.

Candidatures à adresser à Monsieur le Directeur Général du CIRMF, 28, Bd de Grenelle, 75015 PARIS, avec Curriculum Vitae, références professionnelles et prétentions. (W620)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF ORGANIC CHEMISTRY

SYSTEMS MANAGER

Applications are invited for an experienced person to manage the database in the LHASA program being carried out in the Wolfson CADOS Unit in the Department. This work is being undertaken in collaboration with LHASA UK (LHASA UK is a non-profit making consortium comprising the University of Leeds and leading pharmaceutical and agrochemical companies in the UK to develop the database for a program for computer-aided design of organic synthesis). The Appointee will co-ordinate these activities and help in the provision of a bureau service to users.

Applicants should possess a PhD or equivalent qualification, a good knowledge of synthetic organic chemistry, an interest in computers in chemistry and the ability to work with people.

The appointment will be for a fixed term of one year. It is hoped that further funding may become available from which subsequent appointments may be made.

Salary in the IA Range (£7,190 — £11,615). Appointment may be made at the upper end of the scale for a person with appropriate experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 45/30, to whom applications should be addressed. Closing date for applications 22 February 1984.
(972)A

OREGON STATE UNIVERSITY
GEOPHYSICS

Applications are invited for a 12-month, tenure track position for an Assistant or Associate Professor of Geophysics in the College of Oceanography to complement the present eight member Geophysics faculty. The applicant must have a PhD or equivalent, a demonstrated ability to conduct independent research in theoretical or observational geophysics, and to obtain research funding. Applicants will be considered in most areas of solid earth geophysics. Duties include teaching graduate courses in geophysics, supervising graduate students, and developing a program of grant-funded research.

Interested candidates should submit a résumé, names of three references and a brief statement of research plans by 1 March, 1984 to G Ross Heath, Dean, College of Oceanography, Oregon State University, Corvallis, Oregon, 97331.

Oregon State University, An Affirmative Action/Equal Opportunity Employer, complies with Section 540 of the Rehabilitation Act of 1973. (NW212)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)

CLINICAL PHARMACOLOGY RESEARCH ASSISTANT/ TECHNICIAN

is required to join a multidisciplinar team to work on a number of researcl projects related to the biological rol of prostaglandins thromboxance and leukotrienes. The successfu candidate will be involved in th preparation of specimens fron humans and experimental animal for gas chromatograph-mass spec trometry. The applicant must hav some experience in genera biochemical and/or analytica chemistry techniques to complemen our range of sophisticated equipmen (CG, HPLC, GC/MS) but trainin will be given. The applicant shoul possess an HNC, degree o equivalent in an appropriate subject

The position is supported by th MRC until July 1988. Salar depending upon qualification an experience from £5,087 to £5,20 plus £1,042 London Allowance year.

Application forms and furthe particulars are available from th Personnel Office, Royal Post graduate Medical School, 15 Ducane Road London W12 0HS quoting reference number 20/LD/N

Closing date for the receipt o applications: 3 February 1984.
(958)A

UNIVERSITY OF OXFORD

UNIVERSITY LECTURERSHIP IN ECONOMIC GEOLOGY

(Petroleum Geology/ Geophysics) in Association with St Cross College

Applications are invited for th above post, to be filled from October 1984 or as soon thereafter a possible, within the fields o reflection seismic data processing seismic interpretation and well-lo analysis. Stipind will be according t age on the scale £7,190 to £15,085 pa together with membership of th Universities' Superannuatio Scheme. The lecturership, open t men and women, may be held i conjunction with a non-stipendiar Fellowship at St Cross College.

Full particulars may be obtaine from Professor E A Vincent Department of Geology an Mineralogy, Parks Road, Oxfo OX1 3PR, to whom applications (te copies, or one in the case of oversea candidates) including curriculun vitae, a statement of experience an research interests, a list of publica tions, and the names and addresses o three referees, should be sent so as t arrive before 31 March 1984.
(987)A

Pharmacy/ Life Science Graduate

...for an important role in Product Registration

Greenford, Middlesex

We are currently going through a concentrated phase of introducing new products on to an increasing number of markets and this has created a need for an additional registration officer within our busy Regulatory Affairs Department.

You will help to obtain approval for the marketing of Glaxo pharmaceutical products within Central and South America, Eastern Europe, Africa, Middle and Far East Territories. This will necessitate identifying current regulatory requirements and ensuring that all documentation submitted by the Company to the various national authorities conforms with these requirements.

You should be a pharmacy or biological science graduate with at least one year's postgraduate experience, preferably within the pharmaceutical industry. Experience in product registration would be an advantage, but is not essential. You should be able to present scientific information clearly and concisely, orally and in writing, and have the capacity to work under pressure.

The Company offers excellent conditions of employment, including non-contributory pension scheme, bonus schemes, a subsidised canteen and an active sports and social club.

Please write or telephone for an application form to: Mr. R. W. Jones, Personnel Officer, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex, UB6 0HE. Tel: 01-422 3434 ext. 2707 quoting reference number U/510.

Glaxo Group Research Ltd.

(964)A

POSTDOCTORAL POSITIONS

ailable after May 1, 1984 to vestigate aspects of ribosome ucture and function in procaryotes d eucaryotes utilizing crosslinking, onoclonal antibodies and in vitro utagenesis. Experience in protein chemistry, hybridoma techniques d/or recombinant DNA chniques desired.

Send curriculum vitae and ferences to: Robert R Traut, partment of Biological Chemistry, hool of Medicine, University of lifornia, Davis, CA 95616, (916) 2-3354.

The University of California is an firmative Action/Equal Opportity Employer. (NW185)A

UNIVERSITY OF LONDON THE JOHN ASTOR CHAIR OF PHYSIOLOGY

enable in the Joint School of Medicine based on the Middlesex Hospital Medical School and University College London

e Senate invite applications for the ove Chair. Applications (11 pies) should be submitted to the ademic Registrar (N), University London, Malet Street, London C1E 7HU, from whom further rticulars should first be obtained.

Closing date for receipt of plications: 15 February 1984. (959)A

UNIVERSITY OF LONDON CHAIR OF MEDICAL ENTOMOLOGY AT THE LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

The Senate invite application for the Chair of Medical Entomology which became vacant on the resignation of Professor W W Macdonald on 31 March 1980.

Applications (10 copies) should be submitted to the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained. Closing date for receipt of applications: 24 February 1984. (961)A

KINGSTON POLYTECHNIC

SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES

RESEARCH IN POLYMERS

Applications are invited from honours graduates in chemistry for a research assistantship to synthesize new polymers, evaluate their physical properties and determine their structure. Registration for a higher degree is expected: (Supervisor: Dr G Pritchard). Salary range £5,592—£6,294 inclusive of London allowance.

Application forms can be obtained from the Personnel Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. Closing Date: 3 February 1984. (978)A

BIOMEDICAL RESEARCH OPPORTUNITIES NATIONAL INSTITUTES OF HEALTH NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

The Intramural Research Division of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) has openings for highly qualified applicants in biomedical research. The NIADDK, one of the 11 Institutes comprising the National Institutes of Health (NIH), offers a unique research opportunity for scientists from both the U.S. and abroad.

The majority of the research positions are filled by postdoctoral and sabbatical-leave scientists for periods of up to several years. For further information about specific research in the areas listed below, send inquiries to:

Recruitment Program, Dr. Jesse Roth
Director of Intramural Research
NIADDK
National Institutes of Health, 9000 Rockville Pike
Bldg. 10, Rm. 9N222/Bethesda, MD 20205

Chemistry	Enzymology
Biochemistry	Pharmacology
Molecular biology	Genetics
Mathematical biology	In-born areas of metabolism
Physical biology	Cell structure and differentiation
Neurobiology	Chemical physics
Immunology	

NIH is an Equal Employment Opportunity Employer

(NW216)A

HAGEDORN RESEARCH LABORATORY

MOLECULAR BIOLOGY OF DIABETES

The Hagedorn Research Laboratory has a scientist position available to conduct an active and innovative research program in the etiologi and pathogenesis of insulin-dependent diabetes, a disorder associated with autoimmune phenomena.

Responsibilities include the direction of graduate students and junior visiting scientists. Applicants should have training and strong interest and capacity to pursue research into eukaryotic gene expression with molecular cloning techniques. The position is a three-year appointment with possibilities for renewal.

Send curriculum vitae, name of three references and a statement of research interest to:
The Directors of Research,
Åke Lernmark and Bruno Hansen,
Hagedorn Research Laboratory,
Niels Steensensvej 6,
DK-2820 Gentofte,
Denmark.

The Hagedorn Research Laboratory is together with the Steno Memorial Hospital a WHO collaborating centre for research and training in the pathogenesis of diabetes. (W652)A

UNIVERSITY OF CAMBRIDGE

ASSISTANT LECTURESHIP IN TECTONICS OR MARINE GEOLOGY/ GEOPHYSICS

University Assistant Lectureship in Tectonics or Marine Geology/Geophysics to be filled as soon as possible. This is a full academic teaching appointment usually held by persons under the age of 30 on appointment and corresponding to the younger end of the national Lecturership scale. Stipend is age linked and ranges from £7,630 aged 25 to £9,425 aged 29.

Further information and applications (10 copies) together with a CV and names of 3 referees to be sent to Dr. P. F. Friend, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ by 10 February, 1984. (946)A

UNIVERSITY OF KEELE

VICE-CHANCELLOR

The Council and the Senate of the University of Keele wish to appoint a Vice-Chancellor to succeed Dr. D. Harrison, who will take up his appointment as Vice-Chancellor at the University of Exeter on 1st October 1984.

Applicants are invited to write in confidence to the Pro-Chancellor, Lord Rochester, c/o The Registry, University of Keele, not later than 29th February, 1984.

Suggestions of persons suitable for consideration would also be welcome. The University reserves the right to appoint to the post by invitation. Information about the University and further particulars of the post may be obtained from the Registrar, The University, Keele, Staffordshire ST5 5BG. (967)A

TEXAS A&M UNIVERSITY

DEPARTMENT OF BIOLOGY

HEAD

Applications and nominations are invited for the position of Head of the Department of Biology at Texas A&M University.

The Department of Biology, with a faculty of 38, is an administrative unit of the College of Science offering BS, MS and PhD degrees in Biology, Botany, Microbiology and Zoology.

Candidates for the position must have an earned doctorate, a distinguished record in research and teaching, and other professional accomplishments. Candidates are sought who have demonstrated leadership abilities and a commitment to developing strong academic and research programs in Biology.

Candidates should be available to assume the position by September 1, 1984. Nominations and applications, with résumés and names and telephone numbers of references, will be accepted until February 15, 1984 or until a suitable candidate is identified. Materials should be sent to: Dr C J Maxson, Associate Dean, College of Science, Texas A&M University, College Station, Texas 77843. Phone (409) 845 7361.

Texas A&M is an Equal Opportunity Affirmative Action Employer. (NW190)A

POSTDOCTORAL Position available July 1, 1984 for NIH-funded project studying ion transport by liver plasma membrane vesicles. Applicant must have recent PhD and a strong background in membrane transport. Salary support will be provided for two years. Send curriculum vitae and names of three references to: Bennett L Blitzer, MD, Director, Liver Study Unit, University of Cincinnati College of Medicine, 231 Bethesda Ave. (Room 6560), Cincinnati, Ohio 45267. (NW211)A

UNIVERSITY OF EDINBURGH CHAIR OF ANIMAL PRODUCTION

In the Edinburgh School of Agriculture

Applications are invited for the Chair of Animal Production, vacant following the appointment of Professor J H D Prescott to the Directorship of the Grassland Research Institute, Hurley.

The Professor will be responsible for teaching and research in Animal Production Science both within the University Department and the East of Scotland College of Agriculture.

Further Particulars may be obtained from the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, with whom twelve copies of the application should be lodged not later than 12th March, 1984. Overseas applicants need supply only one copy of application. Please Quote reference No 12/83. (956)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

POST-DOCTORAL RESEARCH ASSOCIATE

Applications are invited for the post of Research Associate to work on a project, funded by The Wellcome Trust, on the properties and regulation of dopamine D_2 receptor-effector mechanisms in the central nervous system. The project includes analysis of intra- and extracellular cyclic nucleotides, inositol phospholipid hydrolysis and receptor labelling techniques.

The successful candidate should possess a PhD (or shortly expect to receive one) and have some experience in biochemical pharmacology/neurochemistry. The appointment will be for three years on the 1A Scale for Research Staff (starting salary £7,630 pa).

Applications (with curriculum vitae and the names of two referees) to and further details from Dr S F Nahorski, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester LE1 7RH, UK (0533) 551234 ext 361

Closing date for applications 15th February 1984. (892)A

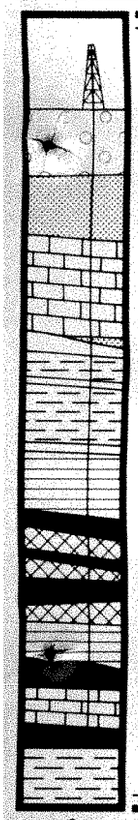
POST-DOCTORAL position available immediately to study mechanism of interferon action using recombinant DNA and gene transfer techniques. Previous experience of cell culture and/or molecular biology an advantage. Write to Dr John A Lewis, Department of Anatomy and Cell Biology, Downstate Medical Center 450 Clarkson Avenue, Brooklyn, NY 11203, USA with cv and one letter of recommendation. (NW214)A

nature classified

LONDON OFFICE
Keith Walesby
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

NEW YORK OFFICE
Miss Gene Fein
15 East 26 Street
New York, NY 10010
Tel: (212) 689 5900

TORONTO OFFICE
Peter Drake Associates
32 Front Street West
Suite 200, Toronto,
Ontario M5J 1C5
Tel: (416) 364 1623



Research in Earth Sciences

Opportunities for scientists with 3 years+ research experience

London based

BP Exploration Limited's exploration and development programme is one of the largest, most successful and ambitious in the oil industry. We are now operating in 23 countries including China, Venezuela, Canada, USA, Australia, New Zealand, Egypt and the UK North Sea.

We require scientists with a minimum of 3 years' research experience for the computer modelling groups of our London based geophysical research organisation. These groups undertake research and technical service work in various aspects of seismic prospecting and in less traditional areas such as basin modelling, fluid flow and palinspastic reconstruction.

Applicants must have studied to good honours degree level in geology or physics, but preferably both. They must be able to demonstrate a high level of numeracy, be skilled computer users and be experienced in using large quantities of observational data to validate hypothetical models. A post graduate or doctoral qualification is desirable.

Salaries and benefits are excellent and include non-contributory pension and relocation assistance, where appropriate.

Please write with details of qualifications and experience, quoting ref. U.119, to: Mrs. J. Wilkerson, Recruitment and Placement Branch, The British Petroleum Company p.l.c., Britannic House, Moor Lane, London EC2Y 9BU.

(995)A

BP

BP Exploration Limited

SILVICULTURE — ASSOCIATE Professor or Professor (tenure track, 12-month). Provide instruction (60%) and research (40%) in silviculture. Teach undergraduate and graduate silviculture course. Conduct research related to extensively managed forest. Opportunity for team research on cooperative programs with industry and federal and state agencies. Earned PhD in silviculture or closely related discipline with one degree in forestry required. Demonstrated teaching and research ability required. Send application, résumé, transcripts and three letters of recommendation to: Arnett C Mace, Jr, Director, School of Forest Resources and Conservation, University of Florida, Gainesville, Florida 32611. Closing date is February 2, 1984. (NW146)A

ASSISTANT PROFESSOR OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Applications are invited for a faculty position in the Department of Biochemistry and Molecular Biology, Harvard University. Distinction in research and teaching is essential.

Send curriculum vitae, three letters of recommendation, and a brief outline of future research plans before the end of April to Susan Young, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, Mass. 02138.

Harvard University is an Equal Opportunity/Affirmative Action Employer. (NW199)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF ZOOLOGY

Postdoctoral position in Neurobiology available immediately to study the physiology, morphology or pharmacology of local interneurones in the insect central nervous system. The project is expected to be funded by N.I.H. for three years. Salary from £7630 pa depending on qualifications and age.

Send C V and names of two referees to Dr M Burrows, Department of Zoology, Downing Street, Cambridge CB2 3EJ. (927)A

KINGSTON POLYTECHNIC HEAD OF DEPARTMENT OF GEOLOGY

Grade V: £16,035 — £17,736 inclusive of London Allowance

Applications for the above post are invited from geologists who have had substantial experience in research and/or the professional practice of Geology as well as in the teaching of the subject to degree level. The primary task of the person appointed will be to provide academic leadership for the discipline of Geology within the Faculty of Science.

Application forms and further details from the Personnel officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. Closing date 30th January 1984. (979)A

FACULTY POSITIONS IN ASTRONOMY AND ASTROPHYSICS

COLUMBIA UNIVERSITY

The Department of Astronomy and the Astrophysics Laboratory of the Department of Physics at Columbia University plan to make at least four (4) appointments at the assistant professor level, to begin in the summer or fall of 1984. One position in the Astrophysics Lab is for an experimental physicist or an astronomer with an extensive background in instrumentation development to work initially, on funded and planned X-ray and gamma-ray astronomy experiments which will fly on the Space Shuttle and future satellite missions. A second position in Physics is available for anyone with a background and/or interest in high energy astrophysics; research activities currently include analysis of *Einstein* and EXOSAT data, complementary radio and optical observing programs, as well as interpretive and statistical investigations. The Department of Astronomy also has two assistant professor positions open. Candidates with interests in any field of observational and theoretical astronomy are encouraged to apply. Current work in the Department of Astronomy includes observational and millimeter radio astronomy and theoretical investigations in star formation, galactic structure, and the atmosphere and winds of early-type stars. At least one tenure appointment is expected within the next five years, and successful applicants can expect to be considered for this position in due course.

Applicants should include a résumé and letter of interest together with a list of three persons from whom recommendations can be obtained upon request; the letter should state their preference (if any) among the above positions. Columbia University is an equal opportunity employer with a strong affirmative action program, and encourages applications from women and members of minority groups. Mail applications to either Prof. R. Novick, Director, Columbia Astrophysics Laboratory or Prof. L. Lucy, chairman, Search Committee, Department of Astronomy, 538 West 120th Street, New York, New York, 10027.

(NW175)A

UNIVERSITY OF CRETE
FACULTY OF MEDICINE
HERACLIO, CRETE, GREECE

The new School of Health Sciences of the University of Crete announces that the first forty-five positions in the Faculty of Medicine are expected to be filled by the summer of 1984. Application deadlines will be set through the official notification to the published in the Government Gazette and in the Greek daily press, probably in January or February, 1984. This preliminary announcement aims to maximize the opportunities for timely submission of applications by Greeks working abroad as well as within Greece.

The positions are to be filled at all four academic ranks (17 Full Professors, 14 tenured Associate Professors, 11 Assistant Professors and 3 Lecturers), and in all ten departments, namely: Basic Medical Sciences (9), Radiology and Medical Physics (3), Clinical Laboratory Medicine (2), Morphology (4), Social and community Medicine (6), Mother and Child Care (5), Internal Medicine (5), Surgery (5), Neurology and Neurosciences (3) and Psychiatry (3).

Like the successful Schools of Sciences and Humanities of the University of Crete, the School of Health Sciences will place considerable emphasis on excellence in research. The School intends to emphasize both the basic sciences and clinical medicine, and to develop strong programs in community and preventive medicine. Its mission also includes commitment to improved health care in Crete. Construction of a 700-bed University Hospital has already begun.

For further information, please write: Faculty of Medicine, University of Crete, Pervolia, Rethymnon, Crete, Greece or call 3-0831-24069 (Mr. S. Kokkinakis) or 3-01-3610477 (Dr. G. Christakis). (NW210)A

ARC ANIMAL BREEDING RESEARCH ORGANISATION
RESEARCH ANIMAL GENETICIST

A statistical or quantitative geneticist is required to join a research team studying genetic aspects of reproduction and growth in farm animals, with related work on mice. Particular objectives are the study of the causes of genetic variation in reproductive rate and embryo survival in sheep and pigs, and the genetic relationships of voluntary feed intake with rate and efficiency of lean growth in pigs.

The appointee will be expected to conduct experiments on either reproduction in pigs and sheep, or on growth in pigs and mice. The opportunity to play a major part in establishing a new programme of fundamental genetic research with mice may be offered to a suitable candidate.

ABRO adopts a multi-disciplinary approach to genetic improvement and includes geneticists, statisticians, physiologists, biochemists, immunologists and molecular biologists on its staff. Excellent computing facilities are available.

Candidates should have a first or upper second class honours degree in a Biological or Statistical subject with a minimum of two or four years relevant post-graduate experience for appointment at Higher Scientific Officer or Senior Scientific Officer respectively. Salary according to qualifications and experience on the scales £7,149 to £9,561 (HSO) and £8,970 to £11,476 (SSO).

Applications giving full curriculum vitae and names and addresses of two referees to: The Secretary, Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ. (955)A

UNIVERSITY OF STRATHCLYDE
Department of Physiology and Pharmacology

LECTURESHIP

Applications are invited for a lectureship in the Department of Physiology and Pharmacology from graduates in physiology, pharmacology or a related discipline. The person appointed will be required to teach physiology and/or pharmacology to students of various disciplines, to participate in research, and to supervise postgraduate students. Preference will be given to candidates with experience in one of the following research fields: neuromuscular transmission, cardiovascular system, inflammatory mechanisms, smooth muscle, and blood glucose regulation. Expertise in electrophysiology or computing may be an advantage.

Salary scale £7,190 — £14,125 per annum. USS benefit.

Application forms and further particulars (Ref: 69/83) are available from Academic Staff Office, McCance Building, 16 Richmond Street, Glasgow G1 1XQ.

Closing date for applications: 9 February 1984. (968)A

ST MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Norfolk Place, London W2 1PG
DEPARTMENT OF BIOCHEMISTRY
LECTURER OR SENIOR LECTURER

required from 1.10.84. The person appointed will take part in the large teaching programme and must be able to attract research funding. The majority of research in the Department is orientated to clinical, evolutionary or environmental problems; the appointee need not duplicate these interests but should complement them.

Enquiries to Bob Williamson (Tel: 01-723 1252 ext 119).

Applications in form of full cv in duplicate with names and addresses of two referees to Madeleine Jinkinson (from whom further written particulars should be obtained) at above address by 17 February 1984. (960)A

BLOND McINDOE CENTRE FOR MEDICAL RESEARCH
Queen Victoria Hospital
GRADUATE OR POST DOCTORAL SCIENTIST

Applications are invited from graduates in the biological sciences for a position based mainly in the tissue culture laboratory and aimed mainly at defining structure-function relationships of human leucocyte membrane glycoproteins. Experience in tissue culture, particularly in relation to cellular immunology and cell fusion, would be an advantage. Initial appointment is for 4 years. Salary in range £7,190 — £10,710.

Applications (cv and names of two referees) to Professor John Fabre, Blond McIndoe Centre, Queen Victoria Hospital, East Grinstead, Sussex RH19 3DZ. Telephone (0342) 313088. (977)A

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS
POST DOCTORAL RESEARCH ASSISTANT

required to work on cell-mediated immune mechanisms in rheumatoid arthritis. Experience in cloning and hybridoma techniques would be desirable but not essential.

The post is funded by the Arthritis and Rheumatism Council until 30 June 1985 and salary will be on the scale of £7,190 — £8,530 plus London Weighting, depending upon age and experience.

Applications in writing, including Curriculum Vitae and names and addresses of three referees, should be sent to the Deputy Secretary, United Medical and Dental Schools, Guy's Hospital Medical School, London Bridge, London SE1 9RT, quoting Ref DM6. (970)A

UNIVERSITY OF MINNESOTA
DEPARTMENT OF ANIMAL SCIENCE
MOLECULAR GENETICIST ASSISTANT PROFESSOR

to develop a gene transfer system i farm animal species; 15 percen undergraduate and graduate teachin and advising; 85 percent research 12-month tenure-track appointment PhD in a Biological Science Research in molecular genetics an recombinant DNA technology Technical competence i microscopy, microinjection cytogenetics and cell cultur desirable.

Submit résumé, transcripts reprints, 3 reference letters an research goals by February 8, 1984 t Dr Alan G Hunter, 495 AnSc/VM University of Minnesota, St Paul MN 55108. Phone 612-376 5676. Th University of Minnesota is an equa opportunity educator and employe and specifically invites an encourages applications from wome and minorities. (NW209)A

DUKE UNIVERSITY
SCHOOL OF MEDICINE
RESEARCH ASSOCIATE

Position available for study of th pharmacology and physiology o hippocampal neurotransmission Candidate must have 2-3 year postdoctoral experience wit intracellular and extracellula recording from brain slices. positio also requires knowledge of amin acid transmitter pharmacology Salary range: $15,500-$16,300.

Send curriculum vitae and thre letters of recommendation to Dr Victor Nadler, Department o Pharmacology, Box 3813, Duk University Medical Center, Durham NC 27710. EOE A/A. (NW163)A

THE QUEEN'S UNIVERSITY OF BELFAST
DEPARTMENT OF PURE AND APPLIED CHEMISTRY
RESEARCH ASSISTANT

Applications are invited fro candidates with a good honou degree or a PhD to work wit Professor R Grigg on synthetic an kinetic studies of new prototrop reactions. Applicants must ha experience in the application of hig field nmr to kinetic problems i organic chemistry and in the use multipulse techniques in structu elucidation.

Salary scale: £6,312 — £8,5 initial placing depending on age an qualifications. Closing date: 1 February 1984.

Applications enclosing a fu curriculum vitae and the names an addresses of two referees should b sent to the Personnel Officer, Th Queen's University of Belfas Northern Ireland BT7 1NN. (Pleas quote Ref 84/N). (983)A

UNIVERSITY OF PENNSYLVANIA
DEPARTMENT OF SCHOOL OF MEDICINE
POSTDOCTORAL FELLOW CELLULAR/STRUCTURAL BIOLOGY

Applications are invited for a post-doctoral fellow, to join our research on the structure, function, and development of microtubule containing organelles and membrane-microtubule complexes. The position is supported by an NIH Training Grant. Facilities and expertise in all aspects of modern structural and cellular biology are available. Send curriculum vitae and names of 3 references to J. M. Murray, Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.
(NW180)A

UNIVERSITY OF CALIFORNIA, BERKELEY
DEPARTMENT OF ASTRONOMY AND ASTROPHYSICS
ADJUNCT ASSISTANT PROFESSORSHIP

Pending final budgetary approval, the Department of Astronomy of the University of California at Berkeley expects to make one non-tenure track appointment renewable up to five years in astronomy/astrophysics at the Adjunct Assistant Professor level, which can start as early as July 1, 1984. Applicants should send a curriculum vitae, bibliography and a brief statement of research interests, and should arrange to have three letters of recommendation sent to Jonathan Arons, Chairman, Search Committee, Department of Astronomy, University of California, 601 Campbell Hall, Berkeley, CA 94720. All materials should be received by February 1, 1984.

The University of California is an Equal Opportunity, Affirmative Action Employer. (NW181)A

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY
Applications are invited for a
POSTDOCTORAL RESEARCH ASSISTANT IN MOLECULAR GENETICS

in a research group examining the distribution and interrelationships of Type II restriction-modification systems. Preference will be given to applicants with a sound training in the techniques of either protein chemistry, or bacterial culture, or recombinant DNA work.

The appointment is for three years from 1st March 1984. The starting date may be held open for an excellent candidate. The salary will be in the Range 1A (commencing between £7,190 and £8,530 pa depending on age).

Applications including cv and the names of two academic referees should be made as soon as possible to Dr N L Brown, Department of Biochemistry, University of Bristol, University Walk, Bristol BS8 1TD UK. (893)A

ASSOCIATE Chief of Staff for Research position available at the Portland VA Medical Center, affiliated with the Oregon Health Sciences University. Responsibilities include administration of growing multidisciplinary research program, coordinating move to new hospital under construction, and maintaining liaison with affiliated programs. Candidates should have a record of nationally recognized ongoing research, background in basic and clinical sciences, and previous administrative experience. MD's preferred. Application deadline March 1, 1984. Send letter of interest, CV and list of 3 references to Daniel E Casey, MD, Chairman, ACOS/Research Search Committee, Psychiatry Service (116A) 3710 SW US Veterans Hospital Road, PO Box 1034, Portland, OR 97207. Equal Opportunity Employer.
(NW187)A

UNIVERSITY OF BIRMINGHAM
FACULTY OF SCIENCE AND ENGINEERING
CHAIR OF GEOPHYSICS AND HEADSHIP OF DEPARTMENT OF GEOLOGICAL SCIENCES

Applications are invited for the Chair of Geophysics and Headship of the Department of Geological Sciences, which will become vacant from 1 October 1984 on the retirement of Professor D H Griffiths. The office of the Headship need not necessarily be linked with appointment to the Chair.

Salary in the Professorial range, with superannuation.

Further particulars available from the Registrar, University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (12 copies: 1 from overseas applicants) should be sent by 13 February 1984.
(900)A

RESEARCH ASSOCIATE OR POSTDOCTORAL POSITIONS

Two positions available immediately for persons with training in molecular biology and/or protein and nucleic acid biochemistry to work on the development of subunit vaccines for important livestock viral diseases. Projects will involve the use of recombinant DNA technology, in particular, the expression of cloned cDNA in prokaryotic and eukaryotic vectors. Salary: $17,000 to $25,000 depending on qualifications.

Send curriculum vitae and three letters of reference to: Tilahun Yilma, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040; phone (509) 335-6011. Washington State University is an affirmative action/equal opportunity employer. Women and candidates from minority groups are encouraged to identify themselves as such.
(NW206)A

YOU'VE GOT A BRIGHT IDEA

for new biological or chemical compounds.

WE HAVE THE CAPITAL

to develop new drugs.

We are not a pharmaceutical firm but a Swiss-based, independant financial group which is prepared to let you participate in a capital venture for the exploitation of your project.

Our objective: the development of new biological or chemical products having previous pharmalogical screening up to the stage of clinical tolerance and efficacy required to permit licensing negotiations.

To obtain all the information required for initial selection, please write in English, French or German to:
DEBIOPHARM S.A. Petit-Chêne 38 – 1001 LAUSANNE (Switzerland).
(W656)A

SAHA INSTITUTE OF NUCLEAR PHYSICS
Calcutta

invites applications from

SENIOR/ JUNIOR PLASMA PHYSICISTS

both in theory and experiment, for a hot plasma program with a medium size research tokamak machine. Areas of interests include researches in the atomic collisional processes, R-F heating and diagnostics in a tokamak plasma.

Send résumé to the Registrar, Saha Institute of Nuclear Physics, 92, A.P.C. Road, Calcutta, India.

D.N. BHATTACHARJEE
REGISTRAR
(W662)A

The Max-Planck-Institute for Psychiatry, Department of Neurochemistry, Martinsried/Munich, Federal Republic of Germany, offers a

POSTDOCTORAL POSITION for Research in Molecular Cell Biology

Applications are invited from scientists interested in post-translational modifications of proteins, particularly tyrosine sulfation, and its role in the sorting and processing of secretory proteins. Preference will be given to applicants with a background in molecular biology/molecular genetics. Appointments starting March 1, 1984, or later will be for two years initially. Salary according to Federal Regulation (BAT IIa).

Applications with curriculum vitae, publication list and names of referees should be sent to Dr Wieland Huttner, MPI für Psychiatrie, D-8033 Martinsried bei München, West-Germany.
(W660)A

UNIVERSITY OF OXFORD
NUFFIELD DEPT. OF PATHOLOGY

Post-doctoral or graduate Research Assistant

Grade 1A or 1B.

A graduate with either a doctorate or a good honours degree is required to participate in a research project on the local and distant spread of human and animal cancer. The work focuses on cell biology and involves cell and tissue culture studies, with reinoculation of cells into animals. Experience of above techniques would be an advantage but is not essential. This work is supported by a grant from the Cancer Research Campaign, at this stage for a period of three years. Salary is up to a maximum of £8,080 dependent on experience and qualifications.

Research Technician

A research technician is required to assist with the work described in the above advertisement. In addition to laboratory procedures such as transplantation and intra-venous innoculation, the post shall involve cell and tissue culture techniques and experience in any of these procedures would be an advantage, but is not essential.

The project is supported for three years at this stage, and the salary shall be on the Whitley Council Scales for Medical Laboratory Scientific Officers, with a maximum starting salary of £6,263 dependant on qualifications and experience.

Applications for either of the above posts, together with names of two referees should be sent to Dr D Tarin, Nuffield Reader in Pathology, Department of Pathology, John Radcliffe Hospital, Headington, Oxford OX3 9DU. Tel Oxford 817797, by 31st January, 1984. (991)A

MEDICAL RESEARCH COUNCIL
Toxicology Unit

TECHNICIAN

Required to work in Laboratory concerned with metabolism of both drugs and natural compounds. The work involves chemical, biochemical and physiological methods.

Qualifications: Degree, HNC or equivalent. Salary according to age, qualifications and experience within the range £5,491 — £7,747 plus London Weighting.

Apply in writing, with curriculum vitae and the names of two referees to: Dr. J. A. Hoskins, Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM54EF.

MRC
Medical Research Council

(936)A

THE MATHILDA AND TERENCE KENNEDY INSTITUTE OF RHEUMATOLOGY
Cell Enzymology Unit

POST DOCTORAL SCIENTIST

A recently qualified Post-doctoral Scientist is required for 3 years to work with Drs I Olsen and H Muir on the mechanism of lysosmal enzyme transfer. Experience of sub-cellular techniques and protein/membrane biochemistry would be an advantage.

Salary on the Universities Research and Analogous Staff scales depending on age. London Weighting paid in addition.

Applications with curriculum vitae and names of two referees to The General Secretary, The Mathilda and Terence Kennedy Institute of Rheumatology, Bute Gardens. London W6 7DW. (990)A

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
PHYSIOLOGY DEPARTMENT

RESEARCH ASSISTANT

required to work on a project investigating the secretion of corticosteroids by the perfused adrenal gland. The post is funded by the MRC and is tenable for a period of two years. Applicants should have a degree in physiology or a related subject.

Salary £6,310 per annum plus £1,186 pa London Allowance.

Further details are available from Dr B J Whitehouse, Department of Physiology. Applications stating qualifications, experience and the names of two referees should be sent to Mr J Staight, Assistant College Secretary, Queen Elizabeth College, Campden Hill Road, Kensington, London W8 7AH. (993)A

RESEARCH ASSOCIATE

Position available for a person with at least one year of postdoctoral experience or extensive graduate training in molecular biology and/or protein and nucleic acid biochemistry to work on an immunologic project involving the characterization of intracytoplasmic proteins regulating nuclear DNA replication and the genes coding for these. Salary $15,000 — $20,000 depending on qualifications.

Send a copy of curriculum vitae, bibliography and three letters of reference to: Dr Stanley Cohen, Professor of Pathology, University of Connecticut Health Center, Farmington, CT 06032.

An Affirmative Action/Equal Opportunity Employer M/W/H. (NW186)A

UNIVERSITY OF OXFORD
UNIVERSITY LECTURESHIP IN BIOCHEMISTRY AND TUTORIAL FELLOWSHIP AT TRINITY COLLEGE

Applications are invited for a lecturership to be held in the Department of Biochemistry in the field of Physiological Biochemistry. A medical qualification is desirable but not essential. Stipend according to age on the scale of £7,190 — £15,085. The successful candidate may be offered a stipendiary Tutorial Fellowship at Trinity College. Details of the post from Professor J Mandelstam, Department of Biochemistry, South Parks Road, Oxford OX1 3QU, to whom completed applications (8 typed copies, two from overseas candidates) and the names of two referees should be sent by 25 February 1984. (974)A

THE UNIVERSITY OF CALGARY

Department of Chemistry
Graduate Studies

Chemistry: Analytical, Inorganic, Organic, Physical and Theoretical Chemistry, and Applied Chemistry in projects dealing with Energy Conversion, Oil Sands Recovery, Sulphur Chemistry, and Synthetic Membrane Technology.

Programs leading to PhD and MSc degrees; Excellent laboratory, instrument, and library facilities; Minimum stipend of approximately $10,000 p.a. for Teaching and Research Assistantships; Maximum stipend of approximately $13,000 p.a. for outstanding students holding Scholarship combined with part-time Teaching Assistantships; Program fees remitted for full-time teaching appointments; travel allowance available.

For further information and descriptive brochures write to the following, indicating field of interest:

Chairman of Graduate Studies
Department of Chemistry
The University of Calgary
Calgary, Alberta T2N 1N4
Canada (NW192)A

Creative BioMolecules

SCIENTIFIC STAFF

Biotechnology firm is seeking candidates for the following positions:

Molecular Biologist with proven experience in the area of expression of cloned DNA in coli, bacillus or yeast.

Research Associates with one or two years experience in basic Molecular Biology techniques such as Plasmid preparation, restriction mapping and DNA sequencing.

Research Associates/Scientists with experience in protein isolation and characterization, RIA, gel electrophoresis and in vitro translation.

We offer a scientific challenge and equity participation with competitive salaries and fringe benefits. Forward résumé to: Director of Personnel, Creative BioMolecules, 385 Oyster Point Blvd., Suite No 4, South San Francisco 94080. (NW195)A

Please mention

nature

when replying to these advertisements

BIOLOGY TEACHING OVERSEAS

The Department of Biology of the American University of Beirut invites applications for four tenure-track positions at assistant professor level in plant anatomy, plant taxonomy, plant physiology, plant or animal biochemistry, plant or animal cell biology, developmental biology or vertebrate zoology. Appointments are normally for a three-year period. Faculty members are expected to teach undergraduate and graduate courses and conduct a research program.

Send curriculum vitae and three letters of recommendation to Dr. Michael J. Arslanian, Chairman, Biology Department, American University of Beirut, Beirut, Lebanon. An Affirmative Action/ Equal Opportunity Employer.

(NW201)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF CHILD HEALTH
MEDICAL LABORATORY SCIENTIFIC OFFICER

Duties involve assistance with research projects in Nutrition, Allergy and Developmental Biology. Good prospects for MPhil/PhD Study. Applicants should hold degree or equivalent. Salary in range £5,491 — £7,747 pa plus £1,042 pa London allowance.

Application form from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.

(985)A

THE INSTITUTE OF ZOOLOGY
THE ZOOLOGICAL SOCIETY OF LONDON
LABORATORY TECHNICIAN

required to join a project studying lactation and reproduction in seasonally breeding mammals, based at Whipsnade Park Zoo. Applicants should possess a BSc (or equivalent technical qualifications) in Agriculture/Nutrition/Zoology and experience in handling large mammals would be desirable. Driving licence essential. The appointment is for 18 months initially and salary, according to age, qualifications and experience, will be within the range £5,781 to £6,567 inclusive. Please apply in writing by providing CV and names and addresses of two referees to the Establishment Officer, Zoological Society of London, Regent's Park, London NW1 4RY. (980)A

FELLOWSHIPS

UNIVERSITY OF ALBERTA
DEPARTMENT OF ZOOLOGY

Applications are invited for an
NSERC UNIVERSITY RESEARCH FELLOWSHIP POSITION

Applicants should have a PhD degree or equivalent, preferably with postdoctoral experience. The Department is interested in an individual with training and research experience in the field of population biology, specifically aspects of regulation and/or modelling of boreal vertebrate populations. Position will involve some teaching responsibilities appropriate for the specialization of the appointee. Salary commensurate with experience, equivalent to Assistant Professor level, 1983-84 base, $29,720.

Submit complete curriculum vitae with names of three referees and reprints by March 7, 1984 for consideration in the November 1984 NSERC competition to: Dr R E Peter, Acting Chairman, Department of Zoology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada.

The University is an equal opportunity employer but, in accordance with Canadian Immigration and NSERC requirements, priority will be given to Canadian citizens and permanent residents of Canada.

(NW203)E

Fellowships cont on page 22 and 23

POST Doctoral Research Associate. Available immediately to study growth factors and protein kinase. Experience with handling of protein and tissue culture desirable. Send résumés and names of 3 references to: James Tam, Box 294, Rockefeller University, 1230 York Ave, New York City 10021. Equal Opportunity/Affirmation Action.

(NW183)A

MOLECULAR BIOLOGIST

The Divisions of Clinical Immunopathology and Experimental Pathology of the Department of Pathology, University of Pittsburgh School of Medicine, seek a molecular biologist to establish an independent research program. Potential areas of clinically oriented collaboration include human tissue transplantation, lymphoproliferative disease, autoimmunity (emphasizing insulin-dependent diabetes), ageing and immune deficiency diseases. Collaboration is also sought in defining structural and functional properties of the rat major histocompatibility complex.

Please send a curriculum vitae and 3 letters of recommendation to: Bruce S Rabin, MD, PhD, Chairman of the Search Committee, Division of Clinical Immunopathology, Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261. (NW198)A

Environmental
Contamination of Food

Scientific Adviser

This is an opportunity to join a small multi-disciplinary team which provides scientific advice within the Ministry on related topics ranging from nuclear power and radioactive waste management in relation to food, to assessment of the effects of nuclear fall-out on plants and animals. Members of the Atomic Energy Unit are also involved in extra-mural research contracts and are expected to keep abreast of national and international developments in the areas in which they are engaged.

Candidates should have a good honours degree or equivalent in an appropriate scientific subject and experience of the nuclear industry and radiological protection. Familiarity with computer applications would be advantageous.

This London-based appointment will be as Principal Scientific Officer £12,590-£16,180 (including £1250 Inner London Weighting) with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 3 February 1984) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). **Please quote ref: S(A)677.**

Ministry of Agriculture, Fisheries and Food

(982)A

Applied bioTechnology Inc.

A MOLECULAR BIOLOGIST AND A VIRAL IMMUNOLOGIST

sought for young firm in Cambridge, Massachusetts concerned with exploitation of recombinant DNA techniques for creation of immunogenic proteins for use as viral vaccines. Research involves development and use of a range of techniques in molecular biology, protein chemistry, virology and immunology. Located near several excellent academic research departments. Two years of post-doctoral experience in related areas desired.

Applied Biotechnology
Attention: Personnel
80 Rogers St.
Cambridge, MA 02142

(NW213)A

CONFERENCES and COURSES

JAPANESE BIOTECHNOLOGY STUDY TOUR

An in-depth series of site visits, conferences and seminars with some of Japan's leading industrial scientists and executives in biotechnology.

This exclusive study tour is designed to give western scientists and executives an opportunity to become acquainted with the Japanese marketing and research capacity. The mission will be led by Christopher G. Edwards, Editor in Chief of BIO/TECHNOLOGY, and organized by the Technology Transfer Institute. Visits will be conducted throughout Japan from March 31–April 15.

For further information, contact: Technology Transfer Institute—New York, One Penn Plaza, Suite 1411, 250 W. 34th St., New York, N.Y. 10119. 212-947-2648 telex 420057TTIUI. Attention: Mr. Hashizume.

(NW174)C

MOLECULAR ENDOCRINOLOGY AND TECHNIQUES FOR HORMONE ACTION
19 to 25 February, 1984

Course will consist of lectures in methodology with comprehensive syllabus covering receptors, gene transcriptions, mRNA and protein synthesis, recombinant DNA, protein hormones, and cyclic nucleotides.

Co-directors: W.T. Schrader and B.W. O'Malley. Fee: $295/$195 (fellows).

For further information contact: Claudia Payne, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030. Tel: 713-799 6200. Deadline 6 January 1984. (NW115)C

CHELSEA COLLEGE
(University of London)
MSc in BIOPHYSICS AND BIOENGINEERING

This is an interdisciplinary course covering the areas in which the physical sciences overlap with medicine and biology. It is open to students with a first degree in a physical or biological science, engineering or medicine. The course, which is approved and supported by the Medical Research Council, is of a two-year duration.

Further details and forms of application for entry in October 1984 can be obtained from: The Secretary, Department of Biophysics and Bioengineering, Chelsea College, University of London, Manresa Road, London SW3 6LX. Tel: 01-351 2488 ext 2382. (992)C

CONGRESS

SAVANNAH '85

FIRST INTERNATIONAL CONGRESS OF PLANT MOLECULAR BIOLOGY
OCTOBER 27 - NOVEMBER 2, 1985
SAVANNAH, GEORGIA, USA

Organized by
The International Society for Plant Molecular Biology

SCIENTIFIC COMMITTEE

J.L. Key (Athens) Chairman	T.J. Higgins (Canberra)	O. Nelson (Madison)
M-D Chilton (Raleigh)	E.J. Jaworski (St. Louis)	J. Peacock (Canberra)
G.B. Collins (Lexington)	T. Kosuge (Davis)	I. Takebe (Nagoya)
N-H Chua (New York)	A. Kondorosi (Szeged)	G. Toennessen (New York)
K. Hahlbrock (Cologne)	C.J. Leaver (Edinburgh)	D.P. Verma (Montreal)

The Congress intends to provide a forum for current research in plant molecular biology in its broadest sense encompassing all of its subdiscipline. It will feature invited plenary speakers, sessions devoted to the various subdisciplines and poster sessions. Anyone conducting research in the scientific areas covered by the Congress is eligible to present a talk and/or poster. Please write: Congress Secretary, 1st ICPMB/The Georgia Center/Athens, GA 30602/USA

THE UNIVERSITY OF GEORGIA BICENTENNIAL 1785 1985

Dr. Fred C. Davison
President of The University of Georgia
Honorary Congress President

(NW218)Q

INSTITUTE OF MICROBIOLOGY UNIVERSITY OF DÜSSELDORF FEBS ADVANCED COURSE ON METHODS IN YEAST MOLECULAR GENETICS
June 25-July 10, 1984

The course will cover methods of classical and molecular genetics as used for the study of gene regulation and the expression of foreign genes in yeast. It will include isolation and characterization of mutants, tetrad analysis, use of different yeast vectors, selective and non-selective transformation, construction and use of fusion plasmids. Participants should have some experience in recombinant DNA techniques.

Teaching staff will include: P. Philippsen (Basel), R. S. Zitomer (Albany), K. Breunig, M. Ciriacy, S. Das, J. Gafner, C. P. Hollenberg, Z. Janowicz, R. Roggenkamp (Düsseldorf).

The course will be concluded with two days of discussions including contributions by: G. R. Fink (MIT), D. Gallwitz (Marburg), B. D. Hall (Seattle), F. Messenguy (Brussel), K. A. Nasmyth (Cambridge), F. K. Zimmermann (Darmstadt) and the teaching staff.

Applications including curriculum vitae, publication list, letter of recommendation, and research interests should reach: C. P. Hollenberg, Institut für Mikrobiologie, Geb. 26. 12, Universitätsstr. 1, Universität Düsseldorf, 4000 Düsseldorf, F.R.G., by March 1st, 1984. Applicants will be informed before May 1st, 1984. No accommodation fee will be required but applicants will have to bear the costs of travel and meals. A limited number of travel fellowships will be available. Accommodation will be arranged. (W653)C

GRANTS and SCHOLARSHIPS

UNIVERSITY OF LONDON

EDMUND DAVIS SCHOLARSHIP IN MEDICINE, LAW OR ENGINEERING

Applications are invited from suitably qualified graduates of any university for the award of an Edmund Davis Scholarship tenable from October 1984. Under the terms of the benefaction preference will be given to applicants born in the geographical areas formerly known as Northern and Southern Rhodesia.

The Scholarship is awarded for full-time graduate work at a School of the University for an approved course leading to a Higher Degree of the University. The award is tenable for a period not exceeding three years, and the value will not be less than £2,995 per annum with fees. Travel costs and assistance towards sundry other expenses may also be paid in the case of an overseas scholar.

Detailed information and application forms may be obtained from the Secretary, Scholarships Committee, University of London, Room 21A, Senate House, Malet Street, London WC1E 7HU, with whom completed applications and references must be lodged by no later than 1 March 1984.

(975)H

COMMISSION OF THE EUROPEAN COMMUNITIES TRAINING IN BIOMOLECULAR ENGINEERING

SCOPE: Applications are invited for training contracts (1-2 years) or for short training periods (up to 2 months) from scientists who are citizens of a Member State of the European Communities and who wish to undertake, in a laboratory located within the European Communities, specific research in the field of enzyme or genetic engineering.

CLOSING DATES: Applications should arrive by
- April 1, 1984, for contracts commencing on October 1, 1984;
- July 1, 1984, for contracts commencing on January 1, 1985.

TRAINING CONTRACTS

ELIGIBILITY: Applicants must hold a PhD degree or its equivalent. The training period cannot take place in the applicant's country of citizenship or in the country in which he or she normally resides.

ALLOCATION: In the case of non-employed scientists, the comprehensive monthly allowance to be received by the grant-holder presently approximates 1340 ECU (1). In addition, the Commission will pay an allowance for dependants and will reinburse travel expenses to and from the site of the host-laboratory.
In the case of employed scientists on leave of absence, the Commission will provide the grant-holder with a monthly allocation which complements the salary received by the beneficiary during the training period and includes an additional allowance for expatriation approximating 470 ECU (1) per month.

SHORT TRAINING PERIODS

ELIGIBILITY: Short training periods are designed to offer an opportunity for industrial research scientists (PhD level) to improve their knowledge or to learn a specific technique in public laboratories.

ALLOCATION: The Commission will pay to the grant-recipient a daily allowance of approximately 36 ECU (1) during the length of the training period and will reimburse travel expenses to and from the site of the host-laboratory.

BENCH-FEES

The laboratories undertaking to host the holder of a training contract or of a grant for a short training period will receive a bench-fee, calculated pro-rata to the length of the training period, at 10,000 ECU (1) per year.

INFORMATION

Further details, application forms and lists of host-laboratories may be obtained from Dr. D. de Nettancourt (Tel: (2) 235.40.44) or Dr. E. Magnien (Tel: (2) 235.93.47), Commission of the European Communities Directorate-General for Science, Research and Development, Rue de la Loi 200, B-1049 Brussels, Belgium.

(1) Indicative amounts. Actually, the allocations provided take into account the cost of living in the country where the host-laboratory is located.
As per 1.12.1983, 1 ECU is approximately equivalent to FB/FLUX 45,91; DKR 8,17; DM 2,26; DRA 81,34; FF 6,88; IRL 0,73; LIT 1380; HFL 2,53; UKL 0,57. (W650)H

SEMINARS and SYMPOSIA

Continued on page 24

ROYAL POSTGRADUATE MEDICAL SCHOOL
in collaboration with
GLAXO GROUP RESEARCH LIMITED
BASIC SCIENCE IN GASTROENTEROLOGY SYMPOSIA
Seventh Symposium of the Series

GASTROINTESTINAL CARCINOGENESIS

London — 24 April 1984

Organisers: J. M. Polak, S. R. Bloom, N. A. Wright, A. G. Butler

Invited speakers include

D. Britton, UK
W.W.L. Chang, USA
P. Correa, USA
M. Hill, UK
J. Jass, UK
M. Lipkin, USA
B. Morson, UK
R.R. Peto, UK
C.D. Potten, UK
R. Riddell, USA
J.P. Sunter, UK
J. Weisburger, USA
R.C.N. Williamson, UK

Topics include
Epidemiology, Chemical Carcinogenesis, Cell Proliferation, Environmental Factors, Dysplasia, Pre-cancerous Lesions, Atrophic Gastritis

Registration and Lunch — £10.00

For further details and Application Form please contact:
A.G. Butler, Medical Division, Glaxo Group Research Ltd., Ware, Herts. SG12 0DJ. Telephone No. 0920 3993.

Closing date for applications: 13 April 1984.

(951)M

European Society for Animal Cell Technology
International Association of Biological Standardization

The joint ESACT/ IABS meeting on the PRODUCTION AND EXPLOITATION OF EXISTING AND NEW ANIMAL CELL SUBSTRATES

will be held on MAY 21st-25th 1984
in GARDONE RIVIERA, Italy
The Meeting is organized by:
"Menarini" International Foundation, Florence, Italy
Zooprophylactic Foundation, Brescia, Italy

SUBJECT AREAS: Genetic Engineering or Modification of Animal cells, Characterization of Cell Substrates.

Safety Aspects Relating to the Exploitation of Animal Cells, Oncogenes, Regulatory Proteins, Regulatory Authorities.

Production of Antibody from Hybridomas and the Scale-Up of such Systems.

Developments in Large-Scale Cell Production Equipment.

Medium Developments for Cell and Product Generation.

Serium-Free Media (Pains and Pleasures).
Downstream Processing of Materials Produced from Cultured Animal Cells.

New Product Areas (Lymphokines, Enzymes, Hormones).

Organizing Secretariat: G. F. Panina c/ o FONDAZIONE INIZIATIVE ZOOPROFILATTICHE, Via Bianchi 1, 25125 Brescia — Italy. (W649)M

E M B O

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1984 AWARDS

Next deadline: February 16, 1984

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is February 16, 1984. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on April 27, 1984.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany. (W531)E

VICTORIA UNIVERSITY OF WELLINGTON
New Zealand
POSTDOCTORAL FELLOWSHIPS

Victoria University of Wellington invites applications for these Fellowships, in the following areas in 1984: Chemistry: Carbohydrate medicinal, electrochemistry, liquids, Clay-mineral, Azamacrocycle, Strained-ring compounds; Physics: Condensed matter, geophysics, nuclear physics.

Conditions of Appointment and method of application are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or from the Administrative Assistant (Appointments), Personnel Office, Victoria University of Wellington, Private Bag, Wellington, New Zealand, with whom applications close on 24 February 1984. (W642)E

FOULKES FOUNDATION FELLOWSHIPS 1984

The Foulkes Foundation provides financial support for recently qualified science graduates with research experience who want to study medicine, and for medical graduates who want to take a science degree. In both cases graduates must intend to do medical research after qualification.

Application forms and further information available from The Registrar, Foulkes Foundation Fellowships, 37 Ringwood Avenue, London N2 9NT, enclosing sae. Closing date — 31 March 1984. (953)E

POSTDOCTORAL FELLOWSHIP PhD, or Equivalent — 2 yr position. To participate in active research program on inflammation, arachidonic acid metabolism in blood vessels. Salary $17,000. To begin Jan-June, '84. Send inquiries, curriculum vitae, and 2 references to: Michael A Moskowitz, MD, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114 USA. (NW147)E

THE ARKLETON TRUST

1984 FELLOWSHIPS IN RURAL DEVELOPMENT EDUCATION AND CONSERVATION

The closing date for applications for the Ernest Cook and HIDB Fellowships will be 1st May 1984. For further details write to:

THE ARKLETON TRUST, ENSTONE, OXFORD OX7 4HH U.K.
(988)E

FELLOWSHIPS continued

EMBL

European Molecular Biology Laboratory

PREDOCTORAL FELLOWSHIPS

The European Molecular Biology Laboratory is to award a limited number (6-8) of predoctoral fellowships for graduates wishing to study for a PhD at EMBL Heidelberg or its outstations at DESY, Hamburg or ILL, Grenoble. The areas of research in Heidelberg include membrane biology, cellular differentiation, analysis of the structure of membrane proteins and protein nucleic acid complexes and the structure and regulation of genes in procaryotes and eucaryotes. At the EMBL outstations the research centres on the elucidation of biological structures using either synchrotron radiation (Hamburg) or neutron beams (Grenoble).

To be eligible, candidates must hold, or anticipate receiving in 1984, a first or upper second class honours degree, a masters degree or a diplom in biological sciences, physical sciences or mathematics. Preference will be given to candidates from the member states of EMBL and EMBC namely: Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom.

Short listed candidates will be asked to visit EMBL (travel expenses reimbursed) for an interview. Those awarded predoctoral fellowships will be expected to begin working at EMBL in September 1984. The normal duration of the fellowships will be 3 years subject to annual review.

The closing date for completed applications is February 1st 1984. Successful candidates will be notified by the end of April 1984.

Application forms and a catalogue giving further information about the research at EMBL and the groups participating in the Laboratory's predoctoral programme is available on request from Dr J. Tooze, Predoctoral Programme, EMBL, Postfach 10.2209, 6900 Heidelberg, F.R. Germany. (W511)E

UNIVERSITY OF WARWICK POSTDOCTORAL FELLOWSHIP — PHYSICS

The Postdoctoral Fellowship is available under a programme relating to the JOERS initiative.

The appointment is for 3 years and the research programme will be concerned with new glasses for long wavelength optical fibres. The work will involve preparation of the special glasses and investigation of their optical and other properties. The techniques used will include infrared spectroscopy and high resolution electron microscopy.

Applicants should have a strong interest in the physics of materials and should possess or expect to have a PhD in any area related to materials science or solid state physics. Salary on the Research Range IA scale: £7,190 — £11,615 pa.

Enquiries and applications to Professor P W McMillan, Department of Physics, University of Warwick, Coventry CV4 7AL. Closing date for receipt of applications 2nd February, 1984. (962)E

CITY OF LONDON POLYTECHNIC
DEPARTMENT OF PHYSICS
RESEARCH FELLOWSHIP
Polymers and Colloids Research Group

A Postdoctoral Research Fellow is required to work in the above Group. The programme will include the use and development of mainly optical techniques (eg laser light scattering, precision refractometry and turbidimetry) to probe the motions and interactions of polymer molecules and colloidal particles in both the solid and the liquid phase. The research is of both scientific and industrial relevance.

The appointment is from as soon as possible for two years with the possibility of extension for a third year. Candidates should have a research degree.

Research Fellows are currently paid on the scale £8,202 — £8,646 — £9,078 per annum including London allowance.

Further information can be obtained from the Department of Physics, City of London Polytechnic, 31 Jewry Street, London EC3N 2EY. (963)E

PATERSON LABORATORIES CHRISTIE HOSPITAL & HOLT RADIUM INSTITUTE MANCHESTER
DEPARTMENT OF MOLECULAR BIOLOGY
Two Cancer Research Campaign
POSTDOCTORAL RESEARCH FELLOWSHIPS

tenable for three years from 1 April 1984 are available in the new Department of Molecular Biology. The fellows will join a group investigating the molecular biology of Epstein-Barr virus with particular emphasis on the state and expression of the viral genome in EBV-associated tumour cells. Applicants should have a background in a relevant biological science and possess a keen interest in human tumour virology.

Requests for further details and applications, including the names and addresses of two referees should be sent as soon as possible to: Dr J R Arrand, Department of Molecular Biology, 2nd floor, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Withington, Manchester M20 9BX, Lancs. (814)E

Vacant Research Appointments

The Council of the Royal Society invites applications for appointments tenable from 1 October 1984:

1. Two Mr. and Mrs. John Jaffé Donation Research Fellowships, one for research in chemistry and one for research in one of the other physical sciences (including geology).
2. Two Pickering Research Fellowships, one for research in chemistry (especially physical or inorganic), and one for research in plant sciences.
3. The Rosenheim Research Fellowship for research on the biochemistry of plants and the simpler forms of animal life. preference will be given to candidates wishing to study the biochemistry of higher plants.
4. An Eliz. Challenor Research Fellowship for research in physics.

The Head Bequest Committee of the Royal Society invites applications for:

5. The Henry Head Research Fellowship, tenable from 1 October 1984, for original research in neurology in its widest aspects.

The Warren Research Fund Committee of the Royal Society invites applications for:

6. A Warren Research Fellowship, tenable from 1 October 1984, for research in metallurgy, engineering, physics or chemistry or for the use of application of such research or its results in or for industry and industrial development.

Appointments are subject to the Society's General Regulations governing Royal Society Research Appointments, or, for 6, the regulations governing Warren Research Fellowships.

All candidates must have a PhD or equivalent research experience. There are no restrictions on nationality of applicants or proposed places of tenure for 1, 2, 4 or 5. Candidates for 3 and 6 must propose to hold the appointment in any appropriate university department or research institution in the United Kingdom (or, for 6, an industrial research laboratory). Candidates for 6 must be British subjects.

Salaries and conditions of tenure:

1.-4. Salary scale £7,783 × £446 to £10,013 per annum. Tenure for three years in first instance, possible renewal for two further years only.

5,6. Salary scale £10,829 × £466 to £13,159 per annum. Tenure for three years in first instance, possible renewal for two further years only (5). Tenure for five years in first instance, possible renewal for one further year only (6).

For all appointments superannuation benefits will be provided and there will be some provision for research expenses. The point of entry on the relevant salary scale will be determined by Council or the Head Bequest Committee (for 5) or the Warren Research Fund Committee (for 6).

Preferred age limit (on 1 October 1984):
1-4 — Under 26. 5,6 — Under 33
Method of application: Apply (by letter) for application form and further details (*stating for which appointment*).
Address: The Executive Secretary (UMAM), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.
Closing date: 13 February 1984.

THE ROYAL SOCIETY

Royal Society 1983 University Research Fellowships

The Council of the Royal Society invites applications for about 20 appointments to be supported from the Society's parliamentary Grant-in-aid and tenable from 1 October 1984 as Royal Society 1983 University Research Fellowships.

All candidates must have a PhD or equivalent research experience; they must be at least 26 but should not have passed their 33rd birthdays by 1 October 1984. They must be British Citizens (of the United Kingdom) and must hold the appointments in departments of science (including agriculture, medicine, mathematics, engineering and technology) in universities in the United Kingdom. Persons already holding substantive posts in universities in the United Kingdom will not normally be considered.

Appointments, which will be subject to the Society's General Regulations governing Royal Society Research Appointments, will be for up to five years with the possibility of renewal for two successive periods of up to three and two years. Salary will be on the University Lecturers' scale for non-clinical academic and related staff with entry point related to age and London Allowance where appropriate. A basic grant of £900 per annum will be provided for research expenses.

The primary criterion for selection will be the scientific merit of the individual applicant based on his or her record so far and on future potential. Account will be taken of the facilities and intellectural environment being offered by the prospective host department. preference will be given to applicants who wish to hold their appointments in departments which at present have few or no young (i.e. under 35) staff. Each application will need to be accompanied by a statement from the head of the prospective host department who will be asked to confirm that the applicant could be expected to be a very strong candidate for a permanent post at the end of the Fellowship period.

Similar posts may be offered in each of the next three years but the number available cannot yet be indicated.

Candidates who were unsuccessful in 1983 should not be deterred from applying in 1984 if they are still within the required age range. They should, however, make a fresh application on the new (redrafted) application form and arrange for updated statements from referees and the prospective head of department to be provided.

Apply (by letter) for forms for completion by applicant and head of department and further details.

Address: The Executive Secretary (UMAM), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

Closing date: 13 February 1984.

(949)E

WORKSHOP

Three Intense Laboratory Workshops In Nucleic Acid Technology

Chemical Synthesis of DNA Oligonucleotides

Students will synthesize oligos of their own design of length 12-14 bases. The liquid triester condensation method will be employed. The product will be sequenced for verification.

Tuition: $1,000

May 14-23

To apply: Send a brief description of your research interests and the relevance of our course to your plans. Include a brief resumé of your training and institutional affiliation. Please include a contact telephone number. Respond to: Dr. John Newbold, Workshop Coordinator, Program in Molecular Biology & Biotechnology, The University of North Carolina at Chapel Hill, 125 MacNider Building 202H, Chapel Hill, North Carolina 27514. Telephone: 919/966-4161. Application deadline: March 5, 1984. Acceptance notification by March 30, 1984. Inexpensive air conditioned dormitory housing is available.

The Carolina Workshops, May Through July, 1984

Human Genetics/General DNA Technology

General DNA techniques such as subcloning, vector and genomic DNA preparation, electrophoretic analyses, Southerns and nick-translations. Techniques special to human genetics such as in situ chromosome hybridization, linkage analysis, chromosome library construction, and the use of biotinylated probes. Intensive lab work and lectures on the applications of DNA technology to human genetics.

Tuition: $1,200

June 4-20

DNA Sequencing

Extensive experience in chemical cleavage and dideoxy sequencing techniques. High resolution gel electrophoresis (including gradient gels) will be used in sequence analysis. Cloning into plasmid and M 13 phage vectors. Generation of libraries for rapid sequence analysis. Instruction will include computer-assisted analysis of the sequence data utilizing public-access data banks. You may bring your own cloned DNA sample to sequence.

Tuition: $1,200

July 2-18

University of North Carolina at Chapel Hill

Program in Molecular Biology & Biotechnology

(NW193)V

EMBO LABORATORY WORKSHOP ON MICROINJECTION
April 8-13, 1984

Subjects: Microinjection of macromolecules into culture cells, biological assay, reextraction of injected DNA and blot analysis, liposomes, and reconstituted Sendai virus envelopes as carriers for DNA.

Lectures will include invited speakers and course participants. The course is limited to 12 participants. An advance fee of US$ 100, for registration is required.

Applications should be sent to A. Graessmann not later than February 20th, 1984, Institut fuer Molekularbiologie und Biochemie, Freie Universitaet Berlin, Arnimallee 22, D-1000 Berlin 33. (W654)V

INTRODUCTORY WORKSHOPS ON TECHNIQUES IN MOLECULAR BIOLOGY
September 1984

Separate laboratory-based workshops on
(a) PROTEINS
(b) NUCLEIC ACIDS

Details and application forms from Dr. J. Walker, Biological Sciences, Hatfield Polytechnic, Herts. AL10 9AB England. (948)V

GRANTS and SCHOLARSHIPS
continued

MULTIPLE SCLEROSIS RESEARCH

Applications are invited for Grants funded by the Society for research related to Multiple Sclerosis. Support will be provided for approved clinical or epidemiological work as well as for laboratory-based studies within Australia.

Applications from suitably qualified persons will also be considered for Post-Doctoral Fellowships.

Intending applicants for project grants should write for further information to the Executive Director, National Multiple Sclerosis Society of Australia, 616 Riversdale Road, Camberwell, Vic. 3124.

Applications close on Saturday 31st March 1984. Dr E L French (Chairman), Prof D R Curtis (Deputy Chairman), Research Advisory Board, National Multiple Sclerosis Society of Australia. (W647)H

THE NATIONAL KIDNEY RESEARCH FUND

invites applications for renal research grants for consideration at the Meeting on April 17th 1984.

Application forms for these and travel grants available from N.K.R.F. 184B Station Road, Harrow HA1 2RH (Tel: 01-863 4460).

Completed forms required by 5th March, 1983. (920)H

WANTED AND FOR SALE

Scientific and Technical Reviews.
Please inquire for runs or single volumes.
SANTO VANASIA,
58 Via M. Macchi,
20124 MILANO, Italy. (W547)L

APPOINTMENTS WANTED

advertise your qualities and qualifications through the most influential science weekly in the world at a special reduced cost of *25p a word.* (Personal Box Numbers £1). Advertisements must be pre-paid and sent to: Nature Classified (Appts Wtd), 4 Little Essex Street, London WC2R 3LF. (890)B

HYDROGEOLOGIST (MSc; Dip Hydro; BSc). Aged 26, Free to travel. Fully Experienced. Short Term Contracts Considered. Write, J M Ashworth, 82A Lower St, Merriott, Somerset TA16 5NW. (989)B

VOUS en avez assez d'écrire vos articles en anglais? Ecrivez les en français; je vous en ferai la traduction. Highly qualified English scientist (PhD, London) presently at ENS, Paris, will undertake technical translations. Reasonable rates. References available. Box W655 c/o Nature, 4 Little Essex Street, London WC2R 3LF. (W655)B

STUDENTSHIPS

THE HANNAH RESEAR INSTITUTE POSTGRADUATE STUDENTSHIPS (Biological, Chemical o Food Science)

Applications are invited for studentships tenable at the Inst for three years leading to a PhD c University of Glasgow.

For the Agricultural and Research Council (AF Studentship, the research area w be selected from: (i) mammary differentiation and turnover; monoclonal antibodies to adipocyte plasma membrane; regulation of glucose utilizatic lactating ruminants; (iv) mole mechanisms of regulatior lipoprotein lipase in mam tissue; (v) colloidal stability of c micelles.

For the United Kingdom Industry Studentship (terms ider to AFRC Studentships) the resc topic would be either: (i) mecha of gelation reactions in milk, c changes in the properties of protein induced by dehydration

Applicants should be citizer the United Kingdom and are req to have, or should expect to obt 1984, a first or upper second honours degree in Biolog Chemical or Food Science.

Further particulars car obtained from the Secretary, Hannah Research Institute, Ayr 5HL, to whom applications, v should contain the names addresses of two academic refe should be sent by 14th Febr quoting ref: PG/84. (96

KING'S COLLEGE LONI RESEARCH STUDENTSH

Applications are invited now research studentships leading t PhD degree in Biophysics for the beginning in October 1984. Biophysics Laboratories, comp the King's College Departme Biophysics and the MRC Biophysics Unit, offer five areas of research: Macromole studies of contractile syst Cellular and developmental n biology; DNA and molec genetics; Cell behaviour and mo Crystallographic and comp graphics studies of biolo molecules.

Applicants need a good hor degree, most likely in biochem biophysics, biological science molecular biology, chemistr physics. A full list of research t and supervisors can be obtained Mr D M Drummie, King's C London, 26-29 Drury Lane, Lo WC2B 5RL, or by telephc 01-836 8851 and asking for Crick. (97

MISCELLANEOUS

PRIVATE Beach Cottages. E sive coral reefs, birds, tropical Snorkel, fish, swim, scuba from front door. Discount for scie work & student groups. Broc Charles F Turpin, Charlotte Tobago, West Indies. (NW34

Typesetting: J.W. Arrowsmith Ltd, Bristol and Outline Graphics Ltd. London EC4Y 0HP. Printing: Kingsdale Press Ltd, Reading, Berks, in association with Godfrey Lang Ltd. London WC1R 4EB and The William Byrd Press Inc., Richmond, Virginia, USA Published by MACMILLAN JOURNALS LTD

At 65¢ a Volt...



...it's the best buy in Constant Power Supply

The $1295 EC500 was designed specifically to meet the need for a 2,000 Volt Constant Power Supply in special electrophoretic applications such as gel electrofocusing, gel sequencing, SDS gels, and standard slab and tube gels. Check these important features and benefits:

- maximum output: 2,000V; 150mA; 300W
- 3-mode control: Constant Power, Voltage, or Current
- optional voltage limit control for sensitive gels
- dual-range meters and high-low switching without effect on set points
- high voltage safety interlock
- positive electrical protection
- adapts easily to any cell

Call Technical Service *collect* at 813-344-1644 for more information or write. E-C Apparatus Corp., 3831 Tyrone Blvd. N., St. Petersburg, FL 33709

E-C

Circle No.05 on Reader Service Card.



graphy, it pays to match Olympus microscopes with something equ outstanding: The Model PM-10AD The PM-10AD faithfully records th high image resolution, definition a clarity of Olympus microscopes. it does so with the convenience o fully automatic exposure.

The time and effort you save are markable. Thanks to a built-in mic computer and a Silicon Blue Cell (S that relays information to it quickly accurately, you need no longer wo about complicated adjustments.

The automatic exposure range is tremely wide, from 1/5000 of a sec to 2 hours. The microcomputer a matically corrects the reciprocity failure characteristics of the film are using and operates continuou to yield perfect exposures.

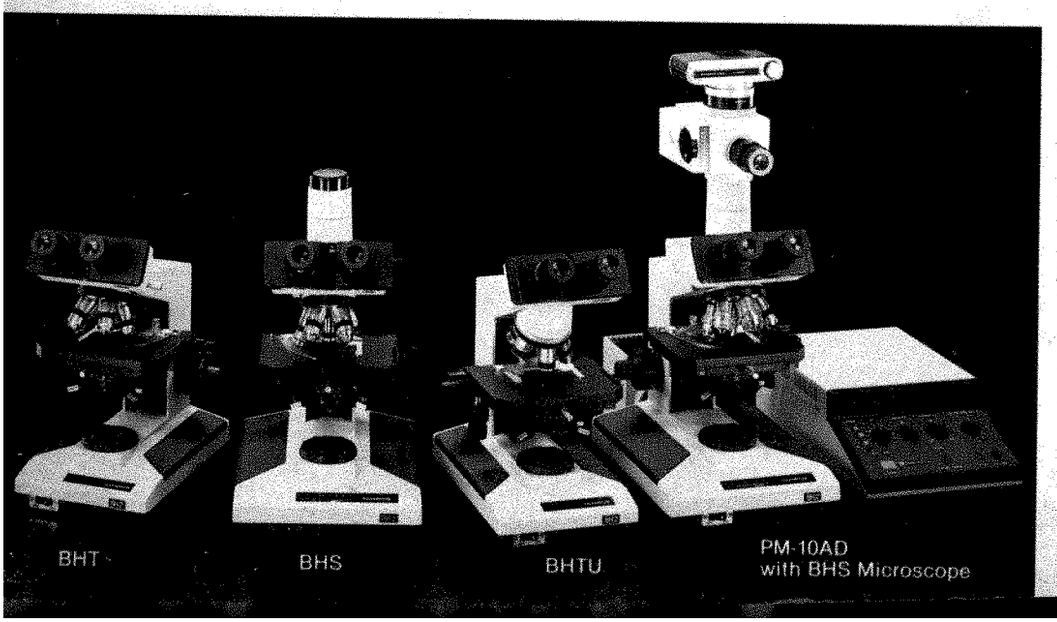
The PM-10AD allows automatic a manual operation. In either mode can be used not only for conventic purposes but also for multipl exposures of double-stained fluor cence specimens and panorama photography, in which case unifor density is assured.

Other features:

- Clear digital displays include estimat and actual exposure times, time remai to complete exposure and recall of th previous exposure time.
- Unique vibration-free electromagnet shutter. The contact-free design provi a shutter life of at least 100,000 operat with absolutely no adverse effects on image quality.
- Optional PM-CTR color temperature meter module for simple checking an adjustment of color temperature.

A TRIUMPH OF INSPIRED SIMPLICIT

PM-10AD Photomicrography and Cinemicrography Syste



The PM-10AD is a triumph of inspi simplicity—a simplicity that mean optimum results.

OLYMPUS

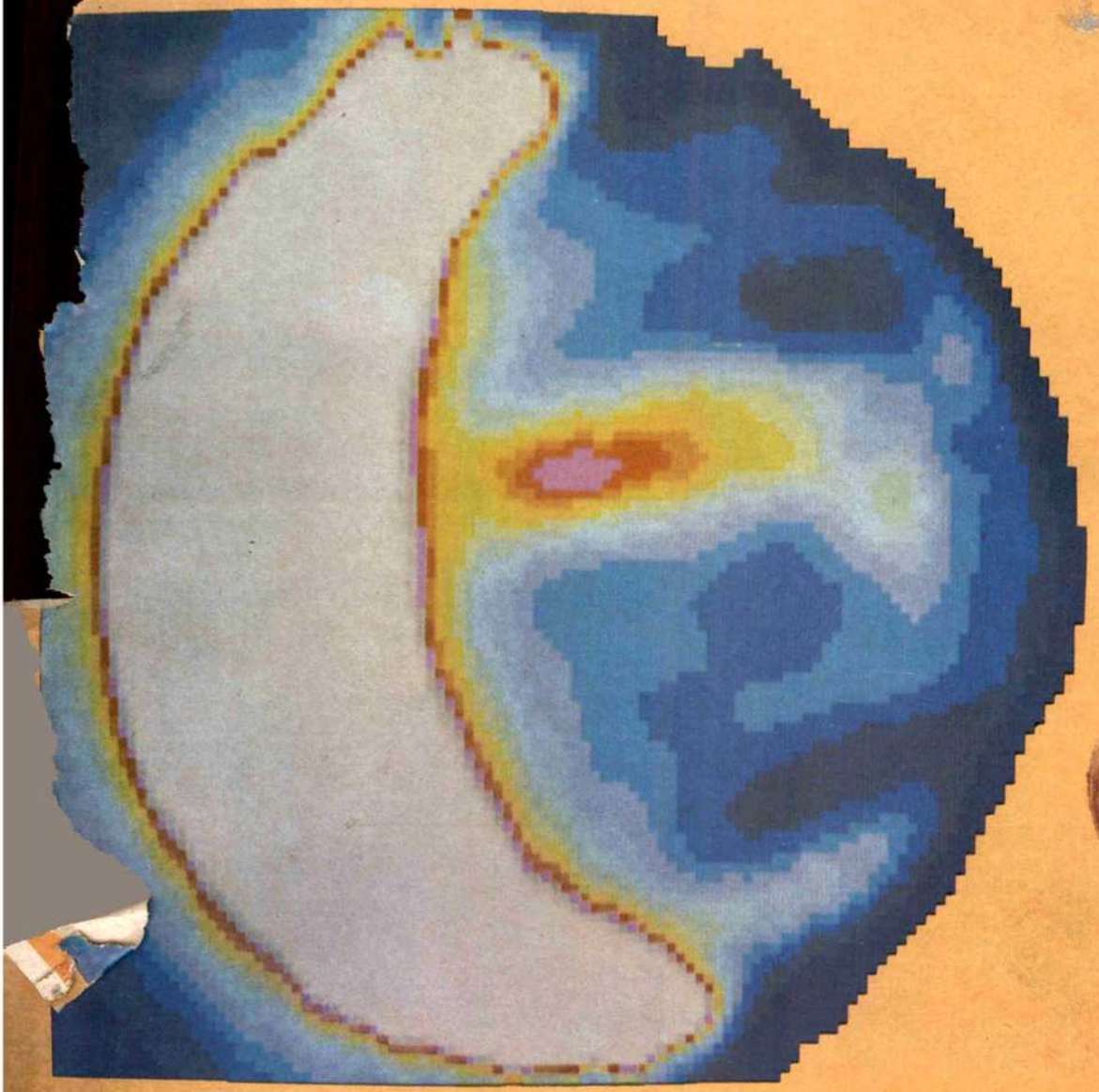
Match your skills with ours.

OLYMPUS OPTICAL CO., LTD.
San-Ei Building, 22-2, Nishi Shinjuku 1-chome, Shinjuku-ku, Tokyo,
OLYMPUS OPTICAL CO. (EUROPA) GMBH
Postfach 104908, Wendenstrasse 14-16, 2 Hamburg 1, West Germ
OLYMPUS CORPORATION
4 Nevada Drive, Lake Success, N.Y. 11042-1179, U.S.A.

Circle No. *54* on Reader Service Card.

nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
307 No 5948 19-25 January 1984 £1.80 $4.50

Author Index



LENDING COPY

CLOUD STRUCTURE ON THE DARK SIDE OF VENUS

T.. FRAC 100
Fraction Collector



The FRAC-100 does what a
small fraction collecto
collects fractions, P

The FRAC-100 will fulfill all you
for basic fractionation requiremen
conventional collector the FRAC-
unequaled in performance and relia
Simplicity in design (3 moving pa
control (flexible fractionation pa
accessed by a multifunction k
accomplish fractionatio
previously attainable only
expensive i

The FRAC-100, as a sophist
fraction collector presents unique
handling and integration capabilities.
input of a 0-10mV detector sig
processed by the FRAC-100 to repor
digital display such sepa
parameters as peak height, peak w
peak retention and peak area
addition of a PSV-100 solenoid
enables selective fractionation of onl
peak material as judged by a
detector defined by a programm
threshold v

If what you're looking for is a small, simple, inexpensive fraction collector - the FRAC-100 is your best choi

If what you're looking for is a small sophisticated collector with power and abilities far beyond anythi
presently available - the FRAC-100 is your only choice!

Pharmacia Fine Chemicals AB
Box 175
S-751 04 Uppsala 1
Sweden

Pharmacia
Fine Chemi

Circle No.14 on Reader Service Card.

...e to m...t, to ...now, to understand.

A world-wide scientific research organization for human health and welfare.
A center for promotion of scientific and cultural activities

SERONO SYMPOSIA

Serono Symposia is an independent foundation, created in 1971, to promote scientific research in all disciplines which contribute towards improving human health.
This aim is pursued by means of congresses, courses, seminars and specialized studies.



...f the international congresses scheduled for 1984 are:

...opharmacology
...a, March 8-10
...ic Organization: P.M. Miescher (CH)

...ndocrine Physiology of Pregnancy ... Peripartal Period
...a, April 11-13
...ntific Organization: R.B. Jaffe (USA) – ...ell'Acqua (I)

...roid Disorders Associated with ...line Deficiency and Excess
...iburg, April 24-26
...entific Organization: J. Koebberling (D) – ...Hall (GB)

...tives in Fetal Diagnosis
... May 2-4
... Organization: A.M. Kuliev (USSR) – ...ell (GB)

Cytobiology of Leukemias and Lymphomas
Siena, May 24-26
Scientific Organization: D. Quaglino (I) – F.G.J. Hayhoe (GB)

The Adrenal Gland and Hypertension
Padua, June 22-23
Scientific Organization: E.G. Biglieri (USA) – F. Mantero (I)

Reproductive Medicine
San Juan, October 4-6
Scientific Organization: E. Steinberger (USA)

Development and Function of the Reproductive Organs
Rehovot, October 22-24
Scientific Organization: A. Tsafriri (IL)

SERONO SYMPOSIA
CONGRESS CARD
This card entitles the bearer to attend the Serono Symposia throughout the world. Its presentation gives access to all services provided by the registration fee. Valid for 1984

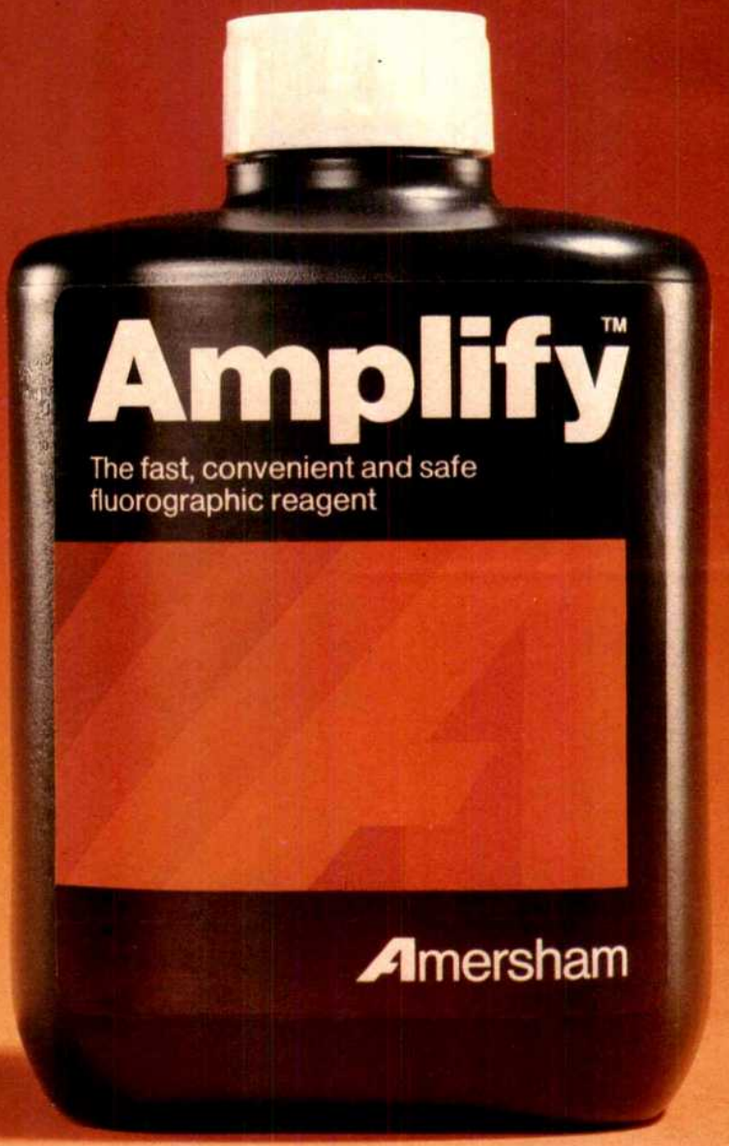
Do you know all the advantages that the Serono Symposia Congress Card offers? Please fill in this form and mail to Serono Symposia for information.

swissair Official Carrier

I would like to receive information about:
☐ Serono Symposia Congress Card ☐ All the above Congresses
☐ In particular the Congress on .....
.....
☐ Serono Symposia publications
Name .....
Address .....
Institution .....
Please send to Serono Symposia, Via Ravenna 8 - 00161 Rome - Italy

Please send to Serono Symposia, Via Ravenna 8 - 00161 Rome - Italy

A quantum jump in fluorography



Please contact Amersham or your local representative for further details.

Amersham International plc
Amersham England HP7 9LL
telephone Little Chalfont (024 04) 4444

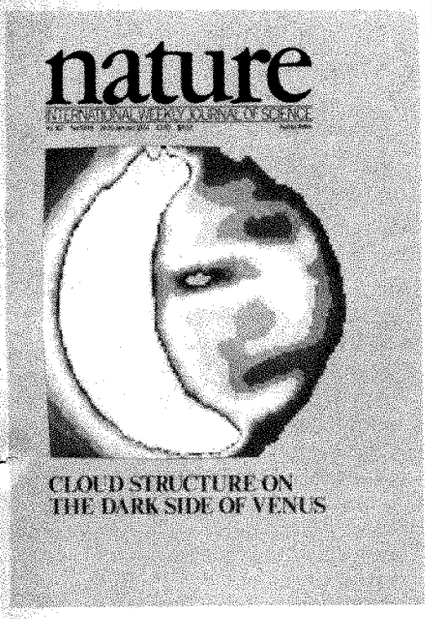
Amersham

Amersham Australia PTY Limited Sydney **Amersham Belgium SA/NV** Brussels **Amersham Buchler GmbH & Co. KG** Braunschweig W. Germany
Amersham Corporation Arlington Heights USA **Amersham Denmark ApS** Birkerød **Amersham France SA** Paris **Amersham Japan** Tokyo **Amersham Nederland BV** Utrecht

Circle No.50 on Reader Service Card.

nature

COVER



CLOUD STRUCTURE ON THE DARK SIDE OF VENUS

The dark side of Venus is unexpectedly bright at infrared wavelengths below 3 μm, and observations from the Anglo-Australian Telescope show structured cloud patterns (above) with very long rotation periods (see page 222).

OPINION

Transparency at Stockholm 197
Pork-barrel supplement 198
Examination fever
You are not what you eat 199

NEWS

Fast breeder reactors 200
Asian academy established
French fast breeder reactors
US nuclear arsenal 201
Nuclear reprocessing
The falling index
Franco-Chinese collaboration 202
Nature conservancy
Molecular biology 203
UK public health
US research computers 204
French research
Budget prospects

CORRESPONDENCE

Publication by conference/Indian scientists/Antibiotic misuse/etc. 206

NEWS AND VIEWS

Exotic nuclear decay discovered
John Maddox 207
Mapping the sea floor by satellite
Roger N Anderson 208
The origin of gas in quasars — reverse stellar evolution?
Martin Gaskell 210
The AIDS epidemic: continental drift
Jerome E Groopman & Paul A Volberding 211
Gene amplification and drug resistance
Margaret Fox 212
Viruses and tissue injury
Bernard N Fields 213
Chalky submarines
Paul D Calvert 214

BOOK REVIEWS

Supersymmetry and Supergravity *by J Wess and J Bagger*
Abdus Salam 297
Physiological Plant Ecology II, III and IV *O L Lange et al. eds*
Peter D Moore 298
Natural Selection, Heredity and Eugenics *J H Bennett ed.*
Cedric A B Smith 299
The Biology of Cyanobacteria *N G Carr and B A Whitton eds*
Sergey V Shestakov 300
Vectorial Astrometry *by C A Murray*
P K Seidelmann

ARTICLES

Observation of stellar remnants from recent supernovae
D J Helfand & R H Becker 215

Cloud structure on the dark side of Venus
D A Allen & J W Crawford 222

Trace element patterns at a non-marine Cretaceous–Tertiary boundary
J S Gilmore, J D Knight, C J Orth, C L Pillmore & R H Tschudy 224

Coupled Sm–Nd and U–Pb systematics in late Caledonian granites and the basement under northern Britain
A N Halliday 229

Molecular cloning of cDNA for murine interleukin-3
M C Fung, A J Hapel, S Ymer, D R Cohen, R M Johnson, H D Campbell & I G Young 233

A molecular map of the human major histocompatibility complex class III region linking complement genes C4, C2 and factor B
M C Carroll, R D Campbell, D R Bentley & R R Porter 237

Transcriptional interference in avian retroviruses — implications for the promoter insertion model of leukaemogenesis
B R Cullen, P T Lomedico & G Ju 241

LETTERS TO NATURE

A new kind of natural radioactivity
H J Rose & G A Jones 245

Power spectrum of long-period solar oscillations and 160-min pulsations during 1974–82
A B Severny, V A Kotov & T T Tsap 247

Early Solar System aqueous activity: Sr isotope evidence from the Orgueil CI meteorite
J D Macdougall, G W Lugmair & J F Kerridge 249

Meteoritic $^{138}Ce/^{142}Ce$ ratio and its evolution
H Shimizu, T Tanaka & A Masuda 251

Carbon isotope composition of low molecular weight hydrocarbons and monocarboxylic acids from Murchison meteorite
G Yuen, N Blair, D J Des Marais & S Chang 252

Stable isotope compositions of gases and vegetation near naturally burning coal
J D Gleason & T K Kyser 254

Persistence of organic phosphates in buried soils
J H Ottaway 257

Presence of NADPH-cytochrome P450 reductase in central catecholaminergic neurones
L Haglund, C Köhler, T Haaparanta, M Goldstein & J-Å Gustafsson 259

Effect of impulse blockage on cytochrome oxidase activity in monkey visual system
M Wong-Riley & E W Carroll 262

Contents continued overleaf

Nature (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Annual Index (mailed in February). Annual subscription for USA and Canada US $230 (for subscription prices elsewhere, see next page). USA and Canadian orders only to: *Nature*, Subscription Dept PO Box 1018, Manasquan, New Jersey 08736, USA. All other orders to *Nature*, Brunel Road Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY10010 and additional mailing offices. US Postmaster send form 3579 to: *Nature*, 15 East 26 Street, New York, NY 10010. Published in Japan by Macmillan Shuppan K.K., 5th Floor Eikow Building, 1-10-9 Hongo, Bunkyo-ku, Tokyo, Japan. 1984 Macmillan Journals Ltd

Advertisement

BRL® PRODUCT REVIEW

Bethesda Research Laboratories, Life Technologies Inc. P.O. Box 6009 • Gaithersburg, MD 20877
Bethesda Research Laboratories GmbH Offenbacher Strasse 113 • 6078 Neu Isenburg • West Germany
Bethesda Research Laboratories (UK) Ltd. P.O. Box 145 • Science Park • Cambridge • United Kingdom CB4 3BE
Bethesda Research Laboratories SARL F-95220 Herblay France

Two Superior Color Indicators

Bluo-Gal

For cloning into the lac gene, choose Bluo-Gal, a histochemical substrate for β-D-galactosidase. As with the widely-used substrate X-Gal, lac$^+$ bacterial colonies are blue in its presence. However, Bluo-Gal contains a unique chromophor that rapidly produces a darker blue color than X-Gal. BRL's exclusive Bluo-Gal is the ideal indicator for M13 cloning and whenever β-galactosidase activity is altered. Already an integral part of the BRL M13 Cloning System, Bluo-Gal now is available separately for the first time.

Circle No. 40 on Reader Service Card.

AMPSCREEN™

Use BRL's exclusive AMPSCREEN, and detect ampicillin-sensitive recombinant DNA-containing colonies in only 10 minutes without replica plating. AMPSCREEN is a sterile disk, sized to fit into a standard 85 mm petri dish, and treated with a chromogenic substrate. After the colonies growing on a petri dish are blotted onto AMPSCREEN, recombinant DNA-containing colonies remain blue, while non-recombinant ones change from blue to yellow. The complete color change takes place in less than 10 minutes.

Circle No. 41 on Reader Service Card.

DNase I, RNase free

BRL DNase I is free of contaminating ribonucleases, making it ideal for isolating RNA. Isolated from bovine pancreas and purified to homogeneity by affinity chromatography, DNase I degrades DNA to oligonucleotides without degrading RNA or proteins. With each lot of DNase I, BRL provides exclusive lot-specific functional data specifying the amount of DNA digested by one μg of DNase I in one minute at 37°C.

Circle No. 42 on Reader Service Card.

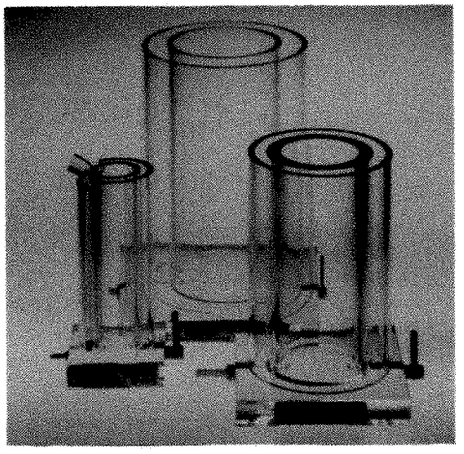
Dpn I

$$5'\text{-}G^{Me}A\downarrow T\quad C\text{-}3'$$
$$3'\text{-}C\quad T\uparrow A^{Me}G\text{-}5'$$

The newest addition to BRL's line of high-quality restriction enzymes is *Dpn* I. *Dpn* I cleaves its recognition sequence *only* when the adenine is methylated, and is especially useful in isolating unmethylated DNA from a mixture of both methylated and unmethylated DNA. Isolated from a variant strain of *Diplococcus pneumoniae*, BRL *Dpn* I is purified free of contaminating nucleases and is qualified as a cloning-grade enzyme.

Circle No. 43 on Reader Service Card.

Save 15% on BRL Gradient Formers January 1 Through March 31, 1984



BRL Gradient Formers provide an inexpensive, easy-to-use means of generating accurate, highly reproducible gradients of aqueous fluids. The compact, concentric reservoir design makes it easy to match fluid heights and requires no cumbersome supports for precise leveling. Each gradient former is individually calibrated and adjusted to assure accurate linear gradients (± 1% of absolute linearity). In addition, concave, convex and exponential gradients can be readily generated. BRL Gradient Formers are available in three sizes, 150 ml, 750 ml, and 2 liter total capacities.

Circle No. 44 on Reader Service Card.

In-House Mycoplasma Detection and Identification

With MycoTect and MycoSpec, available *exclusively* from BRL, you can detect and identify species of mycoplasmas quickly, easily, and inexpensively, right in your laboratory.

MycoTect is cytotoxic to mycoplasma infected cells. After incubation of cell samples with MycoTect and exposure to crystal violet, cell monolayers in uninfected wells stain more intensively and can be easily distinguished from the sparse cell layers found in mycoplasma-infected wells. The entire process takes only five days—less than one fifth the time needed for detection by conventional methods. The cost of a MycoTect assay is only a fraction of the cost of current tests, and the only equipment needed is a 24-well plate.

MycoSpec is a rapid, simple and inexpensive assay for the five major mycoplasma species that account for more than 95% of all laboratory mycoplasma infections. Mycoplasma samples are incubated separately with monoclonal antibodies to each of the five species, then with a biotinylated secondary antibody and finally with a streptavidin-horseradish peroxidase complex. The sample that stains when diaminobenzidine is added contains the monoclonal to the infecting species. With MycoSpec, identification is complete within five hours.

Circle No. 45 on Reader Service Card.

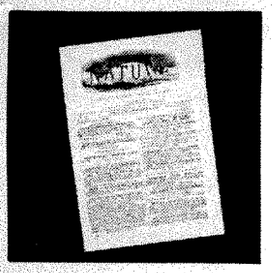
T4 Polynucleotide Kinase

Prepared by an exclusive new method, BRL Kinase is entirely free of contaminating endonucleases and exonucleases and is functionally free of RNase. Each lot of BRL Kinase is functionally assayed for its ability to end-label DNA fragments in both exchange and forward reactions, and data from these tests are provided with every order. BRL provides Kinase in soluble form and, for the researcher's convenience, guarantees the concentration of each lot of enzyme at 10 units/μl.

Circle No. 46 on Reader Service Card.

CONTENTS

Fascinating reading and an unusual gift



A high-quality, 40-page facsimile of the first issue of Nature is available, including articles on:

* **The recent total eclipse of the sun** by Sir Norman Lockyer
* **Science Teaching in Schools** by W. Tuckwell
* **Triassic Dinosauria** by T. H. Huxley

Prices and address for orders are set out below.

Annual Subscription Prices

UK & Irish Republic		£98
USA & Canada		US$230
Australia & NZ	Airspeed	£150
Continental Europe	Airspeed	£120
India	Airspeed	£115
Japan	Airspeed	Y85000
Rest of World*	Surface	£120
Rest of World*	Airmail	£180

(not USA, Canada, Europe & Japan)

Orders (with remittance) to:

USA & Canada
Nature
Subscription Dept
PO Box 1018
Manasquan
NJ 08736
USA

UK & Rest of World
Nature
Circulation Dept
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to:
Japan Publications Trading Company Ltd
2-1 Sarugaku-cho 1-chome
Chiyoda-ku, Tokyo, Japan
Tel: 03-292-3755

Personal subscription rates
These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
15 East 26 Street
New York,
NY 10010, USA
Tel: (212) 689-5900

UK & Europe
Felicity Parker, Nature
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

Credit card orders only (in USA):
Call toll-free: (800) 824-7888 (Operator 246)
In California: (800) 852-7777 (Operator 246)

Back issues
UK, £2.50; USA & Canada, US$6.00 (surface), US$9.00 (air); Rest of World, £3.00 (surface), £4.00 (air)

Binders
Single Binders: UK, £4.50; Rest of World, $10.00
Set(s) of 3 Binders; UK, £12.00; Rest of World, $25.00

Annual indexes (1971–1982)
UK, £5.00 each; Rest of World, $10.00

Nature First Issue Facsimile
UK, 75p; Rest of World (surface), $1.50; (air) $2.00

Nature in microform
For Information:
University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

Community food webs have scale-invariant structure
F Briand & J E Cohen 264

Monoclonal antibody that identifies subsets of neurones in the central visual system of monkey and cat
S H C Hendry, S Hockfield, E G Jones & R McKay 267

Muscarinic inhibitory transmission in mammalian sympathetic ganglia mediated by increased potassium conductance
A E Cole & P Shinnick-Gallagher 270

Specific depletion of immunoreactive growth hormone-releasing factor by monosodium glutamate
B Bloch, N Ling, R Benoit, W B Wehrenberg & R Guillemin 272

Astrocytes present myelin basic protein to encephalitogenic T-cell lines
A Fontana, W Fierz & H Wekerle 273

Hydroxyl radical scavengers inhibit human natural killer cell activity
M Suthanthiran, S D Solomon, P S Williams, A L Rubin, A Novogrodsky & K H Stenzel 276

Viral perturbation of endocrine function: disordered cell function leads to disturbed homeostasis and disease
M B A Oldstone, M Rodriguez, W H Daughaday & P W Lampert 278

Homologous cell-derived oncogenes in avian carcinoma virus MH2 and murine sarcoma virus 3611
H W Jansen, R Lurz, K Bister, T I Bonner, G E Mark & U R Rapp 281

Cell lineage-specific undermethylation of mouse repetitive DNA
V Chapman, L Forrester, J Sanford, N Hastie & J Rossant 284

Regulation of *Antennapedia* transcript distribution by the *bithorax* complex in *Drosophila*
E Hafen, M Levine & W J Gehring 287

Activation of extra copies of genes coding for nitrogenase in *Rhodopseudomonas capsulata*
P A Scolnik & R Haselkorn 289

Transposition of Tn*554* does not generate a target duplication
E Murphy & S Löfdahl 292

MATTERS ARISING

Sedimentation in Loch Earn and Loch Lubnaig, Scotland
A G McKay
Reply: R W Duck & J McManus 295

Control of fertility in red deer
S D Albon & G R Iason
Reply: A S I Loudon, A S McNeilly & J A Milne 296

MISCELLANY

Author index: Vol. 306 ix
100 years ago 214
Books received 301

NATURE CLASSIFIED

Professional appointments — Research posts — Studentships — Fellowships — Conferences — Courses — Seminars — Symposia: Back pages

Next week in *Nature*:

- Hubble constant from 21 cm linewidth
- Volcanic eruption of AD 536
- Twentieth century magnetic secular variation
- 3,450 Myr argon date
- Activation of translocated *myc* oncogene
- Peptide helix termination
- Cultivation of *Plasmodium* parasites in human liver cells
- γ-interferon, immune response and gene expression
- Bushcricket courtship
- Gradual decline of dinosaurs denied

GUIDE TO AUTHORS

Authors should be aware of the diversity of *Nature*'s readership and should strive to be as widely understood as possible.

Review articles should be accessible to the whole readership. Most are commissioned, but unsolicited reviews are welcome (in which case prior consultation with the office is desirable).

Scientific articles are research reports whose conclusions are of general interest or which represent substantial advances of understanding. The text should not exceed 3,000 words and six displayed items (figures plus tables). The article should include an abstract of about 50 words.

Letters to *Nature* are ordinarily 1,000 words long with no more than four displayed items. The first paragraph (not exceeding 150 words) should say what the letter is about, why the study it reports was undertaken and what the conclusions are.

Matters arising are brief comments (up to 500 words) on articles and letters recently published in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted.

Manuscripts may be submitted either to London or Washington (decisions being made only in London). Manuscripts should be typed (double spacing) on one side of the paper only. Three copies are required, each accompanied by copies of lettered artwork. No title should exceed 80 characters in length. Reference lists, figure legends, etc. should be on separate sheets, all of which should be numbered. Abbreviations, symbols, units, etc. should be identified on one copy of the manuscript at their first appearance.

References should appear sequentially indicated by superscripts in the text and should be abbreviated according to the *World List of Scientific Periodicals*, fourth edition (Butterworth 1963–65). The first and last page numbers of each reference should be cited. References to books should clearly indicate the publisher and the date and place of publication. Unpublished articles should not be formally referred to unless accepted or submitted for publication, but may be mentioned in the text.

Each piece of artwork should be clearly marked with the author's name and the figure number. Original artwork should be unlettered. Suggestions for cover illustrations are welcome. Original artwork (and one copy of the manuscript) will be returned when manuscripts cannot be published.

Requests for permission to reproduce material from Nature *should be accompanied by a self-addressed (and, in the case of the United Kingdom and United States, stamped) envelope.*

TWO NEW reliable, up-to-date and complete biotechnology information resources.

Essential aids for everyone concerned with both the old and new biotechnologies — will save you valuable time and money.

EXAMINE ON FREE APPROVAL WITHOUT COMMITMENT

Detailed information on every aspect of biotechnology worldwide

An indispensable guide to:

- ★ Products, services and research worldwide.
- ★ Companies worldwide.
- ★ Government and international agencies worldwide.
- ★ Information sources worldwide.

This **new** world directory, compiled by the eminent biotechnologist Dr. Jim Coombs, provides a mass of authoritative information about **the entire biotechnology industry worldwide.**

Unlike other directories, THE INTERNATIONAL BIOTECHNOLOGY DIRECTORY 1984 —

- is thoroughly international
- features a unique BUYER'S GUIDE to products, research and services
- covers both the old and new biotechnologies

The Directory tells you:

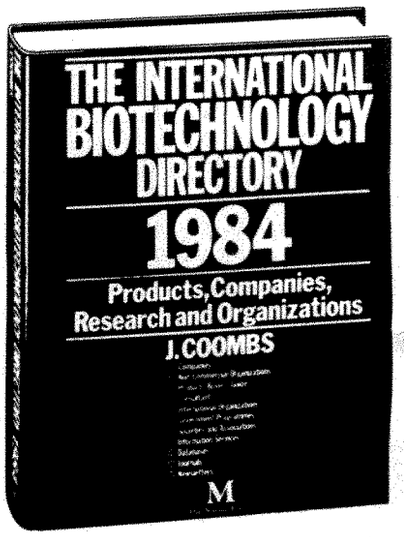
- ★ Who your competition is, and what they are doing.
- ★ Who are potential customers and suppliers.
- ★ Where to buy equipment, chemicals and services.
- ★ Where culture collections are located and what cultures they hold.
- ★ Where to get plants built.
- ★ Who imports/exports biotechnology products in over 20 countries.
- ★ What consultancy services are available.
- ★ Which government departments are associated with biotechnology, and their programmes.

Authoritative author

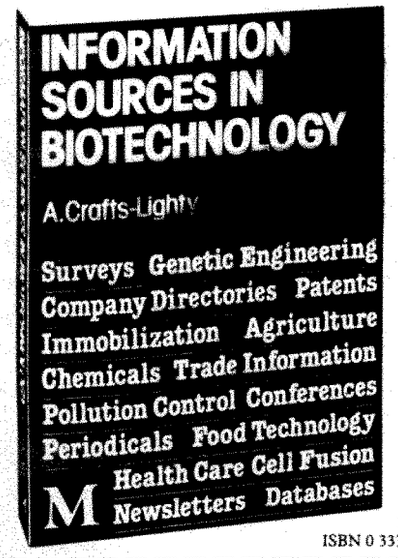
Dr. Jim Coombs is a Member of the Organising Committee of Biotech '83 and of Bio-Energy 1984, and Scientific Secretary of the Third European Community Energy from Biomass Conference 1984. He is editor of *Biomass* and Joint Secretary of the BABA.

The Directory is designed for use across the whole biotechnology industry by scientists and researchers, sales managers, buyers and consultants. Here you will find: over 2,000 organisations — companies, universities and research centres — listed by country. Government agencies and programmes — listed by country. Societies and associations — listed by country. Databases, journals and newsletters — listed alphabetically. Products listed under more than 1,000 headings.

All compiled by Dr. Coombs from a vast array of sources then meticulously cross-checked to ensure the reliability of the data. Now available to you on FREE APPROVAL.



Macmillan Hardcover 400pp
November 1983 ISBN 0 333 35140 1
£50.00/$100.00



The ONLY guide to the selection of cost-effective biotechnology information sources

Evaluates and assesses the whole range of information sources available.

- ★ covers both old and new technologies.
- ★ covers technical and commercial literature.
- ★ covers difficult and hard to trace sources.
- ★ includes over 20 at-a-glance evaluation tables.
- ★ provides an Index of publishers and their addresses.

Dr. Crafts-Lighty, Head of Information and Library Services at Celltech Limited, systematically and critically reviews all the important information sources:

- books • conferences • trade periodicals • research periodicals
- computer databases • patents
- market surveys • directories
- organisations • secondary sources

'Information Sources in Biotechnology' is an invaluable guide to current technical, business and commercial biotechnology information sources for Researchers, Librarians, Information Officers, Businessmen, Investment Analysts, Investors, and Academic Researchers.

Send no money now. Examine on FREE APPROVAL.

You can take both these new books for 30 days examination without commitment. Decide to keep either or both, then simply pay the accompanying invoice. If you are not completely satisfied, just return the book(s) in good condition within 30 days and that will be the end of the matter. Ensure prompt delivery by completing and returning the order form **now.**

Macmillan Paperback
320pp **September 1983**
ISBN 0 333 36178 4 **£40.00/$80.00**

Globe

Globe Book Services Limited,
Brunel Road, Houndmills,
Basingstoke, Hants RG21 2XS, England.
Telephone: Basingstoke (0256) 26222
A DIVISION OF MACMILLAN PUBLISHERS LTD.

The Nature Press,
15 E 26 Street, New York, N.Y. 10010.
Telephone: 212-532-4811

FREE APPROVAL ORDER FORM

To: Ian Jacobs, Globe Book Services Ltd., FREEPOST, Brunel Road, Houndmills, Basingstoke, Hants RG21 2BR, England.

The Nature Press, 15 E 26 Street, New York. N.Y. 10010. Credit card callers call toll-free 800-221-2123 (or in New York: 212-532-4811)

Please send me on 30 days FREE APPROVAL _____ copies of *The International Biotechnology Directory 1984* at £50.00/$100.00 each, and/or _____ copies of *Information Sources in Biotechnology* at £40.00/$80.00 each.

If I am not entirely satisfied, I may return the book(s) in good condition within 30 days and you will cancel my invoice.

Postage and packing
UK subscribers £2.00 (Overseas £3.00)
US Subscribers $1.50.

Tick preferred method of payment

☐ Bill me. I need send no money now.
☐ I enclose cheque for £/$_____
☐ Charge my credit card on publication £/$_____
☐ Visa ☐ Access/Mastercharge ☐ Am. Express ☐ Diners

Card No._____

Expiry Date_____

Signature _____
(Orders cannot be accepted without a signature.)

Name _____ (PLEASE PRINT)

Address _____

Postcode/Zip Code_____
Books will be delivered to you on publication. N1

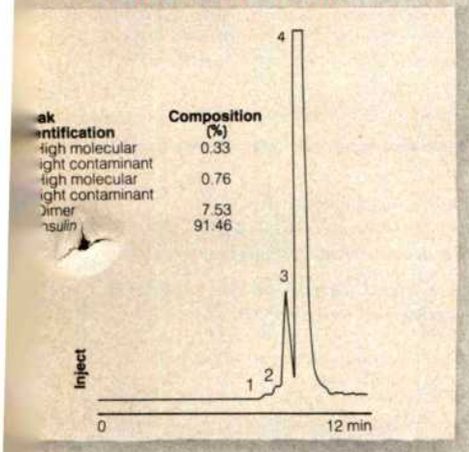
UK Subscribers POST TODAY — NO STAMP NEEDED

Circle No.91 on Reader Service Card.

Now isolate and analyze proteins faster, with higher resolution and recovery.

Waters HPLC systems with new ion exchange, gel filtration and hydrophobic packings for biological separations.

High speed gel filtration separation of insulin.

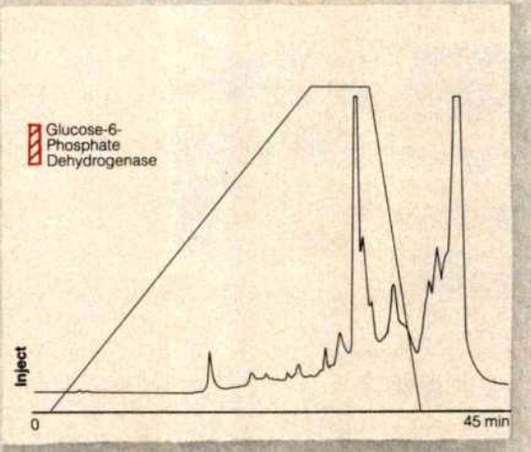


Separation time: 12 minutes.

Waters new high speed gel ration methods allow rapid ctionation of protein samples. is same gel filtration method lets u profile sample composition, olate all components and verify e purity of collected fractions.

mple: 10 µl Insulin, 5 mg/ml
n: PROTEIN-PAK 125
0.1% Trifluoroacetic Acid, pH 2.1
Rate: 1.0 ml/min
tection: 214 nm, 0.5 AUFS

Total recovery of enzymatic activity from a crude bacterial extract using ion exchange.

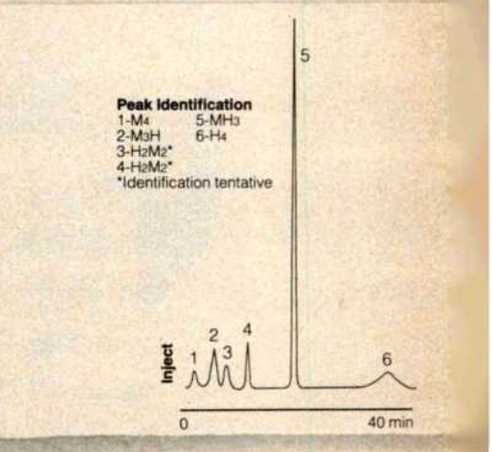


100% recovery of biologically active protein.

The large pore size of Waters polymeric ion exchange packings (1,000 Å) gives you recoveries of enzymatic activity approaching 100% for even high molecular weight enzymes.

Sample: 100 µl Bacterial Extract, 3 mg/ml
Activity: Glucose-6-Phosphate Dehydrogenase
Column: PROTEIN-PAK DEAE 5PW
Gradient Conditions
Eluent A: 0.02M Tris HCl, pH 8.5
Eluent B: 0.02M Tris HCl, pH 8.5 + 0.5M NaCl
0-100% B, curve 6, 25 min
Flow Rate: 0.5 ml/min
Detection: 280 nm, 0.1 AUFS
Specific Activity Increase: 4.9
Activity Recovery: 100%

High resolution ion exchange separation of isoenzymes.



Complete resolution of LDH isoenzymes.

The resolving power of Waters high performance ion exchange packings provides resolution of the closely related isoenzymes of lactate dehydrogenase.

Sample: 100 µl LDH Isoenzymes, 1 mg/ml
Column: PROTEIN-PAK DEAE 5PW
Gradient Conditions
Eluent A: 0.02M Tris Acetate, pH 8.0
Eluent B: 0.02M Tris Acetate, pH 8.0
+ 1.0M NaOAc
0-100% B, curve 6, 45 min
Flow Rate: 0.5 ml/min
Detection: 280 nm, 0.05 AUFS

Add the performance of Waters HPLC to your separations. And achieve greater success in protein isolation and analysis.

For a demonstration or additional information, contact your local Waters representative or call 617-478-2000 (X 2665).

Waters

MILLIPORE

Waters The Liquid Chromatography People®
34 Maple St./Milford, MA 01757/(617)478-2000

For a demonstration circle No. 27 on Reader Service Card
For further information circle No. 28 on Reader Service Card

THE ULTIMATE IN ION-SELECTIVE MEASUREMENTS

WPI'S FD 223
DUAL/DIFFERENTIAL ELECTROMETER



- input resistance $10^{15}\ \Omega$
- input capacitance 0.1 pF
- differential readings
- leakage current 10^{-14} A
- LCD readout
- independent, simultaneous readings

FD223 puts first-stage amplification close to the source with an active probe headstage *minimizing noise pick up and signal loss.*

FD223 DUAL/DIFFERENTIAL ELECTROMETER
. . . . the ultimate for precision measurements from ion-selective and pH macro- and microelectrodes.

Only offered by

WPI

W-P INSTRUMENTS, INC.

60 Fitch Street • P.O. Box 3110 • New Haven, Conn. 06515 U.S.A. Tel.: (203) 389-2183 • Cables: WPINSTR • TWX: 710-465-2638

Circle No.08 on Reader Service Card.

EDITORIAL OFFICES

London Office
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024

Washington Office
1134 National Press Building, DC 20045
Telephone: (202) 737-2355

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff: Alun Anderson, Tim Beardsley, Philip Campbell, Isobel Collins, Peter Gambles, Melanie Kee, Tim Lincoln, Naomi Molson, Sara Nash, Geoffrey North, Peta Pickering, Miranda Robertson, Robert Walgate, Charles Wenz, Nigel Williams, Guil Winchester

Washington Editor: Peter David
Washington Correspondent: Stephen Budiansky

Art and Design: Joan Godwin, Joel Chernin, Theresa George, Paul Norris, Marie Xeridat

Administration: Mary Sheehan, Jacqueline Cole, Ruth Cutts, Joanna Czechowska, Ruth Goldenberg, Sharon Isaacs and Penny Walker (Washington)

Publisher: Elizabeth Hughes

Marketing Director: Ray Barker

Promotion Manager: Felicity Parker

International Advertising Manager: Andy Sutherland
Features Advertising Manager: Marion Delaney

Group Classified Advertising Manager: John Barnes — Telephone: (01) 240 1101

Nature New York Office
15 East 26 Street, New York, NY 10010
Telephone: (212) 689-5900 Telex: 668497

General Manager: Gary Rekstad

American Advertising Manager: Henry Dale

Classified Advertising Manager: Miss Gene Fein

Circulation Manager: Mirta Soto

Canadian Display and Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1—(416) 690-2423

Display Advertising Representatives

New York: Nature Office

Chicago: Didier & Broderick, Inc.—(312) 498-452(

San Francisco: Jobson/Jordan/Harrison/Schulz, Inc.—(415) 392-6794

Los Angeles & Pasadena: Jobson/Jordan/ Harrison/Schulz, Inc.—(213) 796-9200

Philadelphia: Daniel Adams Associates, Inc.—(21 353-6191

Boston: CEL Associates—(617) 848-9306

Fort Lauderdale: Brinker & Brinker—(305) 771-0064

New Jersey: Mary Grunmeier—(201) 572 9018

Dalton, Pennsylvania: Donald E. Northup Associates—(717) 563 2572

West Germany and Austria: Franz Schrecklinger TMW Top Media Werbegesellschaft GmbH Frankfurt—(611) 726046

Holland and Belgium: Adele Struyck G. Arnold Teesing BV Amsterdam—(020) 2636 15

Switzerland: Werner Stähli Agentur Iff AG Schaffhausen—(053) 45821

Scandinavia: Andrew Karnig Andrew Karnig & Associates AB Stockholm—(08) 51 68 70

Japan: Mashy Yoshikawa Orient Echo Inc Tokyo—541-4923

Australia: Gus Bartel & Associates PO Box 1755, Southport, Queensland 4215 (075) 466 577

Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office

©1984 Macmillan Journals Ltd
Vol.307 No. 5948 19 January 1984

nature

Transparency at Stockholm

The conference opening this week at Stockholm is another opportunity for arms control. It should keep hopeful ambitions in check and concentrate, instead, on mechanisms for monitoring.

ON the principle that nuclear war is too important to be left to the nuclear superpowers, European states must make the most of the Conference on Disarmament in Europe which opened this week in Stockholm. The signs are that the countries concerned recognize the occasion as an opportunity. But so, it seems, do the Soviet Union and the United States, both of which seem to have taken fright at the breakdown of the arms control negotiations at Geneva at the end of last year, and which have been making noises that may be construed as conciliatory in the past few days. (More accurately, the United States has been making contradictory noises, not unalloyed denunciation, while the Soviet Union has lapsed into silence.) Rarely can states which have by muddled mismanagement lost one opportunity (see *Nature* **306**, 111; 1983) so quickly been provided with another.

The sunny climate in which people appear to have assembled in Stockholm does not, however, guarantee a successful outcome. Indeed, the constitution of the conference is hardly that of a gathering at which tangible agreements could be reached on matters that have not already been fully explored. Altogether, 35 countries will be represented at Stockholm, all of them signatories of the Helsinki Agreements signed in 1975. The gathering is in this sense a kind of relic of a quite different epoch, that in which East–West detente was the touchstone for most international relationships. Almost as they were signed, the Helsinki agreements came to seem more trouble than they were worth. Various people in the Soviet Union, Brailovsky for example, found themselves locked up because they formed the Helsinki Monitoring Group to ensure that high-sounding language agreed at Helsinki was being translated into reality. (Broadly speaking, people's freedom to move from place to place has not been upheld.) As detente withered, a process that began long before the Soviet hegemony over Afghanistan, the successive conferences held within the framework of the Helsinki agreements became increasingly acrimonious, more like opportunities for threatening the withdrawal of cooperation than the recurrent use of that word in the Helsinki documents implied. The meeting that ended last year in Madrid, and which led directly to this week's encounter in Stockholm, showed how ungainly a forum for negotiation such occasions can be. Variously, it was an occasion for mutual recrimination, for flying adventurous but improbable kites — and for some smaller members, Malta for example, to advertise their presence by demanding satisfaction on some peripheral issue as the price of agreement on the date of the next meeting.

Agreement

Helsinki has, however, produced one tangible understanding which is potentially of great benefit to European security, East and West. This is the agreement between all parties that military manoeuvres within 150 kilometres of the boundary between East and West will be notified in advance, and that observers will from time to time be invited by the government concerned. There have been several occasions in the past five years when this facility has helped to steady people's nerves. The fixed element of the agenda for Stockholm is a plan, derived from what was originally a French proposal, to extend this arrangement to cover the whole land surface of Europe and, at the same time, to ensure that military manoeuvres are open to observers from the other side as of right, and not at the discretion of the countries concerned.

In the absence, perhaps a long one, of an agreement on nuclear weapons based in or aimed at Europe (East and West), the prospect of such a lesser agreement is bound to be attractive. Anything, people will say, is better than nothing — and they will be right. Moreover, if the principle can be agreed that all European governments should know what others are doing with their visible military forces, the way may be opened for a more general understanding that the same principle should apply to the more remote strategic components in the uneasy balance — stragetic missiles, long-range aircraft and even lesser weapons of special kinds, nuclear artillery shells for example. That seems to be the objective in the minds of the governments now advocating the benefits of what they call transparency as a first substantial step towards mutual security. The question that must be tackled seriously in the weeks ahead is whether the mechanisms provided by the Helsinki agreements are durable enough.

Conviction

As with all agreements between mutually distrustful governments, it is dangerous to let good intentions outstrip the means of telling that they have been realized. So much is clear from the professed conviction of the United States Administration that the Soviet Union has violated existing agreements on arms control. The administration has in the past few days been telling people in Congress of these transgressions in ways calculated to ensure that what it has to say finds its way into the newspapers before it formally makes them public next week. The snag, with all these allegations, is that the data published to support them are unconvincing. The claims that South-East Asian yellow rain is a vehicle for delivering mycotoxins to innocent people are, as published, unconvincing — but the US State Department falls back on unpublishable intelligence information said to prove the case to the hilt. Much the same is true of the allegations that the Threshold test-ban treaty of 1974 has been violated by the Soviet Union; the data so far published are not necessarily inconsistent with the threshold limit of 150,000 tonnes of TNT equivalent that specified the threshold — but, ironically, the uncertainties might well be removed if only the two sides would formally ratify the treaty and then, as required, exchange the data about seismic travel times in their nuclear testing grounds on which a more accurate assessment might be based. The moral is that the plans now being hatched at Stockholm should be as much concerned with means of verifying whatever may be agreed as with the objectives of agreement.

This, unfortunately, is where the trouble starts. Even the agreement that military manoeuvres in Europe should be notified has been challenged, perhaps inevitably. The objective of any agreement along these lines is to ensure that one government will not mistake another's decision to exercise its military forces as a sign that some kind of attack is imminent, perhaps prompting pre-emptive measures. But who can tell, simply by looking at what happens, the difference between manoeuvres which must be notified and substantial movements of troops and military equipment in the normal course of business, perhaps because of a strategic decision that forces should be differently disposed? This is why the transparency that has become the objective of many European governments will be persuasive (and safe) only if there is some mechanism for making sure that a government suspecting

0028-0836/84/030197-01$01.00

© 1984 Macmillan Journals Ltd

the worst will have access to means of verifying the facts of what may seem a threatening development and then of coming to an understanding of why that development has taken place.

The obvious mechanism is to arrange that a complainant government should have the right to question any other about its intentions, ideally as part of some framework of regular meetings. The trouble is that there will be natural limits on the frequency with which awkward questions may be raised arising from political considerations and even from the unwillingness of governments implicitly to disclose the weaknesses of their intelligence networks. So why not replicate for the monitoring of European forces a system comparable with the nuclear safeguards inspectorate, which in the past decade has made the Nuclear Non-Proliferation Treaty work without substantial complaint? The result, it is true, would be yet another international body staffed by international civil servants, but the value of such an organization would far outweigh not merely its cost but the irritation it would no doubt cause. Moreover, because such a body of inspectors, individually nationals of member states but collectively impartial, would serve as a model for the supervisory authority there would have to be in any more ambitious programme for transparency in Europe than that now planned, its creation would be a more important gain than any other agreement that could be quickly reached at Stockholm.

Mercifully, such an arrangement would also fit in well with the growing belief, at least in Western Europe, that safety from the threat of all-out war cannot much longer be left to the two principal nuclear powers. Little benefit would result from a broadening of the basis of the negotiations on nuclear arms which have broken down at Geneva, which is not to say that the British and French nuclear forces are irrelevant. For if two powers fail to reach agreement, the chances that four (or even five, with China) would do so must be smaller. Yet the construction of some international mechanism for supervising the movement of soldiers and their equipment (and in due course of counting stockpiles?) would go a long way to satisfy European governments that they have a voice in matters which affect them vitally, but over which they have no direct control. □

Pork-barrel supplement

The US Administration hopes to spend more on competitive grants in agriculture. It must insist.

THE word has gone out from those within the Reagan Administration who support competitive grants for agriculture that good news is coming at the end of the month, when the President will release his budget proposals for fiscal year 1985 (see p.204). The competitive grants programme in the US Department of Agriculture (USDA) has been held to a ludicrously low level — $17 million — by political interests which see perfection in the century-old share-the-wealth system that passes for an agricultural research structure in the United States. Every state gets a piece of the action, no questions asked. Congressmen pat themselves on the back for bringing home the goodies. And mediocre researchers continue to grind away at the same old applied technology that might have made sense once upon a time.

The message has finally begun to sink in, however, that questions must be asked if agricultural research is to remain in touch with twentieth-century science. USDA is said to have asked for $50 million for competitive grants for 1985. The obstacle now appears to be the Office of Management and Budget, which is thought to have ensured that only half of that request will see its way to the budget. The administration last year proposed $21 million, which Congress cut back to $17 million — the same level as for years past. The route to real change is not easy. The administration will have to battle with entrenched interests in Congress, particularly one Representative Jamie Whitten of Mississippi, chairman of the House Appropriations Committee and a stalwart defender of mediocrity when it is in the interest of his home state. A half-hearted effort by the administration will not do. It will have to have the courage of its newly-found convictions. □

Examination fever

British school education is in for another shake-up. The minister in charge should move carefully.

SIR Keith Joseph, Secretary of State for Education and Science, has been in his job for what by British standards is a suspiciously long time. Even if the explanation is that there is nowhere else in the government for him to go, the consequence is beneficial. Familiarity with the educational system he administers has now led Sir Keith into a radical reappraisal of how the job should be done. At a conference in Sheffield earlier this month, he announced that his department plans to establish objectives for the main parts of the school curriculum in British schools for children aged from five to sixteen. By doing so, he has issued the most important challenge so far to the doctrine underlying the 1944 Education Act — that central goverment pays for the cost of public schooling, that local education authorities administer schools and influence the curriculum as they can — but that the final arbiters of what is taught are teachers.

This doctrine has increasingly become a fiction in the past four decades. Teachers have done the best they could in a quickly changing world, but have been given too little opportunity to keep up with what they hope to teach. Local education authorities have sometimes done what they could to help, but their performance has been patchy. Bodies such as the Schools Council, conceived of as an instrument for curriculum development but now demolished, have spent too much of their energy on procedure and not enough on content. Central government has had to be content with seeking to influence the system by the reports of advisory committees appointed for special purposes or by the much diminished influence of its inspectors. So, by default, the school curriculum has in effect been determined by the uniquely British examination system. That, it seems, is the discovery which Sir Keith has now made and the circumstance that he seeks to change. The result could be invaluable, especially in the teaching of science.

As things are, much of the British public is not merely ignorant of even the most basic science but actively suspicious of it. Science is often regarded as a necessary evil that will corrupt the minds of children as they lose the innocence of youth, but that children of primary school age should be exposed to such adult material, heaven forbid, seems quite shocking. Even at secondary level, the notion persists that science is an eccentric pastime or, even more demeaning, a vocational pursuit, — a device to enable clever children to pass difficult examinations but remote from the concerns of everyday life. Such effeteness has persisted because, astonishingly, it has never been thought necessary in Britain to specify the basic concepts and information about the world that the school system should aim to teach. Science, while widely taught, is not an essential part of every student's education. Now that may change.

Sir Keith Joseph's intention seems to be to determine what is taught in British schools by reforming the examinations system. There can be no sensible objection to such an attempt, except perhaps the government's desire at the same time to find short-term economies in public expenditure. The argument that teachers should be allowed to adapt their teaching to local needs is no objection, being an obvious truth and not an alternative. That an education system paid for by the British taxpayer should conform with nationally agreed objectives is self-evident.

Yet as things stand, the system does nothing of the sort. At secondary school level, the curriculum is hagridden by examinations. As things are, there are two tiers of school-leaving examinations, one for those seeking places at universities called A ("advanced") level and taken at age eighteen or thereabouts, and a second layer of two examinations at sixteen, called O (for "ordinary") level and CSE (for "Certificate of Secondary Education") respectively. Three years ago, the government agreed to follow its predecessor's undertaking that the examinations at sixteen would be combined, but the details are still being worked out.

0028-0836/84/030198-01$01.00

Meanwhile, the boards that set examinations for sixteen-year-olds continue to devise examination questions that produce a good spread of results correlating positively with the results of other examination questions and which also satisfy the (counter-productive) demand from employers for a wide spread of grades by cramming in such a range of questions that teachers are hard-pressed to cover the syllabus in time. Not surprisingly, education in general, and science education in particular, suffers: the examinations test stamina and quick-wittedness as much as any skill applicable in real life. At primary school level, science education depends on one thing only: whether there happens to be a teacher in the school who knows anything at all about science. Often there is not.

Not content with challenging the historical basis of the school curriculum, Sir Keith Joseph wants also to introduce public examinations that record absolute levels of attainment ("criterion referencing") to replace the present system, which is based largely on the assessment of performance relative to that of others ("norm referencing"). . Practical difficulties aside (who could decide an absolute criterion for, say, appreciation of English literature?), this proposal could easily become a red herring. The year on year correlations made by examining boards ensure that the present system is not totally lacking in objectivity. But the real reason why the present two-tier system of examinations at 16-plus urgently needs unifying is that it is unjust.

The CSE examinations intended for the middle 40 per cent of the ability range have, over the years, become more and more similar in content to those (O levels) intended for the top 20 per cent of the ability range. There are no examinations designed for the 40 per cent of students at the bottom of the ability range although, under pressure from parents and teachers, many of them do attempt CSE. For similar reasons, many who should be taking CSE take O levels. The interest of the student is not best served by pressing him to take an examination in which he will probably score badly. To add insult to injury, those who do obtain high grades in CSE are often deemed by ignorant employers to be inferior even to failed O level candidates. In Sheffield, Sir Keith Joseph seemed to be hinting that he might back away from a commitment to establish a unified system of 16-plus examinations. The clear need is for a single set of achievement-related tests that would be of real value to future tutors and employers alike. An attempt to compromise on this issue could easily be disastrous.

What is it all for? Sir Keith Joseph says he intends to improve educational standards to the point where 80–90 per cent of students achieve the levels now reached by pupils of average ability at age sixteen. The variation of examination performance between schools suggests that this ambitious goal is feasible. To succeed, however, the goodwill of the teaching profession will be needed. Sir Keith's speech in Sheffield, in which he went out of his way to compliment teachers in general, is a welcome sign that he recognizes that reality.

But teachers will need more than a pat on the back. Science achievement in schools is being constrained by inadequate resources, as shown by studies carried out by the Department of Education and Science's assessment of performance unit, while the annual reports of Her Majesty's Inspectors of Schools show that, for example, 60 per cent of schools are inadequately provided with textbooks and 20 per cent have serious equipment shortages. It is arguable that some local education authorities are not fulfilling their statutory obligations under the 1944 Education Act. Even more important, shortages of teachers in key subjects (especially mathematics and physics) have meant that many vacancies are being filled by inadequately qualified people. Poorly motivated and under-confident teachers are unlikely to be an inspiration to their charges.

The reason for this state of affairs is not hard to find. Two years ago, a committee under a the chairmanship of Dr Wilfred Cockcroft established that qualified mathematics teachers, for example, then earned £1,000–£2,000 less per year than their counterparts in industry and commerce. Since that time, circumstances have deteriorated, and the present salary structure of teachers is made incoherent by the problem of falling school rolls.

It is perhaps a blessing that the long recession is not ending quickly, for then the teachers now in posts requiring special skills would be snapped up by industrialists out head-hunting. Sir Keith Joseph now seems to recognize that this problem will not disappear unless there are more resources in the system to keep teachers at their desks — and seems willing to press for extra. Not before time, it will be agreed.

Part of the trouble with the British school education system is that it is commonly regarded as a system on its own, with nothing in common with educational systems elsewhere. Yet this is precisely the time when, in the United States, the federal government has also woken up to the crisis in the schools, has uttered a series of clarion calls for improvement — and has found that the managers of the school systems are ready to respond (at least for a time). In both countries, the underlying problems are very similar, as they are in industrialized Western Europe as well: increasing professionalism in adult work, and the increased demands of the school curriculum, make teaching seem a backwater profession. If it is underpaid as well, the consequences can be calamitous. Teachers with professional skills outside the classroom will simply melt away — or worse, will be replaced by teachers who claim but in reality lack them. In the United States, many school systems have seen the way the wind is blowing, and have chosen to pay the teachers they need to keep above the odds. In Britain, the system of nationally negotiated salary scales will make the solution more expensive. But there is no choice.

That is one battle the minister will have to fight. Another, closer at home, is the battle he will have to fight within a government which, throughout the past five years, has been even-handed in its meanness towards the different sectors of the educational system but, at the school level, has taken advantage of falling school rolls hugely to economize. Now, as the minister seems to have realized, the process has gone so far that students are being denied opportunities that they, and ultimately the rest of us, would profit from. But at a time when public economy is still all the rage (and economically necessary as well), will he be able to wring the funds he will need from his colleagues, and if so, at whose expense? □

You are not what you eat

Too much cholesterol in blood is bad for people; the same is not true in food.

A NEW study from the National Institutes of Health (NIH) on the benefits of lowering cholesterol levels has predictably set off another wave of bad advice from the dietary pundits who have never been able to distinguish between cholesterol in food and cholesterol in blood. What the study did show, and show conclusively, is that high-risk patients — those with blood cholesterol levels over 265 mg per dl — are significantly less likely to have a heart attack if they can reduce those levels to something closer to the US national average of 210 mg per dl. Almost 4,000 men, all members of this high-risk group, were tracked for more than 7 years; all followed a reduced-cholesterol diet, but half were in addition given a drug to reduce blood cholesterol. The test group, which reduced its blood cholesterol levels by about 17 per cent on average, had 24 per cent fewer fatal heart attacks and 17 per cent fewer non-fatal heart attacks than the control group, which managed only a 3.5 per cent reduction in blood cholesterol.

What the study did not show is that the general population would benefit at all by restricting cholesterol in the diet. Early studies, indeed, have shown that it makes little difference whether one's blood cholesterol level is 210 or 230 mg per dl — the benefits that come from reducing blood cholesterol are far from linear. Dietary cholesterol, according to this and earlier studies, makes too little difference to those who really need to do something about the cholesterol in their blood (and who, the NIH study suggests, may well benefit from drug therapy) and is simply irrelevant to those with average cholesterol levels. □

© 1984 Macmillan Journals Ltd

Fast breeder reactors

Europeans sign joint development programme

THE United States may have abandoned Clinch River, but Europe last week confirmed its commitment to the fast breeder reactor. At least, that is one interpretation of an agreement signed on Wednesday by five European states — the United Kingdom, France, West Germany, Italy and Belgium — to open the way to cooperation on fast breeder research and development and commercialization. A more sanguine view is that the agreement is largely a way to reduce the embarrassing £300-million a year cost of European work on fast breeders.

Britain, for example has already announced a cut in its fast breeder programme. Britain's present spending of £108 million a year (1983–84) will fall by a third by 1986–87, according to a spokesman for the UK Atomic Energy Authority, notwithstanding the new agreement (which does not mention cash figures).

Moreover, Peter Walker, Britain's Secretary of State for Energy, said in announcing the agreement that the fast breeder potential (for energy supplies next century) "can be developed only if Europe . . . . is assured that it would provide an economic supply of energy". The memorandum is directed towards this end, he said, and to assuring safety. At the moment, fast breeder electricity is not economic, the costs being estimated to be about double those of a thermal pressurized water reactor.

Nevertheless, the other major partners are pushing ahead rapidly with their own fast breeder construction programmes. In the south of France near Avignon, the 1,200 MWe Superphénix reactor, cooled by a great basin of liquid sodium, nears connection to the grid, and at Kalkar in West Germany the SNR 300, cooled by a sodium loop, should be completed in 1985, and connected to the grid in 1987. The German Federal Government claims to have solved the problem of financing Kalkar by transferring the bulk of construction costs to industry — but it is still financing the fast breeder research and development team at Karlsruhe. This team should also be supported by industry, the government argues, and it hopes that the companies will agree.

Meanwhile the international agreement signed last week will enable the transfer of know-how, technologies and people among the partners. "It would be absurd", said Mr Walker, "for various countries in the European Community to duplicate the research and development work that is needed. The work can be done more effectively and more economically by working together."

Exactly what economies will follow is not clear, but the agreement signed last week is to be followed at a early date by other more detailed agreements covering research and development, industrial cooperation, patenting and industrial property, fuel cycle arrangements (including transport and processing of plutonium) and an inter-utility agreement "to provide for the joint realization of fast reactor power stations and the exchange of operational experience".

It is not thought likely that Britain, at least, would consider building a successor to Superphénix for upwards of 10–15 years. France, too, may find it difficult to support, with the national utility EDF currently facing international debts of FF150,000 million (£12,500 million) and a surplus of electricity. **Robert Walgate**

Asian academy established

New Delhi

A FEDERATION of Asian Scientific Academies and Societies has formally been established with the long-term aim of "solving regional problems without disturbing the natural ecosystem". The idea of such a federation was formulated at a meeting sponsored by the Indian National Science Academy (INSA) two months ago. Appropriately, the formalities setting up the 10-member federation were completed on 15 January, the day before the golden jubilee celebrations of INSA.

INSA, the oldest of India's science academies, though not of its learned institutions (the Calcutta-based Asiatic Society has just celebrated its bicentenary), is the organization responsible for India's international scientific cooperation programme. Guests at the celebration therefore included representatives from many national academies as well as the American Association for the Advancement of Science and the Royal Society of London, all of whom were to address the two-day symposium following the jubilee session.

The main issue at present facing Indian science — the balancing of the pursuit of excellence against the immediate needs of the country — was, however, the keynote of the jubilee session. Professor A. K. Sharma, president of INSA, stressed the need to ensure the academy's autonomy by guaranteeing it an annual budget that would be stable in real terms.

Vera Rich

French fast breeder reactors

Military doubts in France

COULD the new European agreement on fast breeders help France to make more nuclear weapons? On the face of it, such a military interpretation would fly in the face of the agreement, a "memorandum of understanding" which announced that signatories "confirm that activities taking place as a part of this collaboration will be directed to the peaceful development of nuclear energy".

Nevertheless, simply to maintain the security and future of the French fast breeder programme — the main component of European work — is also to maintain the French weapons programme, a vociferous group of French scientists is claiming.

Jean-Pierre Pharabod of the Ecole Polytechnique, for example, claims that the 40 MWth Phénix reactor — a prototype fast breeder power station now to be succeeded by Superphénix — has been used for military plutonium production. Pharabod also says that this fact has been communicated by France to the director of Euratom, the European treaty organization for nuclear research, based in Brussels.

Certainly, France has not submitted inspection of Phénix to the International Atomic Energy Agency (IAEA) — unlike the UK Atomic Energy Authority's Dounreay fast breeder, the PFR, to which IAEA inspectors have been admitted. According to Pharabod, the French military actually need Superphénix (a 1,200 MWe station due on line next year) to fulfil their planned (and announced) expansion of the French deterrent. The number of submarines, now five, each equipped with 16 missiles each with a single warhead, is to increase to seven submarines with the same number of missiles but each with six warheads; and there will be some 200–400 new tactical weapons with yields of some 100–300 tonnes of TNT. (For example, 120 Hades missiles will replace the present 40 Pluton ground-to-ground weapons.)

These developments will need new plutonium, and the arithmetic shows that only Superphénix can provide it, says Pharabod. Thus it is impossible in France to disentangle peaceful from military uses of plutonium, however much the new memorandum of understanding might appear to do so.

Indeed, according to a statement to the French Parliament by a socialist deputy, M. Georges Benedettit, who represents the nuclear area of Marcoule, "the fast breeder is the best choice for assuring both national energy independence and military independence . . . . The fast breeder is the only means now of making plutonium of more than 95 per cent Pu-239 in sufficient quantities to feed the development of our nuclear forces". **Robert Walgate**

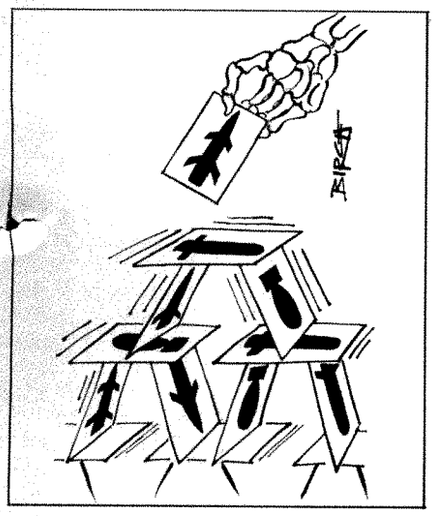
0028-0836/84/030200-01$01.00

US nuclear arsenal

Eight new warheads a day

Washington

MORE than 700 US combat units — over 100,000 troops — are "certified" in the use of nuclear weapons, according to a compendium of US nuclear forces published last week by the Natural Resources Defense Council (NRDC)*. The book, a sort of illustrated catalogue of the US arsenal, offers an unusually complete picture of the full array of tactical and strategic weapons maintained by the United States and of how deeply integrated they are into the US fighting forces. All four military branches — plus the Reserves and National Guard — are trained to use nuclear weapons. Approximately one-half of the 26,000 warheads in the US stockpile are designed for use in battle-field weapons, including antisubmarine and anti-aircraft rockets, air-to-air missiles, artillery shells and even land mines.



Neither the Department of Defense nor the Department of Energy (which has responsibility for production of nuclear warheads) would comment on the accuracy of the data in the book, which was compiled from open sources, including congressional testimony and data obtained under Freedom of Information Act requests. The Department of Energy, however, did advise the authors that publication of the book would be damaging to "the national interest", although it stopped short of taking any legal action to halt publication or distribution.

Some of the most interesting data in the book deal with tactical weapons, which, as co-author Milton Hoenig notes, are not covered even under the stalled SALT and Intermediate Nuclear Forces negotiations. The United States has deployed 5,000 nuclear artillery shells, mostly in Europe, as well as surface-to-surface guided missiles, anti-aircraft missiles, and two varieties of nuclear land mines, known as "Atomic Demolition Munitions" (ADMs). The smaller of these ADMs weighs about 60 pounds and is intended for use by commandos against targets behind enemy lines. It has an explosive yield of as little as 10 tonnes (TNT equivalent). A test of an ADM at the Nevada Test Site in 1955 nonetheless produced a crater 400 feet across and 60 feet deep. Three artillery warheads, for 155-mm and 203-mm guns, are in use, including "enhanced radiation" devices.

The Navy maintains the greatest variety of short-range nuclear arms: ship-launched and submarine-launched antisubmarine rockets, anti-aircraft missiles and surface-to-surface missiles. The Navy is also developing a new air-to-air missile, the Phoenix, to be carried by carrier-based long-range interceptor planes.

The book also provides data on nuclear weapons production, which is now proceeding at the pace of eight new warheads per day; taking into account the warheads retired from service, the result is still a net gain of three per day. According to the book's authors, the US stockpile will grow by 13 per cent over the next five years; by the mid-1990s the arsenal will be approaching the previous peak of 32,000 warheads (reached in 1967) and will in addition have been thoroughly modernized, most warheads having been replaced by new, higher-yield versions.

Future volumes from NRDC will deal with the Soviet nuclear arsenal, arms control and nuclear strategy.

Stephen Budiansky

**Nuclear Weapons Data Book* by T. B. Cochran, W.M. Arkin and M.M. Hoenig (Ballinger, Cambridge, Massachusetts, 1984; $19.95).

Nuclear reprocessing

West Germany plans ahead

THE West German Federal Ministry of the Interior last week reaffirmed its support for the construction of a reprocessing plant for nuclear waste, in particular "to secure national independence in this area".

The organization responsible is the *Deutsche Gesellschaft für Wiederaufbereitung von Kernbrennstoffe* (DWK) which was formed jointly by the 12 electricity companies concerned. The proposed undertaking will be financed by DWK and will receive no money from the federal ministry.

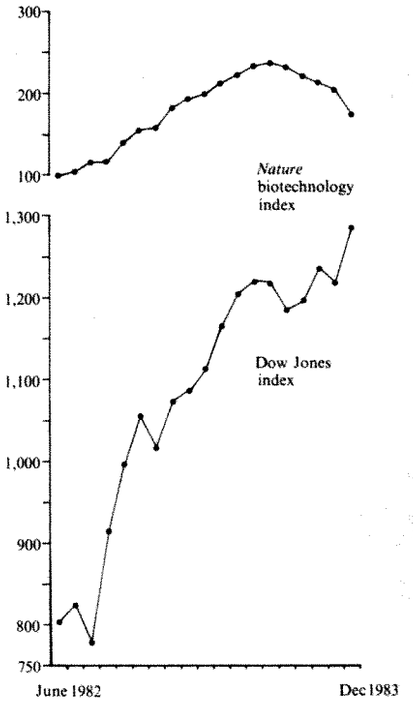
Approval procedures are now under way for two sites: Dragahn in Nieder-Sachsen, not far from the Gorleben underground storage area, and Wackersdorf, Schwandorf in Bavaria. The procedures should end late this year, when DWK will decide which site it wants. Only final approval by the *Länder* ministries will then be necessary before building starts.

The plan is to build a single installation capable of processing an average of 350 tonnes of spent rods per year, beginning in 1986 and coming into service slowly from 1993 at a rate of 50 tonnes and working up to a final 450 tonnes annually. More than 500 tonnes would overstep environmental control limits.

Meanwhile, the question of costs is causing concern. Electricity from nuclear installations, although it is now cheaper than that from coal-fired stations, already carries a small surcharge to the eventual cost of reprocessing. The revenues collected will accumulate in a fund for the construction of the reprocessing plant. While the cost of the plant is currently given as DM4,300 million (£1,100 million), it could easily rise to DM6,000 million or DM8,000 million before completion, with annual running costs of DM1,000 million. "A reprocessing plant is not an economic proposition", the industry's union spokesman asserted, but nevertheless supported the project as a demonstration of industrial expertise.

Sarah Tooze

The falling index



NATURE's monthly biotechnology index tumbled another 21 points in December 1983 (see 12 January, p.102), falling to 175 against the peak of 238 in July 1983. The index started at 100 in June 1982. During the same period, the overall value of shares on the US stock markets, as represented by the Dow Jones index, has risen dramatically (see graph). Moreover, the decline has been spread across the biotechnology board, affecting 15 of the 16 shares from which the index is compiled. □

© 1984 Macmillan Journals Ltd

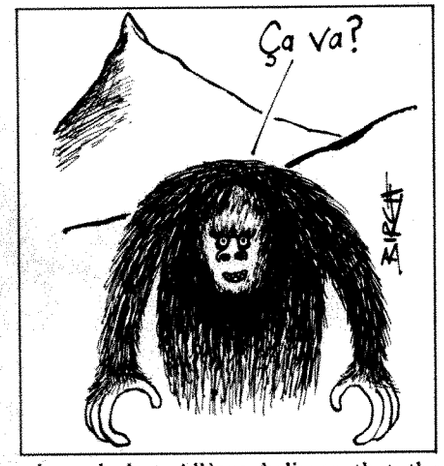
Franco-Chinese collaboration

Tibetan expeditions draw breath

THE spectacular French–Chinese geological expedition to Tibet (see *Nature* 5 January pp.17–36), is unlikely to be repeated — at least for a few years. The problem is not one of politics but of resources according to the expedition's French organizer, Claude Allègre of the Institut de Physique du Globe in Paris.

The expeditions, which have revealed that the high plateau of Central Asia was created by a succession of intercontinental collisions and not just one, "have been a huge effort for both sides" says Allègre. There were three separate expeditions, in 1980, 1981 and 1982. Each involved 50 big trucks in the field, and 150–200 people. France put in some of its best geologists. Chinese resources — both people and cash — were especially stretched.

As a result, no further major French–Chinese expeditions to the area are



planned, but Allègre believes that the Chinese will not be seeking collaboration with other partners either. Meanwhile, however — while both sides gather breath — France and China will mount small joint expeditions each year, the next to the Ping Ling mountains.

In any case, Allègre says, "we've understood what we wanted" about the region. While there is much more to learn, only a third of the results of the expeditions have been published so far and the teams need a few years just to think about the results.

Why had only France been involved in the expeditions, and not other Western countries? Partly, it may be because other geologists were less enthusiastic than Allègre about the exercise. Dan McKenzie of the University of Cambridge, for example, one of the pioneers of plate tectonics, says that while the expedition had been important and useful "I feel one ought to do simpler, cheaper things". If expensive, deep seismic studies were the object then the French–Chinese expedition would have been perfect to do the job, and would have done it well. But there was "no good gravity study" of the Tibetan plateau, for example, a lack that could be rectified much more cheaply. Moreover there were other plateaus in the world which were more accessible to western geologists, says McKenzie: for example eastern Turkey, the Colorado plateau, and the Alteplano. Also, relations with China were improving to the extent that person-to-person partnerships could now be arranged, McKenzie feels, rather than vast government-backed expeditions — and in fact McKenzie is going to China on such a partnership this Easter.

Allègre says he had sought European collaboration when there were plans to continue beyond the first three years, probably with other European countries taking up the running from France. But while individual geologists had shown great interest, none was prepared to organize the collaboration with the Chinese, Allègre claims.

Perhaps this was wise. Allègre describes the negotiations that led up to the expeditions as "terrible", and as having been several times on the point of breaking down. The Chinese drove a very hard bargain, which has led — for example — to the French team leaving several major instruments behind.

Allègre clearly does not relish entering such negotiations again. The result, however, was fair to both sides, he feels. Allègre says he cannot estimate the overall cost, but it had been shared equally, he claimed.

President François Mitterrand of France himself has shown real interest in the expedition, Allègre says, and by no means only in its politics. The President reveals a "greater and greater interest" in science, not only as a means to develop the French economy. Allègre, who accompanied Mitterrand on his 1983 tour of China, says that Mitterrand asked him more about plate tectonics than about science politics.

As a result, Allègre considers that while French science is not as well blessed as promised by the former research minister Jean-Pierre Chevènement, the budget is "protected", and will be in the future. Mitterrand has a very balanced view of the relation between basic and applied research, Allègre believes — more balanced than his ex-minister. **Robert Walgate**

Nature conservancy

UK unit lives hand to mouth

A REQUEST by the British Nature Conservancy Council (NCC) for extra funds to help restore the value of its research budget seems certain to be turned down by the UK Department of the Environment. NCC has asked for £200,000 towards its survey and monitoring programme, but the department has responded by suggesting that research is an area where particularly stringent savings are expected. NCC's grant from the department for next year, now under negotiation, will be announced later this month.

NCC's research programme has been steadily eroded since 1973, when the council was established as an executive body distinct from the Natural Environment Research Council. NCC research is now worth roughly half as much in real terms as in 1974. This is an untoward consequence of the Rothschild customer/contractor principle, by which some civil science research is commissioned by government departments. As, in recent years, the cost of salaries in NCC has, like that in other government departments, consistently outstripped the allowance made for it, the amount left over for research has declined. NCC also labours under an accounting peculiarity whereby it has to meet the cost of its staff pensions from its own funds.

Within a declining research budget, the amount NCC spends on research commissioned from the research councils has declined even faster. According to Sir Ronald Mason's investigation into government commissioned research, carried out for the Advisory Board for the Research Councils (see *Nature* **306**, 102; 1983), the amount spent with the Agricultural and Food Research Council and the Natural Environment Research Council fell from £1.3 million to £0.3 million in the five years to 1982–83 (1983 prices). One reason for the shift seems primarily to have been cost: NCC has found it more economical to employ young researchers on short-term contracts than to hire senior scientists who would often insist on working to a higher standard than was needed. The total annual cost of all NCC research is £1.5 million.

NCC now has to satisfy itself with a very pragmatic approach. According to its chief scientist, Dr Derek Ratcliffe, any possibility of commissioning basic research has long since been abandoned, and there is no chance of turning back the clock to the days when NCC's forerunner ran an experimental station of its own. All that NCC retains is a small group which spends most of its time providing site management advice to NCC local officers, and some survey teams. Most of NCC's resources are tied up in "renotifying" owners of Sites of Special Scientific Interest (SSSIs — see *Nature* **306**, 525; 1983) and survey work aimed at identifying new SSSIs is a long way behind schedule.

Despite the gloomy outlook, NCC's chairman, Mr William Wilkinson, has been working hard to secure more research funds. It is felt that NCC needs hard factual information on sites to be able to make an authoritative case for their notification. **Tim Beardsley**

0028-0836/84/030202-01$01.00

Molecular biology

UK to stay in Europe

BRITAIN'S Medical Research Council (MRC) has advised the Advisory Board for the Research Councils that the United Kingdom should adopt a whole-hearted commitment to the European Molecular Biology Laboratory (EMBL) in Heidelberg, West Germany. The news has come as a relief to EMBL, which has lived under the threat of a possible withdrawal of UK support ever since the Advisory Board for the Research Councils asked MRC to consider the benefits of UK participation in EMBL just over a year ago. The board will consider MRC's advice on 31 January.

The MRC view is based on a detailed assessment of the benefits and costs of UK participation in EMBL and the scientific merits and cost effectiveness of the research carried out there. The assessment, carried out by a group of five senior British scientists, concluded that the case for remaining in EMBL is strong on scientific grounds alone and remains strong even when costs are taken into account.

The group is not persuaded that the £1.2 million contributed by MRC to EMBL in 1983 (£1.5 million is due this year) would have purchased "an equivalent amount of work of equal quality" within the United Kingdom but does believe that EMBL's value for money should be increased.

The group recommends a more effective system of peer review and cost accounting for each of EMBL's scientific programmes but acknowledges that Professor Lennart Philipson has moved in the right direction since becoming director general of EMBL in 1982. It strongly approves of his new system of financial management of programme budgets, hopes and anticipates that costings will be linked to scientific reviews of the programmes but remains doubtful that the procedures for scientific review are adequate.

The problem, the group claims, is that the Scientific Advisory Committee of EMBL, which is charged with advising the laboratory council on EMBL's scientific programme, is in danger of being "seen as a creature of the director general" rather than offering independent advice to which the director general should be free to reply. Again, it welcomes Professor Philipson's moves to avoid such criticism but wants them further discussed and extended.

To judge the cost-effectiveness of EMBL, the MRC group chose to compare it with the MRC Laboratory of Molecular Biology (LMB) in Cambridge. The figures, summarized in the accompanying table, confirm suspicions that staff costs in Heidelberg are double those in Cambridge. The group considers that differential to be inevitable, given the salaries at comparable laboratories, notably the European Organization for Nuclear Research (CERN) near Geneva, and the allowances expected by expatriates on short-term contracts. It also considers the greater recurrent costs at EMBL to be justified and points out that expenditure on consumables is equivalent in the two laboratories. The only way to cut costs, the group implies, would be to reduce staff — if stringent peer review revealed weaknesses.

The MRC group's view of the current scientific programmes at EMBL is that, like the curate's egg, it is good in parts. The cell biology programme excels and the outstation programmes at Institut Laue-Langevin, Grenoble and the DESY synchrotron in Hamburg are good. On the other hand, the recent investment in a differentiation programme is speculative and that on molecular structures is too large. Both instrumentation programmes are thought to be somewhat contrived to meet the original aim of EMBL to develop unique and expensive technology that few national laboratories would touch.

Costs at EMBL compared with LMB

	EMBL 1982	LMB 1982-83
	(£ thousands) per head	
Staff costs		
Salaries	16.2	9.3
Allowances, insurance, etc.	5.5	2.4
Total	21.7	11.7
Extra costs		
Consumables	5.4	5.2
Recurrent	8.4	6.6
Total	32.9	18.1

Total EMBL expenditure for 1982 was £9.4 million of which 54.7 per cent was spent on the cost of 232 "man-years" of staff. Total LMB expenditure for 1982–83 was £4.6 million of which 47.3 per cent was spent on the cost of 186 "full-time equivalents" of staff.

Overall the MRC group argues that the benefits the United Kingdom receives from participating in EMBL outweigh those of withdrawing, even if there would be a short-term financial gain in doing so. Professor Philipson, who is generally pleased with the report, questions whether there would be even a short-term gain, once proper account was taken of the cost to the United Kingdom of redeploying many of the British nationals (about 20 per cent of the total) employed at EMBL. Mr D. Noble, MRC under-secretary, claims that the group's calculations did include that possibility.

Noble also said that MRC would be surprised if the Advisory Board for the Research Councils did not accept its recommendation to continue UK participation in EMBL. He hoped that the board would now see fit to advise the UK Government that its science budget should be adjusted to protect the MRC budget against losses at the expense of increased contributions to EMBL due to changes in the UK economy.

Peter Newmark

UK public health

Pathogens for suburbia?

Two laboratories at the UK Centre for Applied Microbiology and Research (CAMR) at Porton Down, in Wiltshire, England, may be closed in order to reduce costs. The two laboratories, the Special Pathogens Reference Laboratory and the Environmental Microbiology and Safety Reference Laboratory, carry out a varied programme of research in viral diagnosis, the production of vaccines and viral safety testing.

The proposal to close the laboratories is one of the options to be considered at a meeting of the Public Health Laboratory Service Board, on 26 January. Routine diagnostic work and possibly some safety work may be transferred from Porton to the new Central Public Health Laboratory building under construction at Colindale, in North London. Dr C. Gordon Smith, chairman of the Public Health Laboratory Service Board, says that a number of proposals will be discussed but stresses that none has yet been examined in detail and that no decisions have been made.

The news that diagnostic work might be moved to London nevertheless made front page news in the *The Standard* of London, under the banner headline GERM LAB PLAN FOR LONDON. Laboratory diagnosis of viral diseases, including Lassa fever and smallpox, has in fact been carried out at the existing Public Health Laboratory building at Colindale, which includes a high containment laboratory, since 1946. A statement issued by the Public Health Laboratory Service and the Department of Health and Social Security to calm the fears of Colindale residents says that "research involving highly dangerous organisms" will not be transferred to Colindale.

Among the staff of the threatened laboratories at Porton there is concern of a different kind. The work carried out there includes research on Ebola virus, Lassa fever and Marburg disease, and is aimed at reducing reliance on animals in diagnosis and producing vaccines. Botulinum toxoids and anthrax and experimental whooping cough vaccines are also manufactured.

Porton staff hold that closure of the laboratories or partial transfer of some of the work to Colindale cannot be justified financially, and point out that experimental work is probably better carried out in a remote area.

It is far from certain whether the Health and Safety Executive would allow some of the work to be transferred to London. The staff at Porton say that they represent a national asset that becomes invaluable in an emergency and may spawn products of commercial value and complain that CAMR seems likely to be hit harder than other Public Health Service laboratories.

Tim Beardsley

© 1984 Macmillan Journals Ltd

US research computers

Agency expects funds to buy time

Washington

GRANTS from federal agencies allowing US researchers to purchase computer time are about to become respectable again. President Reagan's budget for 1985, to be published at the end of the month, will include substantial new funds with which the National Science Foundation (NSF) can help grant-holders to purchase time on supercomputers — machines such as the various models of CRAY computers. But the new funds will fail to satisfy at least two of the groups that have been urging that academic researchers should have more ready access to computer power.

One of these is the working group of the National Science Board (advisory to NSF) which last summer recommended that $200 million should be spent over the next three years on ten supercomputer centres for university researchers. The new NSF programme will fall far short of that proposal, both in the amount of money available and because it is intended that the new funds should be used to buy time on existing machines.

Budget prospects

DR Edward Knapp, director of the National Science Foundation, refused to comment last week on the other components of the NSF budget, but did say that he expected NSF to do as well as last year. The budget for the current fiscal year, published last February, gave NSF an increase of 18 per cent, the largest increase in a single year in the foundation's history.

Speaking on Monday at the National Academy of Sciences, however, Dr George Keyworth, the President's science adviser, did drop a hint about NSF's plant biology budget in the context of his general optimism about funding for agricultural science. Keyworth said that the plant biology programme at NSF had received a budget increase of 16 per cent last year, up to $50 million. "I'd look for comparable growth again next year", he said.

Keyworth was not nearly so specific in discussing the competitive grants programme at the Department of Agriculture, although he did reveal that Secretary of Agriculture John Block had discussed the programme directly with the President and had received Reagan's personal support.

Even so, the new programme will mark a major shift of NSF policy on computing. Not since 1972 has NSF supported computer centres on campuses, while most federal agencies continue to baulk at paying the full cost of purchased computer time under research grants. During the same period, this policy was abetted by researchers themselves, who preferred to buy their own mini- and microcomputers than to use central computing facilities which, according to the working group, had fallen into disrepute.

Some of the consequences of that trend have been unfortunate. The National Science Board's working group concluded last summer that "important" research is not being tackled for lack of supercomputer facilities in the United States and cited the case of an astrophysicist who had found he could get access to the computing power he needed only by collaborating with a West German group. Under the new budget proposals, researchers will be able to buy time on CRAY and other computers such as those at Los Alamos and Livermore National Laboratories.

The new programme will nevertheless offend those who greeted the National Science Board's report with scepticism, saying that the supercomputer was a solution in search of a problem — and that the important applications of these machines are few. Meanwhile, an alternative policy is suggested by the work of a group of researchers at the California Institute of Technology (Caltech) who are developing what may prove to be cheap supercomputers. According to Geoffrey Fox, the theoretical physicist who directs the Caltech project, and Charles Seitz, a computer scientist, the few hours of computer time that a researcher may be able to buy with NSF funds will not add significantly to the computer power already at his or her disposal.

Fox says that "an IBM personal computer is more cost-effective than a CRAY" and that a few hours of CRAY time will not crack any problem that cannot be solved on a personal computer in a few hundred hours. What worries Fox is that under the new policy, supercomputer time "will be chopped up so that everyone will get a few hours. But you don't chop up Palomar into a pixel for each observer; I've never understood why we do that in computing."

The Caltech approach, according to Fox and Seitz, could give academics access to dedicated supercomputing power by cutting costs dramatically. They have already built a machine using 64 microprocessors of the kind used in personal computers with a power one-tenth of a CRAY machine but at only one-hundredth of the cost (about $80,000).

The Caltech programming philosophy is also different. While CRAY machines have several processors that operate in parallel when the program-compiler identifies steps in a sequential program that can be executed simultaneously, the Caltech approach would parcel out steps in the solution of a problem to its separate processors. In the 64-chip version of the machine, each processor can communicate with six others and thus be kept running almost full-time.

At Caltech, the next step is to expand the desk-top 64-chip machine to a total of 1,024 chips. This would be equivalent to a CRAY machine in computing power but because of the use of mass-produced chips may cost only one per cent as much.

Stephen Budiansky

French research

Academics between millstones

RESEARCHERS in French universities who receive support from the Centre National de la Recherche Scientifique (CNRS), the principal research council, will soon have to "clarify" their position in relation to the university and CNRS. This follows an agreement last week between CNRS and the ministry of national education (MEN) which seeks to disentangle the sometimes complex relationships between research groups and their sponsors that have grown up through years of benign neglect, and a series of individual, idiosyncratic agreements between CNRS and individual groups.

Whether or not this will be seen to aid research depends the observer's point of view. It will certainly aid research management, and it will also aid university management — for in the past, university presidents have not always known what their own groups were doing. A new contract between a group and CNRS might commit the university to new overhead (providing laboratory space, for example) without the university having had any say in the matter.

On the other hand, however, the situation gave individual scientists the chance to play off one part of the bureaucracy against another in the game of raising a grant. Under the new agreement, this will be harder, and there will be fewer corners where researchers not conforming to national policy can find rest.

The outline agreement describes "the ancient links, many and narrow" between CNRS and universities and claims that these justify "an effort of clarification and organization". The two sides have sought "precise texts" and "a common charter", which would be "legal, rational and balanced".

Under the charter, CNRS will draw up a general agreement with each university (or grand école), followed by a series of particular agreements with each group in that establishment, these particulars being placed in the context of the general agreement. The establishment as a whole will bear responsibility for applying the agreement, something that should give a university president more power in his (or her) own house (provided the president can control the new three-committee management structure of the university, a prospect which some French researchers find unlikely).

Robert Walgate

0028-0836/84/030204-01$01.00

Making science a good read

SIR — The leading article "Must science be impenetrable?" (*Nature* **305**, 477; 1983) concluded that little or nothing could be done to improve the situation, and that the authors are the most obvious culprits. I argue that the poor quality of illustrations contributes towards impenetrability, and that on this point the editors are the culprits.

The decipherment of a figure that contains more than one part can be very laborious, because the pertinent information is often buried in an overcrowded legend. This could be avoided if the following rules were applied:

(1) When the graph includes more than one line, identify each by appropriate labelling.

(2) When the figure contains several panels with the same abscissa and the same ordinate, usually identified as (*a*), (*b*) and so on, indicate on each panel its experimental specificity.

(3) Whenever possible, use the same symbol for the same experimental condition in all comparable figures throughout the article.

(4) When several symbols need to be used in a graph, use the open circle for the control condition; for instance, if an experiment has been performed without (o) and with (●) an addition or a treatment, use the symbols as indicated. (The open



circle is the simplest symbol available, and its "emptiness" suggests that nothing has been added.) Rule (4) should prevail over rule (3) when these two rules are in conflict.

(5) Make the labelling of abscissa and ordinate as precise as possible. For instance, it is preferable to write "Time after drug addition" than simply "Time". Also express results in moles rather than in c.p.m. or in absorbency units, sparing the reader the task of doing the conversion. If results are given in per cent, give the value of 100 per cent in standard units in the legend.

(6) The legend should be as short as possible, and could sometimes be restricted to the title since, ideally, the figure would be fully intelligible without making reference to the legend.

From my own experience of the biochemical literature, I know that, if labelling of figures is still very limited, it is because it is systematically discouraged by subeditors, whose policy is to push all the information into the legends. With modern technology, the cost of lettering can no longer be an excuse for this negative attitude.

HENRI-GÉRY HERS
Université Catholique de Louvain and International Institute of Cellular and Molecular Pathology, UCL 75.39, Avenue Hippocrate 75, B-1200 Brussels, Belgium

Nicotine delivery

SIR — The concern of the British Royal College of Physicians (*Nature* 1 December 1983, p.418) with smoking and health is justified and well directed towards the immediate problem of reducing the number of people who may be influenced by cigarette advertising. The problem though, is that cigarette smoking is a means of drug delivery that is both crude and effective. If one assumes that some number of people in any society want to use drugs for a variety of reasons and that smoking is merely a way of delivering an addictive drug, the problem becomes conceptually simpler.

Addiction to the natural alkaloid nicotine is widespread and social tolerance of the drug and its users is such that for years a debate existed over whether chronic users were actually addicted in the same sense as opiate users. Semantic differences apart, smokers mostly smoke to deliver nicotine to their mucus membranes so it can be absorbed into the blood.

Alternative routes of drug administration more cosmetic than chewing tobacco or snuffs should be developed so that the nicotine addict has alternatives to cigarettes. Nicotine chewing gum has had limited success, but may soon become available worldwide. Another alternative might be transdermal application much in the manner of nitroglycerine and scopolomine patches. Nicotine "inhalers" might also be feasible if dosage could be adjusted.

I would appreciate receiving correspondence from individuals or groups actively engaged in finding alternative methods of nicotine delivery in humans.

CECIL H. FOX
8708 First Avenue, Silver Spring, Maryland 20910, USA

Prudent antibiotics

SIR — A leading article in *Nature* in 1981 (*Nature* **292**, 661) referred to the publication of the "Statement Regarding Worldwide Antibiotic Misuse", which was signed by more than 200 physicians and scientists and intended as an alert to the problem of antibiotic abuse.

The article raised the question of how the problem would continue to be addressed. The response generated by the statement enabled the Alliance for the Prudent Use of Antibiotics (APUA) to be established at the end of 1981. Its members include physicians, scientists, medical and public health personnel and members of the general public from more than 50 countries. The alliance has adopted a positive rather than a negative approach by advocating and defining good usage. Its activities include the gathering and dissemination of information and education on antibiotics and their use. The alliance has begun to organize a network of groups and individuals who are working in their own countries to improve antibiotic usage. The *APUA Newsletter* has opened a line of communication among the members. We recognize that it is at the level of the consumer — patient, physician, farmer, gardener, pet owner, beekeeper — that the rational use of antibiotics must be understood and accepted.

It is our long-range objective to implement changes in the use of antibiotics which will preserve their therapeutic effectiveness.

STUART B. LEVY
Alliance for the Prudent Use of Antibiotics, PO Box 1372, Boston, Massachusetts 02117, USA

Gripes of wrath

SIR — I read Walter Gratzer's review of Fowler's *Modern English Usage (Nature* 10 November 1983, p.134) and was both saddened and amused at the examples he quoted of uncouth English.

My own pet irritation is the use of 'in the order of" in place of "of the order of". This has become very common, but I do not recall ever coming across it more than twenty years or so ago. I believe it is an example of a phrase that "escaped" from scientific usage, was mangled by those who do not understand the meaning of order of magnitude, and then was hypnotically readopted by all because of the frequency of its appearance in the distorted form.

Other pet aversions of mine include "anticipate" when "expect" is meant (we anticipate completion by the end of the year), and the introduction of a quite inappropriate conditional qualification (symposium programmes are available in the foyer if delegates care to help themselves).

Finally, an ambiguity in a classified advertisement seen many years ago which never fails to amuse me when I recall it: Miss E. Brown, wardrobe dealer, having cast off clothing of all descriptions, invites inspection.

DENNIS PIPER
Abingerwood, Ewhurst, Cranleigh, Surrey GU6 7SJ, UK

Publishing "in conference"

SIR — I should like to draw attention to an alarming trend in the biological literature — the reporting of scientific data at conferences followed by publication of the abstracts in journals where they acquire the cachet of fully refereed papers.

During a recent literature search (using the BIOSIS 80 system) the key words "Ia antigen", "interferon", "macrophage" and "prostaglandin", in various combinations, elicited a total of 33 references for 1980-82 of which 19 were abstracts from conference proceedings. This struck me as being overbalanced. I do not question the validity of the data or the integrity of the investigators, but it is not possible to assess a piece of work from a 300-500 word abstract which, presumably, has not been refereed.'

The two major "offenders" in my small sample were, coincidentally, both American, in particular, abstracts from the annual meeting of the Federation of American Societies for Experimental Biology published in *Federation Proceedings* and the annual meetings of the American Federation for Clinical Research published in *Clinical Research*. Such abstracts are subsequently extensively quoted in the literature and may be the only source for the work being discussed.

This trend seems to have come about because of the greatly increased numbers of scientists wishing to publish and the consequent increased pressure for space in conventional journals, the pressure on conference organizers to include all submitted abstracts and the increased use of poster sessions — impossible to referee. The overload of submissions is made worse by the apparent tendency of conference organizers to base their acceptance of registration on whether or not the applicant wishes to submit an abstract.

An example of these trends has recently been seen in the British Pharmacology Society, where data used to be presented as a talk and, following discussion, refereed "on site" by a show of hands from the members of the society. If the authors wished it the abstract was then published in the *British Journal of Pharmacology*. With the introduction of poster sessions and the consequent reduction in the possibilities for adequate reviewing, the society now publishes abstracts as a supplement to its journal — a laudable attempt to indicate the comparative lack of refereeing.

The trend towards publication of abstracts only is disquieting. A solution will not be easy to find, touching as it must the "publish or perish" syndrome. The simplest solution would be not to publish abstracts of meetings — perhaps a little drastic. Or the editors of the relevant journals could ask for a fuller abstract of the work presented to be adequately refereed before publication.

While it is not possible to blame scientists (myself included) from taking this easy road to publication, the inclusion of unrefereed conference abstracts in subsequent articles where they acquire the same pedigree as fully detailed and refereed papers should be viewed with some unease as this practice is open to considerable abuse.

MICHAEL A. BRAY
Strassburgerallee 87,
4055 Basel, Switzerland

• Perhaps data-banks should admit as search criteria Boolean specifications such as "not abstract".— Editor, *Nature*

India's bait

SIR — I sympathize with the Indian Government's attempt to lure expatriate scientists and doctors back home through programmes such as TOKTEN and through the proposal to create a sophisticated technological city (*Nature* **305**, 350; 1983). But short-term solutions such as these are not the answers. India needs a coherent science policy and infrastructure to stem the flow of brain-drain and to lure the expatriates. As a first step, I suggest revitalization of the universities with up-to-date curricula facilities and competent indigenous and expatriate teachers.

I graduated from Kerala University in 1965 with a BSc in chemistry, but without having had the opportunity to use even a *p*H meter and without having been exposed to many of the fundamental principles of chemistry. When I joined the Training School of the Atomic Research Centre (Bombay) immediately afterwards I was confronted with advanced chemistry concepts (which at first went over my head), and a plethora of sophisticated instruments. I literally had to burn the midnight oil to bridge the almost inseparable gap between the university and the research centre.

I strongly urge the Indian authorities to rejuvenate science education in universities throughout India (not just in Bombay, Delhi or Madras). When the universities become centres of excellence, the expatriates will return home without the need for baits. One need only recall how Nalanda and Taxila, two excellent centres of learning in ancient India, attracted even foreign scholars to India.

K. S. SUBRAMANIAN
Environmental Health Center,
Health and Welfare Canada,
Ottawa, Ontario K1A 0L2, Canada

SIR — We would like to add a few of comments concerning the creation of a UN biotechnology centre and India's bait for expatriates (*Nature* **305**, 350; 1983). We are of the opinion that many of the Indian scientists and doctors working abroad would like to go back to India. We would like to point that a recent survey by E. Garfield (*Sci. Public. Policy* **10**, 112-127; 1983) has shown that India is the "superpower" of Third World countries in scientific research.

The establishment of a biotechnology centre in India, either at New Delhi or Bangalore, would attract many of the Indian scientists working abroad to return to their country and promote science. For this reason, we urge UNIDO to reconsider India as a site for such a centre.

The announcement by the Prime Minister, Mrs Indira Gandhi, of the plan for a "technology city", where Indian scientists now working abroad would be able to work in conditions to which they have become accustomed, is a constructive step towards bringing scientists of Indian origin back to India. The return of meritorious scientists to India would create a competitive and productive environment for those already working in Indian universities and other research centres.

HARCHARAN SINGH RANU
PANKAJAM K. NAIR
Department of Biomedical Engineering,
Louisiana Tech University,
Ruston, Louisiana 71272, USA
SUSHIL KUMAR JAIN
Louisiana State University
Medical Centre,
Shreveport, Louisiana, 71130, USA

Grains of truth

SIR — D.A. Williams claims in his letter (*Nature* 1 December 1983, p.420), that those who attended the Royal Astronomical Society discussion meeting on "Are interstellar grains bacteria?" were impressed by McDonnell's photographs of interplanetary dust in which silicate crystals were visible. He also claims that Hoyle conceded that interstellar bacteria are not alive. In point of fact Hoyle only said, in response to a question, that he had never claimed that they were alive, which is not quite the same thing.

Related to this point is the argument that bacteria would be photodissociated on scales of the order of 1,000 years. However, similar arguments were put forward by chemists to refute the suggestion that there were molecules in interstellar clouds — a position no longer held as shielding mechanisms ensure the long-term stability of organic interstellar molecules.

McDonnell's photographs do not show any evidence of the depletion of interstellar grains but only of the high velocity impacting interplanetary dust (micrometeorites), and were therefore irrelevant, as McDonnell suggested in his opening comments. Indeed the one thing that was held to be clearly disproved at the meeting was that the 10-micrometre feature was not due to siliceous material.

MICHAEL J. SHALLIS
University of Oxford,
Department for External Studies,
3-7 Wellington Square,
Oxford OX1 2JA, UK

Exotic nuclear decay discovered

The discovery, nearly a century after Becquerel, of a novel mode of radioactive decay is a surprise, but one that confirms α decay as the chief means by which heavy nuclei shed mass.

THOSE who decorate their offices with wall charts showing the isotopes, stable and otherwise, of the elements are in for trouble. As things are, these elaborate diagrams usually show by means of a colour scheme of some kind which radioactively unstable nuclei decay by which means. Decays in which the α– or β-particles (electrons) are emitted predominate, but the diagrams must also make room for other less common processes — positron emission, internal conversion (of an electron in an inner shell) and even fission. Now the same wall charts will have to accommodate radioactive decays in which unstable nuclides emit substantially heavier nuclear fragments than the familiar α–particle.

That is the burden of the discovery (see p.245) reported this week from the University of Oxford by H.J. Rose and G.A. Jones of a handful of unmistakable carbon nuclei among the decay products of radium-223 (^{223}Ra), hitherto marked in the charts as a simple α–emitter with a half-life of 11.2 days. What they have shown is that ^{223}Ra nuclei occasionally, but significantly, decay with the loss not of an α–particle but of a carbon-14 (^{14}C) fragment. Anthropomorphically, from the point of view of the nucleus so to speak, this novel arrangement has obvious advantages. The decay scheme of ^{223}Ra involves the successive emission of three α–particles (to give radon-219, polonium-215 and lead-211) followed by an electron, to give bismuth-211. (This decay scheme, which begins with uranium-235, predominantly leads to lead-207 by the further emission of two α-particles and an electron.) So why should not an unstable ^{223}Ra nucleus shorten this tortuous process by eliminating a substantial part of the charge and mass that must ultimately be lost in a single package? What now emerges is that that option is not as rigorously forsworn as the conventional wall charts suggest.

So how can it be that the best part of 90 years has passed since Becquerel's discovery of radioactivity before it has been recognized that α- and β-emission are not the whole of radioactivity? That is the obvious question that will now arise. The proper response, as a glance at the article by Rose and Jones will show, is that the discovery of ^{14}C emission from ^{223}Ra, important though it is, serves chiefly to emphasize that α-particle emission is the chief means by which radioactively unstable nuclei lose mass. Indeed, in the observations now reported, there are roughly 1,000 million times as many α-particles and ^{14}C nuclei in the decay of ^{223}Ra, perhaps as vivid a proof as there could be of the dominance of the familiar mechanisms of decay.

The rarity of these events is also the explanation why this novel form of radioactivity has not previously been found. So much can be told from the account by Rose and Jones of their observations, which entailed more than half a year of running time with detectors arranged so as to distinguish between single ^{14}C fragments and more frequent occurrence of groups of three α-particles from the nearly simultaneous decay of separate ^{223}Ra nuclei. (The fact that one of the two detectors seems at one stage to have been put out of action by accumulated radiation damage is a telling measure of the numbers of α-particles involved, even if the fault appears to have been an ambition to avoid buying new material for this purpose.) In the circumstances, there is no reason to think that the discovery that ^{223}Ra can decay with the emission of a ^{14}C particle will quickly be followed by similar observations in respect of other nuclei. Rose and Jones say they are looking at another candidate nucleus, but there will be few groups with the stamina that compels competition.

Some, no doubt, will even ask whether this search for exotic and necessarily rare mechanisms of decay can be worth the trouble, now at least that it has been demonstrated that such mechanisms are not entirely excluded. The simple answer, as always, is that the neglect of phenomena on the simple grounds that they are only rare, or that their effects are small, is never justified. On this occasion, several important questions arise, not the least of which is why the first exotic fragment to have been found in the decay of a radioactive nucleus should have been the unstable ^{14}C nucleus and not the stable ^{12}C, itself simply a combination of three α-particles.

Superficially, the calculation of the rate of some specified radioactive decay is a simple problem in elementary quantum mechanics, often found in introductory textbooks. An α-particle, for example, can escape from an unstable nucleus only if it can tunnel through the potential barrier against disintegration caused by the nuclear forces that hold even unstable nuclei together. The chance that if such a particle exists within the nucleus it will then escape, or the rate of the corresponding disintegration process, is thus a function of the height of the potential barrier, its width and of the total decrease of the potential energy of the system once the disintegration has taken place.

This calculation, first made in simple form by Gamow, has more recently been much refined and accounts for the "Gamow factors" used by Rose and Jones as part of their reason for believing that their disintegration products are ^{14}C and not some other isotope of carbon. But even on this simple picture, one huge uncertainty persists — the chance that a particle of the kind that eventually tunnels successfully through the barrier can be held, if only momentarily, to exist. What emerges from what Rose and Jones now say is that the "preformation probability" of ^{12}C would have to be comparable with that of the much simpler α-particle if it were to be produced in a distintegration. That cannot be the case, at least on this simple picture. It is relevant, but sobering, also to recall that the preformation probability of ^{14}C inferred from the results now reported is substantially greater than might have been expected from simple considerations, which is a reminder that the calculation of the absolute rates of even α decay, for example, is still not feasible.

What, in these circumstances, may be made of the discovery now reported? If, in the years ahead, there should accumulate a handful of examples of exotic kinds of radioactive decay in unstable heavy nuclei, it should at least be possible to glean some empirical information on the chance that various groups of nucleons will assemble into different potential disintegration fragments in neutron-rich heavy isotopes such as ^{223}Ra.

Hopes that exotic disintegration routes may have some practical value in other fields, perhaps in some novel technique of geochronology, seem, however, to be slim. Exotic disintegrations of the kind now identified are simply too few compared with the more familiar α disintegrations for their daughter nuclei and their products to be recognizable among the end products of the three principal radioactive series. (And ^{14}C is in any case unstable.) But at this stage it would be rash to rule out such possibilities. Too many confident predictions that new phenomena have no practical significance have too often been falsified for that course to be wise. **John Maddox**

© 1984 Macmillan Journals Ltd

Marine geology

Mapping the sea floor by satellite

from Roger N. Anderson

WHAT may well become one of the most famous satellites ever launched into Earth orbit transmitted for only three months before an electrical short-circuit killed it dead. Clearly, it will not go down in history as one of NASA's most successful engineering exploits. So how is it that the satellite may yet become a scientific legend?

Launched in 1978, with instruments primarily intended to map sea surface conditions, SEASAT also carried an ultra-sensitive altimeter. A continuous monitoring of the flight path of the satellite and the microwave travel time from the satellite to the sea surface and back again, allows the construction of an extremely accurate map of the topography of the sea surface. Much more than just temporal variability of the ocean surface (itself of importance) appears in such a map. For example, the map shows that the sea surface off the UK is several metres lower than the sea level off Iceland. In the Indian Ocean south of India is a huge trough — travelling from Africa eastwards towards Australia, a ship will sink over 100 m downwards and then gradually rise again. Why does this occur? Because excess mass beneath Africa and Australia pulls water away from the central Indian Ocean. Quite literally, the pull of the Earth's gravity is larger towards the edges of the Indian Ocean than at its centre. By mapping the sea surface height, one can map the Earth's gravity field since the ocean will always form a surface that is everywhere perpendicular to the direction of the gravitational pull.

If a satellite flies over a piece of ocean day after day, constantly mapping sea surface height, short-term disturbances will be averaged out and an accurate map of the Earth's gravity field can be generated. But why is such a map important?

For marine geologists the answer can be seen by considering, for example, a remote portion of the South Pacific Ocean west of the southern tip of South America and east of New Zealand (see Fig. 1). In over one hundred years of oceanographic exploration, actual depth measurements to the sea floor have been made only along the widely spaced ship tracks shown at the bottom of the figure. In only three months, SEASAT covered the same area with the satellite passes shown at the top of the figure. Obviously, if we could know how to transform gravity into bathymetry, SEASAT could make existing maps of the sea floor obsolete.

But there are military implications of such a data set too. Missile-equipped submarines, like the Trident, play a vital part in nuclear defence because they could survive a first strike. But submerged submarines must navigate. Of course, they can float radio antennae to the surface to communicate with satellites to determine their position, but how long would satellites last if a nuclear war began? Just as Cruise missiles and Backfire bombers navigate at extremely low altitudes by following the terrain of the continents, submarines could navigate relative to the terrain of the ocean floor — if they knew that topography well enough. A map from SEASAT could be as good as a road map to a submarine commander.

How then to recover the maximum amount of information from the SEASAT altimeter data? The Goddard Space Center, NOAA's National Ocean Service in Maryland, the US Navy and Jet Propulsion Laboratory in California have all had a crack at it. At low latitudes, satellite tracks are as much as 200 km apart — wider than the dimensions of geological features that SEASAT was capable of detecting. The difficulty is to preserve as much information as possible so that accurate maps of the gravity field and sea floor can be generated. Good maps were produced by these organizations (see, for example, refs 1–4), but by sacrificing details, through smoothing the data. With release of the SEASAT data to the academic community new techniques for extracting fine detail were developed. At the bottom of Figure 2 is shown existing bathymetric knowledge of an area in the southern Pacific. At the top is a gravity map produced by William F. Haxby of Lamont-Doherty Geological Observatory. The complete world-wide map is shown in reference 5.

What did Haxby do that those at other laboratories didn't? The secret is that the major geological features of the sea floor are strongly lineated as a consequence of plate tectonic processes. Trends identified in the SEASAT data were used to fill in the gaps in data coverage. The information derived from SEASAT was further enhanced by computing variations in gravitational force from a grid of sea surface heights. Think of driving slowly up a long grade to a precipitous cliff, then plunging down steeply into a valley, then up another long hill. Topography would record the gentle slope up, then the plunge down and the next slope, but the gradient of topography would record gentle slopes as virtually flat (not much change in topography from point-to-point) while cliffs show-up very dramatically because they are surrounded by a flat background, rather than by a 'real background'. Haxby went on to use north–south and east–west gradients of sea surface heights to calculate the actual gravity anomaly by combining them using La Place's equation. The map Haxby produced highlights important features of ocean topography in a way that has never before been achieved.

In the example area alone two important discoveries were made[6]. First, the clear outline of fracture zones, even under sedimentary cover, could be made out. These are scars left in oceanic plates by past plate motions and can be used to map the past movements of the Pacific and Antarctic lithospheric plates. Second, seamounts or undersea volcanoes were discovered in places where no previous seamounts were known. Chains of such volcanoes denote locales on the sea floor where extended volcanic activity has occurred long ago in the geological past. Such 'hot spot' trails not only tell of past motions between plates, but of absolute motions of the plates relative to the ultimate source of the heat, the Earth's mantle.

In the gravity map not only can we see the sigmoidal fracture zones that show gentle shifts in motion between the Antarctic and Pacific Plates over geological time, but also a north–south topographical scar left by a several hundred kilometre jump of the East Pacific Rise to the west 50 million years ago (far right, centre of image).

But there is political danger in scientific success. In October 1984, GEOSAT will be launched to map further the variability of the sea surface. But for the first 18 months of its existence, all data it transmits will be classified. The US Navy states that after 18 months of mission-oriented science, they may operate a scientific effort for the satellite, if it is still working.

In response to strong scientific interest in such satellites, from not only marine geologists, but physical oceanographers as well, there are a number of planning efforts now going forwards in the US within the Federal Agencies, the National Academy of Sciences, and in the academic community coordinated by Joint Oceanographic Institutions, Inc. The efforts are focused on the opportunities for global scientific programmes offered by the new satellite technology and on the challenges presented to all these groups in learning to work together with this new kind of data. The hope is that science will gain a respected place alongside the military in the satellite exploration of our planet — and just as important, that academic scientists will get their hands on the raw data as quickly as those from the government laboratories. □

Roger N. Anderson is in the Lamont-Doherty Geological Observatory of Columbia University, Palisades, NY 10964.

1. Sandwell, D. *EOS* **64**, 35, (1983).
2. Dixon, T.H. & Parke, M.G. *Nature* **304**, 406 (1983).
3. Marsh, J.B., Chaney, R., Martin, T. & McCarthy, J. *EOS* **63** (1982).
4. *National Geographic*, Sept. (1983).
5. Francheteau, J. *Scient. Am.*, Sept. (1983).
6. Haxby, W.F. & Weissel, J.K. *Lamont-Doherty Geological Observatory Yearbook* (1983).

0028-0836/84/030208-01$01.00

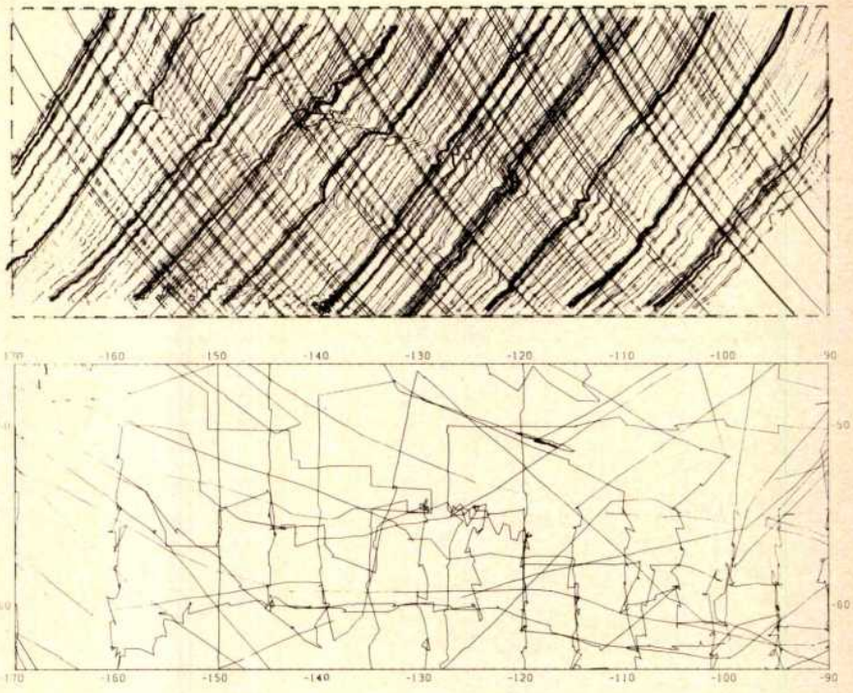


Fig.1 Top: SEASAT orbital tracks for the region of interest. Residual geoid heights are shown along descending tracks (half the total data set). Bottom: distribution of ship tracks in the region for which digital bathymetric data exist. Less than half of these cruises employed satellite navigation (from ref.6).

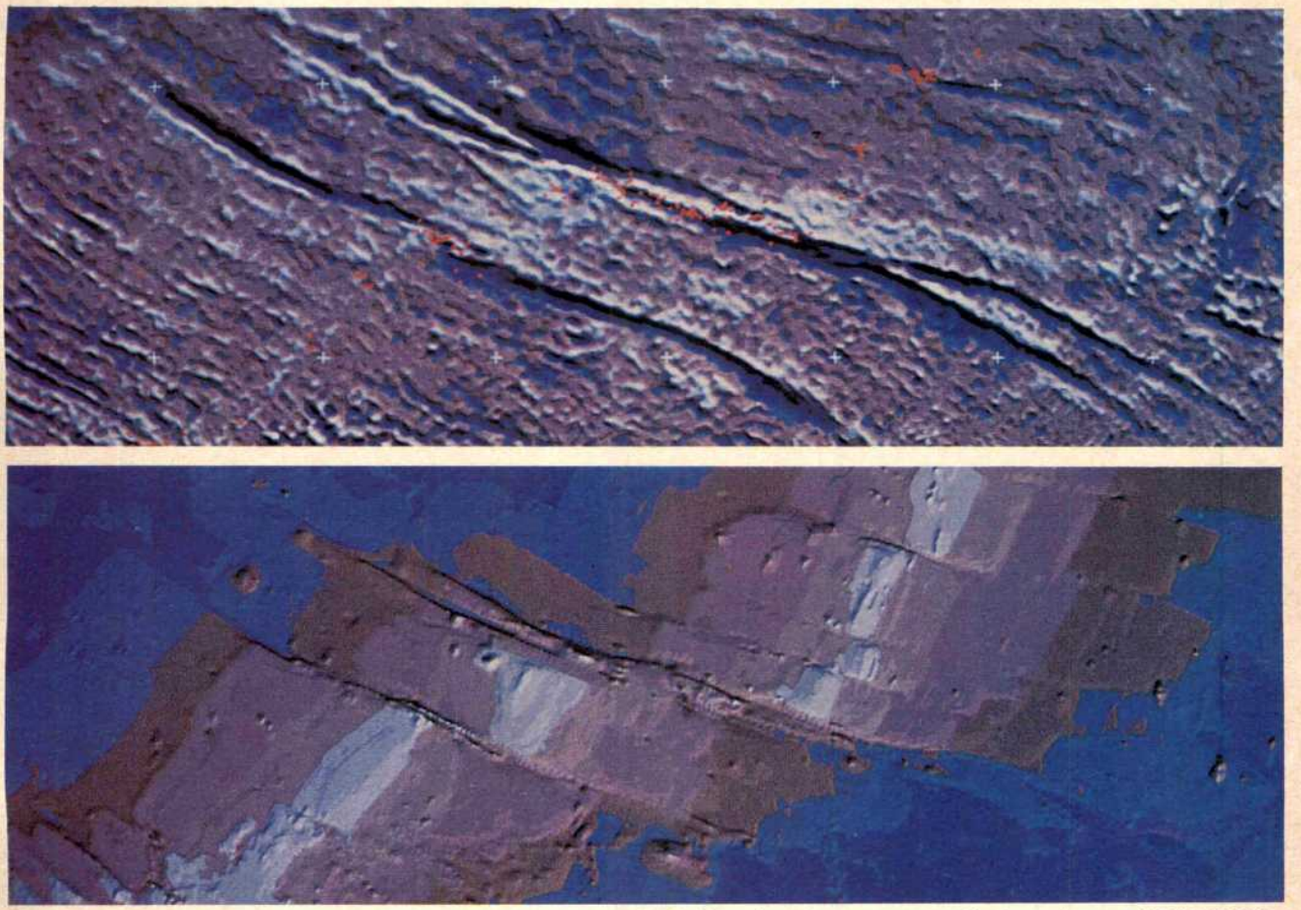


Fig.2 Top: colour relief image of gridded SEASAT-derived gravity anomalies in the region of the southern Pacific Ocean between 170°W, 90°W, 63°S and 46°S, contoured at 4 mgal intervals and illuminated from the NE. The gravity signatures of the major fracture zones (from north to south: Menard, Heezen, Tharp and Udintsev) are well defined as are several smaller fracture zones. Every 10° of latitude and longitude is denoted by a small white cross. The small red dots are epicentres of shallow earthquakes (WWSSN/NOAA sources, depths 0–70 km, $m_b > 4.5$) which occurred between 1961 and 1981. Bottom: colour image of gridded bathymetry in the same region, contoured at 500 m intervals and illuminated from the SE. The digital bathymetric data set for the Southern Hemisphere was prepared at Bay St Louis, Mississippi by scientists of the US Naval Oceanographic Office (from ref.6).

© 1984 Macmillan Journals Ltd

Astrophysics

The origin of gas in quasars — reverse stellar evolution?

from Martin Gaskell

WHAT is going to happen to a star situated next to the central energy source of a quasar as it is blasted by an intense stream of ultraviolet, X-ray and γ-ray radiation? A recent study by W.G. Mathews[1] makes the bold prediction that stars in such an environment will reverse the direction of their evolution!

One of the defining features of quasars is the presence of rapidly moving ($v \geq 10^4$ km s^{-1}) gas clouds with densities greater than 10^8 particles per cm^3 (much denser than normal gaseous nebulae seen around hot stars). Because they produce wide Doppler-broadened emission lines these clouds are called 'broad-line' clouds and the region from which they come is called the 'broad-line' region. From considerations of photoionization equilibrium we can work out the distance of these broad-line clouds from the central energy source (the mean ionization depends on the intensity of ionizing photons and the rate of recombinations which depends on the density). This distance is about a light year for an average quasar. In Seyfert galaxies (a kind of low-luminosity quasar) the broad-line region is expected to be an order of magnitude smaller (light-weeks to light-months) and emission-line variability observations have independently confirmed this size scale[2]. We are very confident, therefore, that the broad-line region is extremely close to the central energy source of the quasar.

The origin of the broad-line clouds is one of the major mysteries of quasars. The gas has a relatively high density, intermediate between normal gaseous nebulae and the density of the outer layers of stars[3], and a chemical composition not very dissimilar from stars such as our Sun[4], but these facts tell us very little. It is the motions of the clouds that are potentially the most important indicators of their origin. In the past all possible kinematic conditions have been given serious consideration — rotation in a disc, random orbits, infall and outflow — but it is the radiative acceleration theory that has been worked out in the greatest physical detail[5-8]. Although the issue is by no means completely settled there is mounting evidence that the broad-line clouds are indeed preferentially outflowing (rather than orbiting or infalling). For example it has been known for many years (from optical absorption lines) that ionized gas is outflowing from many high-luminosity quasars and even Seyfert galaxies[9]. Recently discovered systematic differences in broad-line profiles[10] imply that at least the highest-ionization broad-line clouds are outflowing. Perhaps the most direct evidence in favour of the radiative acceleration model is the correlation between line widths and physical conditions of broad-line clouds[11]. This is readily explicable in radiative acceleration models (since the same radiation which is accelerating the clouds is also photoionizing them) but is hard to understand in models where gravity dominates the dynamics.

Despite the growing evidence in favour of the radiative acceleration model and the unsatisfactory state of alternative gravity-dominated models[12] the problem of the actual origin of the clouds is perhaps more acute than ever. In the radiative acceleration theory a relatively steady volume source of cloud material is essential since it is only gas starting outside some critical radius which is accelerated outwards. Gas clouds created inside that radius (for example, from tidal disruption of stars near the black hole or from the accretion disc) will fall inwards (this is in fact highly desirable since one needs an influx of matter to power the quasar). The stars within a light year or so of the central energy source are the most natural source of the broad-line clouds but it has been realized for some time that normal stellar mass-loss processes (for example, stellar winds) fail by an order of magnitude or two to produce the necessary mass outflow rates if the broad-line clouds are all being expelled from the nucleus. This has led some workers to consider whether the intense radiation of quasars can itself enhance stellar mass loss. An attractive possibility is pushing the gas off the surfaces of the many lower main-sequence red dwarf stars in the galactic nuclei by the same quasar radiation pressure that will accelerate it to high velocities[13]. While this can work for red giant stars, the surface gravities of red dwarfs turn out to be prohibitively high for this sort of radiative ablation and red giants are expected to be much too rare to provide enough mass.

Mathews[1] offers a startling way out of these difficulties by proposing that red dwarf stars close to a quasar have their internal structures changed by the quasar radiation and effectively reverse their evolution by expanding. These 'inflated' stars would have lower surface gravities and could be ablated by radiation pressure. The key fact Mathews points out is that low-mass stars in the broad-line region actually have more radiant energy incident upon them than they produce by nuclear reactions or gravitational contraction. Mathews argues that if more than a few per cent of this energy can be mixed throughout the star a new configuration will be reached with an expanded star having a lower central temperature. Low-mass stars would then evolve in the opposite direction to their original contraction, towards the main sequence (hence the term 'reverse stellar evolution'). The hard X rays can deposit their energy relatively deeply in the stars and from there it can be mixed throughout the star either by convection or by large-scale meridional circulation currents (analogous to the situation in Jupiter where the incident solar radiation could do the same thing). Once the star has been inflated (or rather re-inflated) the radiative ablation process can remove matter efficiently from the surface of the star. After some 10^9 years or so the entire mass of the red dwarf star will have been removed. When the quasar has exhausted its supply of red dwarfs its activity will die down. This is in agreement with observations which show that quasars were far more active 10^{10} years ago when galaxies were young than they are now. Mathews even goes so far as to suggest that a possible deficiency of red dwarf stars in the inner one light year of our own galactic nucleus[14] could be a result of past quasar activity.

The inflation/ablation model has a number of attractive points: it gives the needed enhanced stellar mass-loss rates; the distance from the X-ray source within which stellar inflation is possible is consistent with the size of the broad-line region inferred from completely different arguments; and the gas is automatically produced at the right density and temperature. Inflated stars are also more liable to collide and produce the hot 10^8K intracloud medium. The question of whether the inflation mechanism does actually work will, however, require more detailed studies to settle it. It is certain that a star next to a quasar cannot have a normal cool 3,000K surface, but just how the harsh incident X-ray flux will affect its internal structure is a very difficult thermodynamic problem depending on poorly understood theory. If the inflation process fails and some other adequate mass-loss mechanism cannot be found then the physical model for the quasar that has emerged in recent years will need radical modification. □

Martin Gaskell is on the faculty of the Astronomy Department at the University of Texas at Austin, Austin, Texas 78712.

1. Mathews, W.G. *Astrophys. J.* **272**, 390 (1983).
2. Cherepaschchuk, A.M. & Lyutyi, V.M. *Astrophys. Lett.* **13**, 165 (1973).
3. Davidson, K. & Netzer, H. *Rev. Mod. Phys.* **75**, 715 (1979).
4. Gaskell, C.M., Shields, G.A. & Wampler, E.J. *Astrophys. J.* **249**, 443 (1981).
5. Mathews, W.G. *Astrophys. J.* **139**, 23 (1974).
6. Blumenthal, G.R. & Mathews, W.G. *Astrophys. J.* **198**, 517 (1975).
7. Blumenthal, G.R. & Mathews, W.G. *Astrophys. J.* **233**, 479 (1979).
8. Mathews, W.G. *Astrophys. J.* **252**, 39.
9. Anderson, K.S. *Astrophys. J.* **189**, 195 (1974).
10. Gaskell, C.M. *Astrophys. J.* **263**, 79 (1982).
11. Gaskell, C.M. *Proceedings of XIth Texas Symposium on Relativistic Astrophysics* (ed. Evans, D.S.) (New York Academy of Sciences, New York).
12. Mathews, W.G. *Astrophys. J.* **258**, 425 (1982).
13. Edwards, A.C. *Mon. Not. R. astr. Soc.* **190**, 757 (1980).

0028-0836/84/030210-01$01.00

Acquired immunodeficiency syndrome

The AIDS epidemic: continental drift

from Jerome E. Groopman and Paul A. Volberding

WHERE now to dig for clues to the aetiology and pathogenesis of acquired immunodeficiency syndrome (AIDS) is the major decision facing investigators this year. The study of AIDS has progressed rapidly since the initial recognition of the disorder in 1981 (ref. 1); largely through investigation of cases belonging to accessible high-risk groups such as homosexual men, intravenous drug users and haemophiliacs. The majority of those studied have resided in cities like New York, San Francisco and Los Angeles, the epicentres of the epidemic. But the cause of AIDS has not yet been identified, and digging in geographical areas outside these epicentres and investigation of atypical cases is probably going to be required.

Three types of case are now being most carefully studied: those outside the continental United States; those among persons with no known risk factors; and those that do not fit the original surveillance definition of AIDS of the Centers for Disease Control but are likely to result from the same causative agent(s).

There are currently nearly 300 cases of AIDS (defined as opportunistic infections and/or Kaposi's sarcoma in patients with unexplained cellular immunodeficiency[2]) in Europe. What distinguishes the European cases from those in North America is an intriguing link to Central Africa, particularly Zaire. Nearly all the 40 Belgian cases have had occupational or sexual contact with persons from Zaire or Chad.

A recent visit to Kinshasa, capital city of Zaire, by a team from the Centers for Disease Control and the National Institutes of Health has confirmed the existence of substantial numbers of AIDS cases in urban Zairian hospitals. Although it is still unknown whether AIDS in Central Africa predated the emergence of the disorder in the United States in the late 1970s, the occurrence of the disease outside the continental United States may provide important clues to not only its origin, but also its patterns of spread. During the past decade there appears to have been continental drift of the 'AIDS agent' among Africa, North America and Europe.

Among African cases in Africa and Europe there are about equal numbers of males and females; homosexuality does not appear to be a factor. AIDS may be transmitted among Central Africans through pathways including heterosexual sexual contact and medicinal use of unsterile needles.

The pattern of occurrence of AIDS among Haitians in the United States and in Haiti is more controversial[3,4]. There are claims that it is explicable by covert male homosexuality and that the taboos against homosexuality are so strong in Haitian society that accurate epidemiological information could not be obtained at first. It is important to resolve this controversy and to resolve it in a fashion that avoids any possible stigmatization of a socially vulnerable group like the Haitians and that does not bow to political pressures.

Studies of Central Africans and Haitians are now being carried out with special attention being paid to possible aetiological agents of AIDS. The current leading candidate is the human T-cell leukaemia virus (HTLV)[5-7]. Isolates of HTLV-like viruses have been obtained by both American and European groups from peripheral blood, lymph nodes and spleen, and molecular mapping of the isolates may point to genomic regions important in the pathogenesis of the immune suppression. Despite such endeavours, the prevalence of a number of other viruses in both Africa and the Caribbean may confound rapid identification of a unique aetiological agent.

The situation will probably be very different among persons in the US who have contracted AIDS but have experienced no known risks other than exposure to blood products. Such previously healthy persons do not have lifestyles or travel histories that particularly predispose them to infections with viruses such as cytomegalovirus, Epstein-Barr virus or HTLV. There are currently 38 cases (30 adult, 8 children) of AIDS in the United States that appear to be associated with blood transfusion.

The earliest case of transfusion-associated AIDS occurred in mid-1982 and there has been a rapid increase in cases since that time[8]. The incubation time (time from transfusion to onset of clinical symptoms) of AIDS in such cases ranges from 10 to 45 months in adults with a median of 25 months. Individuals have developed AIDS after blood transfusion with as few as two units of blood but most cases have had multiple units from several donors.

In nearly all the cases studied to date, the Centers for Disease Control was able to identify at least one donor who was 'suspicious' in that he was a member of a high-risk group for AIDS and often had associated immunological abnormalities. It is intriguing that only one 'suspicious' donor actually had AIDS as defined by the Centers for Disease Control while the others had lymphadenopathy, constitutional symptoms, or were entirely asymptomatic. This may have important implications in identifying who is capable of transmitting the disorder, at least through blood donation. There may well be an asymptomatic carrier state of the syndrome analogous to that of hepatitis B. In addition, infection with the putative AIDS agent may be transient while immune injury persists, eventually leading to clinically apparent disease. If this is true, identification of the causative agent, which may be present for only a short period of time, will require large prospective studies of individuals likely to be exposed to the agent and aggressive serial procurement of tissue.

The extraordinarily long incubation period apparent from these transfusion-related cases may be due to unknown host susceptibility factors or to a 'slow virus'. It is of note that the majority of transfusion-related AIDS cases have occurred in those regions in which AIDS is quite prevalent (New York and California) while cases among haemophiliacs does not follow the same geographical pattern. This probably relates to the fact that the factor VIII concentrates used by haemophiliacs are distributed nationwide while more standard blood products are locally distributed. AIDS has now become the second major cause of death (after haemorrhage) in haemophiliacs in the United States.

It is clear that the original surveillance definition of AIDS developed by the Centers for Disease Control was purposely narrow in scope and has excluded from the data base large numbers of individuals who probably have a milder form of the disorder. AIDS-related complex (ARC) is a constellation of clinical findings including unexplained generalized lymphadenopathy, fever of unknown origin, night sweats, weight loss and chronic diarrhoea with associated laboratory abnormalities indicating impairment of the cellular immune system. ARC is occurring in the same groups at high risk for AIDS. Data are just being accumulated on ARC patients, but it appears that their syndrome is transmissible in the same manner as AIDS and is probably caused by the same agent[9,10]. It is estimated that there are 5–10 times as many persons with ARC as AIDS in the United States, or about 15,000–30,000 cases taking CDC statistics on AIDS.

The incidence and prevalence of AIDS in certain areas of the United States is now beginning to be determined. A cohort of 6,500 homosexually active men in San

1. *Centres for Disease Control: Morbid. Mortal. Weekly Rep.* **30**, 250 (1981).
2. *Centres for Disease Control: Morbid. Mortal. Weekly Rep.* **30**, 409 (1981).
3. Viera, J. *et al. New Engl. med. J.* **308**, 125 (1983).
4. Pitchenik, A.E. *et al. Ann Intern Med.* **98**, 277 (1983).
5. Essex, M. *et al. Science* **220**, 859 (1983).
6. Allmann, E.P. *et al. Science* **220**, 862 (1983).
7. Essex, M. *et al. Science* **221**, 1061 (1983).
8. Curran, J. *et al. New Engl. med. J.* (in the press).
9. *Centres for Disease Control: Morbid. Mortal. Weekly Rep.* **31**, 249 (1982).
10. Ioachim, H. L., Lerner, C.W. & Tapper, M. L. *J. Am. med. Ass.* **250**, 1306 (1983).

© 1984 Macmillan Journals Ltd

Francisco has been followed since 1978 as part of an epidemiological study of hepatitis B. The overall prevalence of AIDS in this group is one per cent and, for a subset of men 30–40 years old, nearly two per cent. The yearly incidence of AIDS in this cohort is about 0.5 per cent. This is a remarkably high incidence and prevalence for a communicable disease which is usually fatal. It can be estimated that about 5–10 per cent of this cohort may develop ARC. Should these numbers be accurate, then the United States is facing an epidemic of significant dimension and measures should be taken to contain it. □

Jerome E. Groopman is in the Division of Hematology/Oncology, New England Deaconess Hospital, Boston, MA 02215, and Paul A. Volberding is in the Division of Oncology, San Francisco General Hospital, San Francisco, CA 94110.

Cell biology

Gene amplification and drug resistance

from Margaret Fox

SINCE the original reports in 1978 by Schimke and co-workers of the association of increased dihydrofolate reductase (DHFR) gene copy number with overproduction of DHFR protein and resistance to the drug methotrexate (MTX) many other examples of the association of gene amplification with drug resistance have been documented. Leading workers in the field were recently brought together at a symposium* where the major problems of the relationship between gene amplification and the development of drug resistance were discussed.

At the symposium it was made clear (G. Stark, ICRF, London and G.Wahl, Salk Institute, San Diego) that gene amplification is perhaps something of a misnomer because in almost all cases seen to date varying amounts of flanking DNA sequences are co-amplified with the selected gene to give an amplified unit which can vary from approximately 40 kilobases (kb) to over 1,000 kb in different cell lines selected for resistance to the same drug. The replacement of the term 'gene amplification' by that of 'DNA amplification' was proposed.

Most studies have been made on a variety of *in vitro* cell lines derived from human tumours, and 'normal' and transformed rodent cell lines. It is clear that if an appropriate enzyme inhibitor which binds tightly is available, then cultured cell lines will respond by amplifying the gene coding for the target protein together with varying lengths of flanking sequences. The rate and degree to which amplification occurs is directly proportional to the concentration of the inhibitor used in the case of resistance to N-(phosphonoacetyl)-L-aspartate (PALA) in BKH cells (Stark).

Data from other investigators (R. Kellems, Baylor College of Medicine, Houston and R. Wilson, Institute of Genetics, Glasgow) indicated that the level of protein overproduction was almost unlimited given sufficient selective pressure. R. Kellems, for example, reported that adenosine deaminase (ADA) levels could be increased over 10,000-fold — production then amounting to 50 per cent of total soluble protein — in cells grown in adenosine, alanosine and uridine in the presence of deoxycorfomycin. Similarly, very high levels (approximately 20 per cent of total cellular protein) of glutamine synthetase were achieved by selecting cells for resistance to the inhibitor methionine sulphoximide (Wilson). In the case of ADA, overproduction could be totally accounted for by a corresponding increase in the abundance of mRNA and ADA-coding DNA sequences which were carried on multiple double minute chromosomes.

In other drug-resistant cell lines the increase in gene copy number differed considerably, ranging from a five-copy increase associated with a very large homogeneously staining region (HSR) in a MTX-resistant HeLa cell line (J.M. Trent, University of Arizona) to the production of 600–1,200 copies of DHFR genes in EL4 mouse cells and PG19 melanoma cells (C. Bostock, Mammalian Genome Unit, Edinburgh) and a 200-fold increase in CAD sequences in PALA-resistant lines (Stark).

Sequences flanking the selected gene have always been found to be co-amplified, but the size of the co-amplified DNA varies greatly from one cell line to another. It is not clear what determines the size of the amplified unit but the co-amplified DNA sometimes expresses novel proteins which can be detected on SDS polyacrylamide gel electrophoresis. In cells selected for overproduction of adenylate deaminase (AMP.D) polypeptides other than AMP.D are overproduced (M. Debitasse-Buttin, Institute Pasteur, Paris). Analysis of several clones suggests that different polypeptides are overproduced to different extents in different clonal isolates. The implication is that the co-amplified DNA has undergone a variety of recombination events.

Overproduction of glutamine synthetase and phosphoribosyl pyrophosphate amido transferase was reported in some cell lines selected for overproduction of asparagine synthetase by the use of β-aspartyl hydroxamate and albizzin (I. Andrulis, Hospital for Sick Children, Toronto). In these resistant cell lines a good correlation was found between the level of DNA sequence amplification and abundance of mRNA and protein. Considerable differences in the levels of overproduction of asparagine synthetase achieved were observed in resistant cells selected in the two drugs. Multistep selection in albizzin resulted in 260-fold overproduction of normal enzyme but with β-aspartyl hydroxamine prolonged selection resulted in the accumulation of an apparently mutant enzyme.

In PALA-resistant clones which overproduce the CAD protein (Stark), a 45 kb sequence containing the 24 kb CAD gene was always amplified and the co-amplified DNA also coded for three other RNAs which were overproduced to different extents in different mutants. Overproduction of the multifunctional protein UMP synthetase which is inhibited by pyrazofurin and increases 40-fold in response to selection was reported by P. Suttle (University of Texas, San Antonio). These data indicate that there is not always a direct quantitative relationship between the level of gene amplification and the level of mRNA abundance.

The facility with which genes can amplify in different cell lines and the differences between cell lines with respect to whether the amplification events result in the formation of double minute chromosomes, very large homogeneously staining regions, or abnormally banding regions were much discussed. In human tumour cells and in *in vitro* cultured mouse lines, both double minute chromosomes and homogeneously staining regions have been reported, the former being generally associated with more unstable phenotypes. Amplified sequences contained in homogeneously staining regions can also be slowly lost on prolonged growth in the absence of selection (J. Biedler, Sloan-Kettering Institute, New York) but abnormally banding regions seen mostly in mouse cells appear stable. In Chinese hamster cells double minute chromosomes are rarely if ever observed. There were also suggestions of a tighter control of amplification in differentiated cells (F. Fougère Destrachette and M. Weiss, National Centre for Scientific Research, Gif-sur-Yvett).

The question of permissiveness for amplification was discussed by J.A. Wright (University of Manitoba) who reported that isolation of ribonucleotide-reductase-amplified cell lines from human diploid fibroblasts was much more difficult than from permanent rodent cell lines. Human tumour cells *in vivo* and cell lines derived from human tumour cells appear to amplify their genes with relative ease (Trent).

Several contributors addressed the

*The 18th Paterson Symposium was held at the Paterson Laboratories, Manchester, on 17–19 October 1983.

0028-0836/84/030212-02$01.00

question of the structure of the amplified unit. A model involving translocation and homologous recombination was suggested by W. Flintoff (Health Sciences Center, London, Ontario); Bostock and Stark also discussed several possible models. Considerable evidence in favour of recombination being involved in the generation of amplified DHFR and CAD units was presented by both these authors. Amplified sequences have been assayed for novel fragments (joints) after differential hybridization of control and amplified DNA and subsequent cloning of amplified DNA in λ vectors. In all amplified lines novel sequences have been detected, many of which are unique to individual amplified clones. Stark posed the question as to whether selection procedures act on previously amplified sequences or whether amplification is generated by the selective agent. Data from fluctuation analysis indicated that amplification by the selective agent is of only minor importance in comparison with the action of selection on previously amplified sequences.

The question of whether genes amplify *in situ* or whether translocation and rearrangement are involved was addressed by Wahl. Cloned CAD genes of Syrian hamster were introduced into Chinese hamster cells where they can be recognized because they possess unique sequences. The donated genes were shown by *in situ* hybridization to reside on different chromosomes to the one containing the native gene and, in one case, they amplified spontaneously at a frequency at least 100-fold higher than that seen in three other transformants or wild-type cells. The suggestion is that flanking chromosomal sequences mediate the amplification event although it is conceivable that the hyper-amplifiable structure was created by the insertion of donor genes, for example, by disruption of a replication origin.

Several major questions remain unanswered. First, are normal diploid cells capable of amplifying their genes, in particular do human diploid cells possess this capacity? Human tumour cells appear to be amplification proficient, but is this an initial event in the generation of the malignant phenotype related to overall loss of genomic control or do specific alterations in DNA sequences occur followed by a cascade of events? How frequently is DNA amplification involved in intrinsic and acquired resistance in human tumours *in vivo?* Suggestions that amplification is directly involved in the generation of drug resistance come from studies of oat cell carcinoma with the c-*myc* probe in which some correlation between the presence of amplified c-*myc* sequences and refractoriness to chemotherapy was evident (Trent). Do drugs used in chemotherapy actually trigger amplification and could we prevent it if we knew the mechanism?□

Margaret Fox is in the Paterson Laboratories, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX.

Virology

Viruses and tissue injury

from Bernard N. Fields

IN A report in this week's *Nature,* Oldstone and colleagues (p.278) describe how infection of young mice with lymphocytic choriomeningitis virus leads to changes in growth and glucose regulation[1]. What is remarkable about these findings is that there is no histological evidence of tissue injury in the pituitary glands of the infected mice — even though growth hormone deficiency was clearly detectable, transplantation of growth hormone-producing cells allowed infected mice to grow normally, and the virus was shown to be replicating in the pituitary. The observation makes clear that injury as detected by conventional pathological analysis may be absent in the face of profound functional impairment and should stimulate a closer look for viruses in other diseases where the function of differentiated cells is abnormal.

The work of Oldstone and collegues is one of a number of recent reports demonstrating that viruses are capable of injuring mammalian hosts in a variety of ways not previously suspected.

Originally, it was thought that viruses were the cause of acute or occasionally chronic infections associated with detectable injury to tissues. With the development of cell culture techniques, particularly in the 1950s, viruses were detected and often identified by their characteristic cytopathic effects. The extraordinary diversity of the behaviour of viruses was further revealed by unusual or exotic occurrences such as that of subacute sclerosing panencephalitis (a progressive fatal neurological illness) occurring years after measles infection[2] and the identification of the exotic spongiform encephalopathies (such as Kuru of man and scrapie of sheep) in which a progressive degenerative neurological illness occurs without fever or an inflammatory reaction and is due to a transmissible agent that has a number of extraordinary properties (possibly including the absence of nucleic acids)[3]. Recent studies using animal models have expanded further the possible role of viruses in diseases of unknown aetiology and illustrated new ways that viral injury may be mediated. It is now clear from experiments in mice that viruses can specifically injure endocrine tissue, leading to abnormalities of endocrine secretions[4], and the work reported in this issue shows that lymphocytic choriomeningitis virus can alter pituitary cells, causing them to produce hormonal deficiences, while producing no detectable cytopathic effects[5].

It is especially interesting that the findings with lymphocytic choriomeningitis virus demonstrate how endocrine tissue may be affected since viral infections have long been considered as leading candidates in the causation of insulin-dependent diabetes mellitis[6]. While there are a number of important differences between insulin-dependent diabetes mellitus and the lymphocytic choriomeningitis disease described by Oldstone and colleagues (particularly the presence of tissue injury as manifested by insulitis in the former), insulin-dependent diabetes mellitus serves to illustrate additional ways by which virus infections may result in disease. In particular, the studies of Notkins and co-workers have shown that certain viruses may directly infect pancreatic β cells in mice resulting in chemical diabetes[7]. Viral infections of mice may also result in autoantibodies to growth hormone and a polyendocrinopathy[4]. While the relevance of these mouse models to human illness has not been proven, it is clear that antihormone antibodies and antitissue antibodies are additional important ways that viral injury may be mediated.

Evidence for another route by which virus infection may lead to the production of autoantibodies and tissue destruction has been seen in recent studies of the mammalian reoviruses[8]. Viral receptors were postulated to share structural features (idiotypes) with antibodies directed against the viral haemagglutinin (the protein of the virus responsible for attachment to the cell). Anti-idiotypic antibodies directed against antibodies to the reovirus haemagglutinin could thus bind to cell surface receptors on uninfected host cells in a manner resembling the virus and could destroy cells bearing complimentary receptors. Interestingly, cytotoxic T lymphocytes generated after reovirus infection have been shown to be capable of lysing uninfected B-lymphocyte cells bearing anti-idiotype-like structures on the cell surface[9].

The slow virus infections are among the most interesting and least understood of all infectious diseases[2]. This class of diseases, of which scrapie disease of sheep is one example, is characterized by long incubation periods, a progressive degenerative neurological illness and an unusual agent (termed 'prions' by Prusiner and colleagues[10]). Partially purified preparations of the scrapie agent contain a 'prion' protein (designated PrP) that is unique to scrapie preparations and may be a structural component of the prion[5]. Insight into the nature of the injury caused by scrapie may come from the observation that prion preparations contain numerous rod-shaped particles that, like amyloid, exhibit a green colour and birefringence by

© 1984 Macmillan Journals Ltd

polarization microscopy. As pointed out by Prusiner and colleagues, amyloid deposits have been seen in a number of degenerative disorders of the central nervous system. A direct link between the putative rods associated with scrapie and amyloid would provide a major clue to the aetiology of a number of degenerative diseases. Unfortunately, the association between prions and amyloid is not yet certain as normal cells contain rod-like structures, and 'prions' have not yet been completely purified[5].

Clearly, the means by which viruses can injure their hosts are diverse. These include injury due to subtle effects on cell metabolism without overt pathological change, the production of autoantibodies to tissue components or products, and the deposition of material related to normal cell products. What remains to be determined is which of these strategies of virus-induced injury are important in causing human disease. □

Bernard N. Fields is in the Departments of Microbiology and Molecular Genetics and Medicine, Harvard Medical School, Boston, MA 02115.

1. Oldstone, M.B.A., Rodriguez, M., Daughaday, W.H. & Lampert, P.W. *Nature* **306**, 278 (1984).
2. ter Meulen, V.J. *Gen. Virol.* **41**, 1 (1978).
3. Gajdusek, D.C. *Science* **197**, 943 (1977).
4. Onodera, T. *et al. J. exp. Med.* **153**, 1451 (1981).
5. Prusiner, S.B. *et al. Cell* **35**, 349 (1983).
6. Notkins, A.L. *Scient. Am.* **241**, 62 (1979).
7. Onodera, T., Jensen, A.B., Yoon, J.W. & Notkins, A.L. *Science* **201**, 529 (1978).
8. Fields, B.N. & Greene, M.I. *Nature* **300**, 19 (1982).
9. Ertl, H.C.J. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7479 (1982).
10. Prusiner, S.B. *Science* **216**, 136 (1982).

Natural materials

Chalky submarines

from Paul D. Calvert

MOST of us, if asked to design a submarine, would not think of chalk as a very suitable constructional material. But a recent paper by Birchall and Thomas (*J. Materials Sci.* **18**, 2081; 1983) on the structure of cuttlefish bone illustrates how good design can procure impressive performances from the most unpromising starting materials.

The cuttlefish (*Sepia officinalis*, a relative of the squid) maintains neutral buoyancy at varying depths in the sea by pumping water into or out of the cuttlebone which is divided into small closed compartments. When empty of fluid these cells are at a pressure of about 0.8 atmospheres. Hence they must withstand a high external hydrostatic pressure corresponding to the depth of the fish (1atm per 10 m).

The cuttlebone structure comprises parallel sheets of calcium carbonate (aragonite) about 10 μm thick separated by 200–600 μm. The sheets are held apart by pillars after the manner of a multistorey car park except that each layer is sealed from those above and below. The whole assembly is supported on a 1.5 mm dense bony dorsal shield.

The obvious design for a pressure-resistant vessel is a thick-walled hollow sphere — just as seen in a bathysphere. A simple calculation for a given compressive strength of the material would give this sphere a density of about 75 per cent of either a layered pillared structure or a cylinder. Birchall and Thomas measure the same crushing strength perpendicular or parallel to the layers as the structure has to resist uniform hydrostatic forces. Apparently, the cuttlefish trades the improved depth resistance that would come from a spherical pressure vessel for an elongated cuttlebone which can also act as an internal skeleton. The pillared layer structure bears a strong resemblance to the skin-and-honeycomb panels used to obtain maximum rigidity with minimum weight in aircraft and provides a light and rigid skeleton for the cuttlefish.

A problem with using such an intrinsically brittle material as chalk is that it can be quite strong in compression but relatively small tensile loads can produce failure. Much of the design of the medieval cathedrals can be seen as an exercise in minimising or eliminating tensile loads, but the cathedrals did not have to swim around and fight. One way of circumventing the problem is to ensure that local failure does not lead to catastrophic collapse. Crushing tests show that the cuttlebone does fail by a progressive collapse of the layers rather than imploding as would a hollow structure.

The cuttlefish would also like to get as much tensile strength from the material as possible without sacrificing compressive strength or rigidity. This is also very much a concern in the production of synthetic ceramics both in the context of 'high performance' ceramics, which are the subject of a recent Japanese initiative (*Nature* **305**, 373; 1983), and in the high-strength cements developed by Birchall and his co-workers (*Nature* **289**, 388; 1983). For maximum strength one would like a fine grain size; very small pores, if any; and strong bonds between the grains. The key to the high strength of biological ceramics is their four per cent organic component — a protein–chitin complex which forms a layer covering the carbonate surface. The organic component acts as a nucleating surface for the aragonite crystals and may play a more important part in preventing the crystals from growing too large and/or in improving the bonding between the crystals. S.A. Wainwright and co-authors discussed these ideas well in *Mechanical Design in Organisms* (1976).

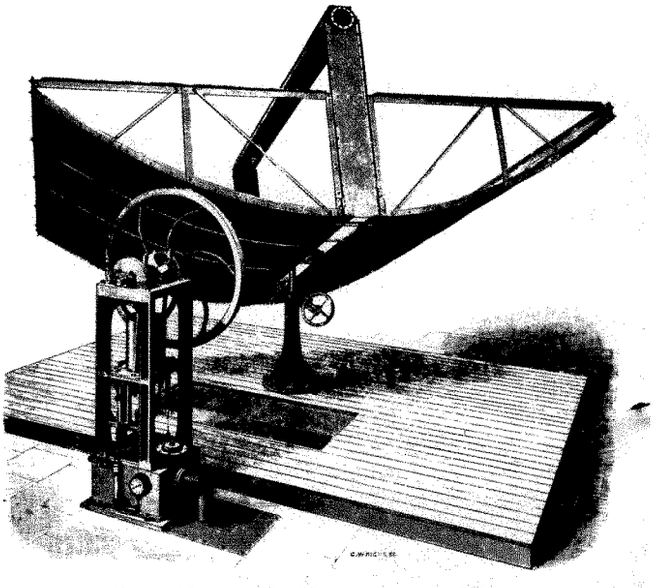
The operating parameters for a cuttlefish seem quite respectable when compared with submarines. The cuttlebone crushes at pressures corresponding to a depth of about 230 m and cuttlefish have been recorded at depths of 200 m. The osmotic pump which controls the gas–fluid exchange in the bone would not work beyond about 240 m. By comparison large submarines are reputed to be limited to operating depths of 300 m, which is only about twice their own length, despite being constructed of far stronger materials. It seems almost sacrilegious that such a sophisticated structure as cuttlebone should frequently end up as a budgerigar's beak cleaner! □

Paul D. Calvert is in the School of Chemistry and Molecular Sciences, University of Sussex, Falmer, Brighton BN1 9QJ.

100 years ago

THE SUN MOTOR

THE illustration represents a perspective view of a sun motor put in operation last summer. The leading feature of the sun motor is that of concentrating the sun's radiant heat by means of a rectangular trough having a curved bottom lined on the inside with polished plates so arranged that they reflect the sun's rays towards a cylindrical heater placed longitudinally above the trough. This heater contains the acting medium, steam or air, employed to transfer the solar energy to the motor; the transfer being effected by means of cylinders provided with pistons and valves resembling those of motive engines of the ordinary type.



From *Nature* **29**, 217, 3 January 1884.

0028-0836/84/030214-01$01.00

Observation of stellar remnants from recent supernovae

David J. Helfand

Columbia Astrophysics Laboratory, Departments of Astronomy and Physics, Columbia University, New York, New York 10027, USA

Robert H. Becker

Department of Physics, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA

The current observational situation regarding the presence or absence of collapsed stellar objects associated with supernova remnants is reviewed. The theoretical expectations and observational evidence for the various possible classes of stellar remnants are discussed. The results of searches for these objects in the radio, optical, X-ray and γ-ray regions of the spectrum are outlined with the conclusion that nine bona fide *candidates are currently known. The implications of these results for the origin and evolution of compact objects are summarized.*

FIFTY years ago Baade and Zwicky[1] established the distinction between common novae and the vastly more energetic supernovae which they argued must bear visible witness to the destruction of a normal star. They also concluded[2] "With all reserve, we advance the view that a supernova represents the transition of an ordinary star into a neutron star.... Such a star may possess a very small radius and an extremely high density". Eight years later, Baade[3] and Minkowski[4] discussed the Crab Nebula as the remnant of the supernova reported by the Chinese in AD 1054, and pinpointed the central star as the compact object powering the Nebula's emission. In Minkowski's words, "Taken altogether, the spectroscopic evidence admits, but does not prove, the conclusion that the south preceding star is the central (exciting) star of the nebula". He went on to place an upper limit on the size of the star of $<0.02\,R_\odot$.

Theoretical work on the final state of collapsed stellar cores continued over the next three decades, although the observational picture remained static. Then, at the end of the 1960s, the suggestions of Baade and Zwicky were dramatically confirmed with the discovery in rapid succession of radio pulsars[5] (isolated neutron stars), X-ray pulsars[6] (accreting-binary neutron stars) and Cygnus X-1 (ref. 7) (presumably an accreting black hole). As the change in gravitational energy for a star between its normal state ($R \sim 10^{11}$ cm) and one of these collapsed configurations ($R \leqslant 10^6$ cm) is $\sim 10^{53}$ erg, the formation of compact objects must be accompanied by a huge release of energy. The explosion itself is, in at least some cases, visible as an optical supernova, while the small fraction of the energy which couples to the surrounding medium leads to an expanding bubble of shock-heated material, compressed magnetic fields and relativistic particles which we observe at later times as a supernova remnant (SNR).

The modern theory of supernova explosions admits several possibilities regarding the fate of the stellar core. Leventhal and McCall[8] originally suggested that a remnant white dwarf might be required to explain the late time light curve of Type I supernovae (SN). While this explanation is no longer thought to be relevant, SN explosion models have recently been constructed in which a core of $\sim 1\,M_\odot$ supported electron degeneracy remains following the ejection of the star's outer layers[9]. The standard models for more massive stars[10] require a central implosion which pushes the electron degenerate pre-supernova core to densities of $\geqslant 10^{14}$ g cm^{-3} and results in the formation of a neutron star. In very massive stars, the neutron degeneracy pressure of this phase may be insufficient to halt the collapse and a black hole will form. Finally, in some instances such as the carbon detonation[11] and pair instability[12] scenarios appropriate for stars in the 4–8 $M_\odot$ and 100–300 $M_\odot$ mass ranges, respectively, the core may disrupt completely along with the rest of the star and no stellar remnant is formed. The applicability of these various models to a specific mass range and evolutionary history of SN progenitors is still quite uncertain. Likewise, the properties of the stellar remnants themselves—mass, surface temperature, magnetic field strength and angular momentum—are not well-determined by the theories and we must look to observations of the sites of relatively recent SN explosions to delimit the range of possibilities.

As noted above, a blue star with a peculiar spectrum in the 1,000-yr-old Crab Nebula was the first suggested candidate for the compact remnant of a SN explosion. This suggestion was confirmed 35 yr after it was first made with the discovery that this star was a radio[13,14] and optical[15] pulsar: a rapidly rotating ($\Omega \sim 200$ rad s^{-1}), magnetized ($B \sim 10^{12}$ G) neutron star. This discovery actually followed by a few weeks the detection of a similar object in the 10^4-yr-old Vela SNR[16]. Despite numerous searches in various wavelength bands throughout the electromagnetic spectrum, however, no further pulsar/SNR associations were discovered between 1969 and 1979, although the circumstantial evidence that a significant fraction of SN must produce neutron stars became compelling. Several hundred more slowly rotating (and presumably older) radio pulsars were discovered, suggesting $\geqslant 10^5$ such objects existed in the Galaxy and leading to a pulsar birth rate currently estimated as 1 per 40 yr (ref. 17). In addition, 100 of the brightest galactic X-ray sources were found to be binary systems powered by accretion of matter from a normal companion onto an orbiting neutron star or, in perhaps a few cases, a black hole. Neutron stars were also implicated in other discoveries of the decade including 100 MeV γ-ray sources, globular cluster X-ray sources, and γ-ray and X-ray bursters. Integrating a 1 per 40 yr birthrate over the lifetime of the Galaxy, a total galactic population of $\sim 5 \times 10^8$ neutron stars seemed probable.

Comparison of these population statistics with the rate of SN occurrence in the Galaxy (variously estimated as 1 per 20 yr from comparison with the rates observed in external galaxies[18] to 1 per 80 yr from the SNR formation rate[19]) suggests that either most SN produce compact stellar remnants or that such collapsed objects can be formed in some less spectacular manner. However, even if all SN do result in the formation of a stellar remnant, there is good reason to doubt that they all result in the formation of a rapidly rotating magnetized neutron star similar to the Crab pulsar. The radio, optical, and X-ray appearance of the Crab Nebula is clearly different from that of most other historical SNR. Its centrally peaked brightness distribution, flat radio spectrum and high degree of linear polarization are understood to result from the injection of relativistic parti-

© 1984 Macmillan Journals Ltd

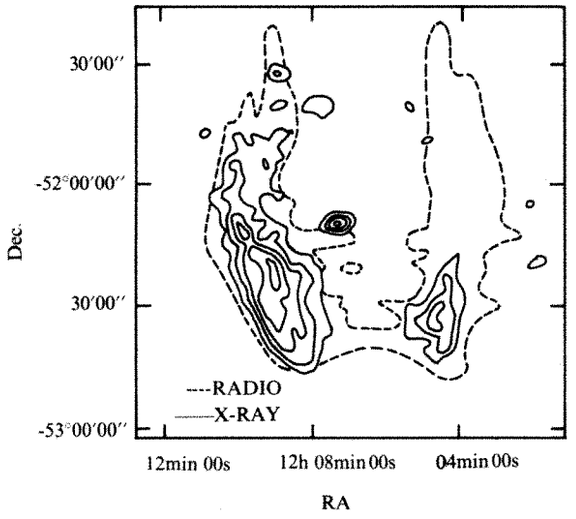


Fig. 1 A radio/X-ray contour plot of the remnant PKS1209−52.

cles accelerated near the neutron star; that is, the presence of the Crab pulsar could have been inferred from the surrounding synchrotron nebula even if the pulsed radiation had gone undetected. Most other historical SNRs show neither a pulsar nor any evidence for pulsar accelerated particles. From this one must conclude that either most SN do not create rotating magnetized neutron stars or that they create such stars in an environment or with characteristics which suppress the formation of a synchrotron nebula.

Examples of this second possibility, that neutron stars exist in SNRs but cannot accelerate particles, include a close binary system with mass transfer, in which the pulsar mechanism is smothered, and a stellar magnetic field outside some allowed range within which the pulsar mechanism operates[20,21]. We know that neutron stars do occur in binary systems from observations of X-ray pulsars but, until recently, none was known to be associated with a SNR; two have now been found[22,23]. The idea that some newly-formed neutron stars may suffer from an intrinsic inability to produce high energy particles is a relatively new one and no clear cases have been confirmed, although the possibility that such stars have been detected through their surface thermal emission is not excluded (see below). Within this context, then, we outline below the current status of the continuing search for the stellar remnants of supernovae.

Searches for compact remnants

Radio: The association of radio pulsars with SNRs has rested on the two cases of the Crab and Vela for over a decade, despite the discovery of over 330 other pulsars and the cataloguing of over 140 SNR. The fact that there are no other coincidences is a cause for concern but not, perhaps, alarm. Several observational selection effects work against the discovery of young pulsar/SNR pairs in most pulsar searches: decreased receiver sensitivity resulting from the added noise of the remnant itself, decreased search sensitivity at short periods, the increased severity of pulse smearing for short-period sources due to large dispersion measures and multipath scattering, the large distances to most SNR (50% of the known pulsars are <2.3 kpc away, whereas only ~10% of the SNR are within this distance), and the fact that perhaps as few as 1 in 5 of the pulsars will have beams which intersect our line of sight. High pulsar proper motions are not a problem, as the mean expansion velocity of even a 20,000-yr-old remnant is $\geq 400\ km\ s^{-1}$, larger than the largest measured pulsar transverse velocities.

A few targeted searches have been conducted on several nearby SNR. Manchester and Taylor[24] catalogue the results through 1976 for 12 remnants ranging in distance from 2 to 10 kpc. The limits are in the range $L_R \sim 5\text{–}10 \times 10^{25}\ erg\ s^{-1}$, only a few times that of the weakest known pulsar and a factor $>10^3$ less than the radio luminosity of the Crab. More recently, Seiradakis and Graham[25] have searched 20 SNR within 8 kpc to a similar sensitivity at 21 cm in an attempt to limit the problems of the SNR background and pulse smearing. However, their lower limit to periods was 300 ms and no new sources were found. Manchester *et al.*[26] have just completed a larger 21-cm search in the Southern Hemisphere covering 55 galactic SNR, 30 Large Magellanic Cloud (LMC) remnants, and several other objects, including two Cos B γ-ray sources. At a limiting sensitivity of 1 mJy out to dispersion measures of 1,800 cm^{-3} pc, they detected three new galactic pulsars only one of which[27] is clearly associated with a SNR—the recently identified X-ray pulsar in MSH15-52.

We need not assume, *a priori*, that all compact sources within SNR will be radio pulsars. In 1973, a variable unresolved, radio source named CL4 was discovered near the centre of the Cygnus Loop[28]. The source was immediately discussed as a possible stellar remnant, although it was also argued that it was extragalactic[29]. Very recently, CL4 was identified as a background quasar[30]. In 1978, a list of nine unresolved radio sources, all located within SNR, were proposed as candidate stellar remnants[31]. The list included two sources previously detected as X-ray objects, SS433 and Cir X-1, as well as CL4. It is now well known that SS433 is associated with the SNR W50 (ref. 22). Cir X-1 is without doubt also a galactic object, but its association with G321.9−03 has never been firmly established. Recent reviews summarizing the remaining sources in the list suggest that the others are all unrelated background objects[30,32]. Thus, the Crab and Vela pulsars, along with SS433, remained until very recently, the only radio-emitting stellar objects associated with SNR.

Optical: Hind and Plummer[33] were perhaps the first to suggest a specific object as the compact remnant of a supernova explosion: an 11th magnitude variable star near the position of Tycho's SN. Baade[3] and Minkowski[4] were the first to identify such an object correctly when they pointed to the 'south-preceding' star near the centre of the Crab Nebula as the stellar remnant of SN1054. Over the next 40 yr, the search continued for 'peculiar' optical objects in the historical remnants and a variety of candidates have been reported, none of which is now thought to be relevant. The most recent such search reviews many of these early attempts but finds no convincing new candidates[34]. Following the discovery of optical pulses from the Crab in 1969, several searches of both galactic and extragalactic SNR using fast photometry were conducted. Horowitz *et al.*[35] set limits ~5 mag fainter than the Crab for any short-period pulsed sources near the centre of Kepler, Tycho, Cas A and 3C58. Papaliolios *et al.*[36] conducted a similar search at the sites of 31 recent extragalactic SN without success. More recently, Pennypacker[37] has extended the search into the IR (0.9–2.4 μm) to take advantage of lower interstellar extinction; he obtained pulsed IR limits 3–4 mag fainter than the Crab for the remnants studied in ref. 35.

In 1980, an optical search of the remnant of SN1006 resulted in the discovery of a hot blue star ~2.5 arc min from its centre[38]. Subsequent observations identified the object as one of only five known sub-dwarf OB stars[39]. The offset between the stellar position and the remnant centre would require an extremely high transverse velocity ($800\ km\ s^{-1}$) for the star, seemingly violating the current proper motion limit for this object[38]. Recently, very broad symmetric absorption lines have been detected in the UV spectrum of this object, confirming that it is an unrelated background star lying behind the remnant[40].

There are thus only three optical point sources associated with the remnants of SN: the Crab and Vela pulsars and SS433. Incoherent synchrotron emission in the outer magnetosphere is thought to be responsible for the pulsar light curves[41] and is expected to scale with the neutron star rotation period P as P^{-10} (ref. 42). Thus, sources with $P > 50$ ms will have $m_v > 20$ for distances of 1 kpc, and are unlikely to be detected even if

Table 1 Properties of compact objects associated with SNR

(1) SNR name (a) Galactic	(1) SNR name (b) Common	(2) Point source position RA (1950)	(2) Point source position Dec (1950)	(3) Distance (kpc)	(4) L_x (0.2–4 keV) (10^{33} erg s^{-1})	(5) Probability chance superposition (a) D (r')	(b) IPC rate (counts s^{-1})	(c) $N(>S)$ (per SNR area)	(6) L_R (10^{28} erg s^{-1})	(7) L_{opt} (10^{31} erg s^{-1})	(8) L_γ (10^{34} erg s^{-1})	(9) Ref. for columns 2	3	5a	5b	6	7	8
		h min s																
G39.6−1.8	W50/SS433	19 09 21.3	+04° 53′ 53″	5.0	100	65	0.3	4×10^{-3} *c*	6,000	2×10^5	<25	96	97	96	85	98	99	66
G109.1−1.0	CTB109	22 59 02.8	+58 36 33	4.2	30	24	0.75	1×10^{-4} *c*	<1	<40	<18	46	100	101	23	101	46	66
G184.6−5.8	Crab	05 31 31.4	+21 58 54	2.0	500 *p*	5	40	1×10^{-8} *c*	14 *p*	400 *p*	20 *p*	102	103	104	50	105	106	107
G263.9−3.3	Vela X	08 33 39.3	−45 00 10	0.5	0.3 *p*	60	0.3	3×10^{-3}	4 *p*	0.01 *p*	4 *p*	108	109	110	57	105	106	107
G320.4−1.2	MSH15-52	15 09 59	−58 56 57	4.2	50 *p*	26	0.07	5×10^{-3}	4 *p*	<1	<18	45	111	111	45	27	45	66
G130.7+3.1	3C58	02 01 51.1	+64 35 23	2.6	0.5	5	0.01 *H*	4×10^{-3} *c*	<5	—	<7	52	112	113	52	114	—	66
G69.0+2.7	CTB80	19 51 02.4	+32 44 52	3	0.2	1	0.02 *H*	5×10^{-5}	<200	<100	<9	52	91	115	52	115	116	66
G332.4−0.4	RCW103	16 13 48.3	−50 55 05	3.3	1.0	9	0.02 *H*	4×10^{-3} *c*	<5 *p*	<1	≤40	94	117	118	119	94	94	66
G27.4+0.0	KES73	08 37 45	−04 59	26	1,500	4	0.25	2×10^{-5} *c*	<50	—	<700	120	91	120	PC	PC	—	66
G296.5+9.7	PKS1209-52	12 07 23.5	−52 09 49	1.1	0.2	81	0.08	0.04	<40	<20	<2	TW	91	121	TW	121	93	66
G6.6−0.2	W28	17 57 55.7	−23 19 59	2.4	0.1	49	0.01 *H*	0.34	<16	—	≤14	122	91	123	122	122	—	66
G127.1+0.5		01 25 08.3	+62 50 59	3.8	0.2	50	0.01	0.35 *c*	2,500	180	<14	125	91	124	TW	63	62	66

p = Limit or detection based on pulsed flux only. Column (2), best available positions for point sources of radiation. Column (3), distances to SNR from reference quoted in column (9); if a range is given, the mean value has been adopted. Column (4), unless quoted directly from the reference cited in columns (9)(5b), the X-ray luminosity in the 0.2–4.0 keV band has been calculated from L_x = IPC rate (5b) $\times 5\times10^{-11}$ erg [cm^2 counts s^{-1}]$^{-1}\times\exp[D_{kpc}/10]\times4\pi D^2$ (column (3)), appropriate for sources at distances $D\geq1$ kpc with a Crab-like spectrum modified by interstellar absorption in a medium of mean density of 1 cm^{-3}. Column (5a), Mean diameters in arc min taken from refs in column (9). b, Einstein imaging proportional counter rates taken from refs in column (9); an *H* following the rate indicates that it was derived from a high resolution imager counting rate and multiplied by a factor of 5, the mean IPC/HRI ratio. c, The number of sources expected *a priori* above the flux in column (5b) within an area the size of the SNR in question, calculated from the expression in the text; a *c* following the expected value indicates that the source is centrally located in the remnant, reducing the probability of chance superposition still further. Column (6), unless quoted directly from the ref. cited in column (9) the radio luminosity has been calculated from $L_R = 4\times10^{27} f_{mJy}\times D^2_{kpc}$ appropriate to a ν^{-1} spectrum integrated over a bandwidth of 3×10^9 Hz. Column (7), unless quoted directly from the ref. cited in column (9) the optical luminosity has been calculated from $L_{opt} = 2.4\times10^{-6}\times10^{-(2m_v - D_{kpc})/5)}\times4\pi D^2$, where m_v is the visual magnitude of the source or the upper limit to the X-ray source counterpart, and an extinction of 1 mag kpc^{-1} has been adopted. Column (8) γ-ray limits calculated using $f[E>100\,\text{MeV}] = 1\times10^{-6}$ photons cm^{-2} s$^{-1}\times4\pi D^2 = 10^{34}\times D^2_{kpc}$ erg s^{-1}; in the cases of RCW103 and W28, fluxes of the nearby γ-ray sources CG333+1 and CG006−0, respectively, have been substituted for the Cos *B* galactic plane survey limit of $\sim1\times10^{-6}$ photons cm^{-2} s^{-1}. Column (9), TW, this work (data published here for the first time). PC, Personal communication from J. Kriss.

their beams are pointed in our direction. If neutron stars are born with very short periods ($P\leq5$ ms) and this scaling holds, however, they could be visible at distances up to 100 Mpc. A source such as SS433 would be visible in any remnant in the nearer half of the Galaxy. Although it would be instantly recognizable at high spectral resolution, its colours are not unusual and similar objects could have escaped detection.

X ray: The launch of the Einstein Observatory with its complement of two X-ray imaging detectors[43] provided a new and powerful tool for the detection of weak cosmic X-ray sources. The high spatial resolution not only increased our sensitivity by several orders of magnitude but also allowed us to discern the presence of point-like sources of emission within extended objects (particularly applicable to SNR). Before 1979, X-ray emission had been detected from the vicinity of all three known stellar remnants within SNR. Images taken with Einstein resulted in the detection of nine additional stellar remnant candidates which are listed in Table 1 along with the original three. Of all these sources, only the objects within the Crab Nebula[44], G109.1−1.0 (ref. 23), and MSH15−52 (ref. 45), have been observed to pulse in X rays, although the upper limit on pulsations in the other objects is restrictive only for the Vela pulsar. This lack of detected pulses leaves room for some uncertainty in the emission mechanism involved; possibilities include blackbody radiation from the stellar remnant's surface, nonthermal emission from the magnetosphere (as is the case for the Crab pulsar), or accretion from a companion star (as is likely for the G109.1−1.0 source)[46]. Unfortunately, most of these sources are quite weak (≤0.01 μJy at 1 keV), and it is unlikely that another opportunity to study them will arise for several years to come.

Despite the success of Einstein in tripling the number of compact sources associated with SNR, it is still true that most remnants contain no point source of electromagnetic radiation. The seven historical remnants (Cas A[47], Kepler[48], Tycho[49], Crab[50], SN1006 (ref. 51), 3C58 (ref. 52) and RCW 86 (ref. 53)) have been particularly well studied, and only the two Crab-like remnants (the Crab and 3C58) show point source emission. In addition, the Einstein programme[54] included 65 other galactic remnants as well as at least 31 confirmed SNR in the Magellanic Clouds[55]. Three of the 25 LMC remnants have centrally-condensed brightness distributions with at least two of the three exhibiting power law X-ray spectra[56]. Limited instrumental resolution does not permit us to resolve any point source components in these objects, but their presence may be inferred by analogy with the galactic Crab-like remnants. None of the other remnants in the Large or Small Clouds shows any signs of an associated point source. In the Galaxy, seven additional Crab-like remants were observed: point sources were detected in two of them (Vela[57] and CTB 80 (ref. 52)), two more were detected as X-ray emitters but no point sources were resolved (G21.5−0.9 (ref. 58) and G29.7+0.3 (ref. 59)), and three were too distant to detect at all. Of the 58 shell-type remnants studied (33 of which are within 5 kpc), point sources have been reported within the boundaries of eight. However, these remnants cover a large area of sky and the number of chance coincidences with foreground (stars) or background (active galactic nuclei) interlopers is non-negligible. Table 1 lists the number of sources at or above the observed flux, *S*, to be expected within the boundaries of each remnant calculated from the known surface density *N* of soft X-ray sources[60].

$$N(>S) = 6.5\times10^{-4}\left(\frac{S}{1\ \text{IPC cts s}^{-1}}\right)^{-3/2}\ \text{sources deg}^{-2}$$

where a conversion factor of 2.5×10^{-11} erg cm^{-2} s^{-1} per IPC count has been assumed. (IPC is the imaging proportional counter on the Einstein Observatory, see ref. 43.) Three sources have a rather high probability of being chance superpositions: W28, PKS1209−52 and G127.1+0.5. The latter source is already known to be the serendipitous alignment of a radio galaxy at redshift $z=0.018$ with the remnant's centre[61,62] (however, see ref. 63 for an alternative view of this object); the sources in W28 and PKS1209−52 (Fig. 1) are significantly offset from the remnant centres. Eliminating the three large nearby remnants which span several square degrees each (Monoceres, Lupus and the Cygnus Loop), we calculate the expected number

Table 2 Sample upper limits on compact objects in SNR

Limits for	Cas A			SN1006			SNR at 5 kpc			Ref. and notes			
Units:	L_{crab}	L_{Vela}	L_{SS433}	L_{Crab}	L_{Vela}	L_{SS433}	L_{Crab}	L_{Vela}	L_{SS433}	Cas A	SN1006	SNR at 5 kpc	Notes
Radio	$0.5p$	$1p$	0.5	$0.001p$	$0.002p$	10^{-4}	$0.1p$	$0.3p$	0.05	24*p*, 127	26*p*, 128	*	‡
Optical	0.005	—	10^{-5}	0.001	30	10^{-6}	—	—	—	129	34	—	§
X-ray	0.03	1	0.01	0.002	0.05	0.0005	0.02	2	0.02	47	130	†	‖
γ ray	0.5	2	—	0.05	0.2	—	1	5	—	66	66	66	¶

p, Limit based on pulsed flux only.

* Assuming (1) a limit on pulsed flux at 400 MHz of 10 mJy, consistent with the sensitivity of recent all-sky pulsar surveys, and (2) a limit of 50 mJy at 1,400 MHz for a radio-point source associated with any SNR and as yet unnoticed.

† Assuming a limit of 0.02 IPC count s^{-1} for a point source associated with any SNR surveyed with the Einstein Observatory and as yet unnoticed.

‡ For comparison with the Crab and Vela pulsed 400 MHz fluxes, we have used pulsed flux limits from the cited references and computed the distance-corrected ratio for the pulsed luminosity limits (denoted by *p*); for comparison with SS433 at 1,400 MHz, we have used limits derived from the 1,400 MHz maps cited and computed the distance-corrected ratio for the luminosity limit. Distances of 3.0 kpc and 1.0 kpc were adopted for Cas A and SN1006, respectively.

§ Optical luminosities were calculated as in Table 1 from magnitude limits in the cited references and compared with those quoted in Table 1 (column (7)) for the Crab, Vela and SS433.

‖ X-ray luminosities were calculated as in Table 1 from IPC (or HRI × 5) count rate limits in the cited references and compared with those from column (4) in Table 1.

¶ γ-ray luminosities were calculated as in Table 1 from the Cos B galactic plane survey limit in the quoted reference and compared with those from column (8) in Table 1.

of chance coincidences for the sample of 60 shell-type remnants as a whole is ~4, in reasonable agreement with the three probably spurious associations found.

The results of the X-ray survey, then, are that point sources are associated with at least 4 of the 9 Crab-like remnants and at most 5 of the 63 shell-like remnants (5 of 30 within 5 kpc where the survey is complete to a limiting luminosity of $\sim 2 \times 10^{33}$ erg s^{-1}). The completeness of our sample for the various types of point source emitters is discussed below.

γ-ray: The Crab and Vela pulsars were both detected in the first survey of the sky at energies >50 MeV (refs 64, 65). The most recent catalogue[66] of localized γ-ray sources from the COS B survey contains 25 entries; the Vela pulsar is the brightest source in the sky in the 0.1–3.0 GeV band and, along with the Crab, is one of only four sources identified with objects known in other spectral regimes. Both sources have also occasionally been reported as detections by ground-based Cerenkov air shower experiments operating at photon energies $>10^{11}$ eV (ref. 67). Despite earlier reports to the contrary, these are the only two radio pulsars that have been detected above 2 keV, although several workers[68–70] have suggested that young pulsars form a significant component of the unidentified γ-ray source population.

The whole galactic plane (and, therefore, most galactic SNR) has been covered in the COS B survey to a limiting flux of $\sim 1 \times 10^{-6}$ photons cm^{-2} s^{-1} ($E > 100$ MeV)[66]. For a source with a Crab-like spectrum at a distance of 1 kpc, this corresponds to a γ-ray luminosity of $\sim 10^{34}$ erg s^{-1} (5% that of the Crab pulsar). We could thus detect any Crab-like source within 4–5 kpc. A semi-empirical prediction of the pulsed γ-ray luminosity from a pulsar magnetosphere is given by L_γ (>100 MeV) ~ $2 \times 10^{32} B_{12} P^{-1.7}$, where B_{12} is the pulsar surface magnetic field in units of 10^{12} G and P is the pulsar period[71]. Thus, even sources with periods as long as 0.1 s may be detectable out to 5 kpc. Without *a priori* knowledge of the period, however, the counting statistics of the γ-ray maps are too poor to establish the pulsed nature of such sources.

In fact, roughly a quarter of the known localized γ-ray sources are, within their large error circles (diameter ~1–2°), positionally coincident with catalogued radio SNR[72,73], a result of marginal statistical significance. Lamb and Markert[74] have examined three of the associated remnants at soft X-ray energies with Einstein and find nothing unusual in any of them. Further progress must await new γ-ray observations with improved angular resolution and sensitivity. Observations at these energies offer a unique window on young pulsars as the γ rays may be produced in a fan beam visible to all observers[75], unlike the narrow pencil beams dominant at lower energies. The very different pulse profiles of Vela at radio and γ-ray wavelengths and the pulse–interpulse morphology of both the Crab and the Vela at high energies suggest that the γ-radiation arises from farther out in the magnetosphere than does the radio emission and thus is likely to have a substantially wider beamwidth. It has been argued that 'Geminga', the brightest unidentified γ-ray source which has a soft X-ray counterpart but no detectable radio emission, is an example of such a γ-ray pulsar whose radio beam is pointed away from us (D.J.H., unpublished data).

We consider the first nine objects listed in Table 1 as highly probable associations of SNR and radiating compact stellar remnants. All are detectable in the soft X-ray band, four are radio sources, three have optical counterparts and two are seen at γ-ray energies. We discuss below the source classes and evolutionary state of these objects; here we summarize the current upper limits to similar sources of radiation in other galactic SNR.

In Table 2 we list, for various spectral regimes, the upper limits on point source emission in several SNR: the youngest (~325 yr) known remnant Cas A, the closest (~1 kpc) historical remnant SN1006, and a typical remnant at a distance of 5 kpc. The limits are quoted as fractions of the luminosity of the Crab, a 10^3-yr-old radio pulsar; of Vela, a 10^4-yr-old radio pulsar, and of SS433, a system which is, to date, observationally unique, but which probably contains an accreting neutron star or black hole.

Perusal of Table 2 leads to a number of interesting conclusions. One can now assert that a pulsar similar to that in the Crab Nebula is not present in any of the nearby SNR. This conclusion holds independent of the radio beaming pattern for two reasons: (1) the theoretical and phenomenological arguments presented above which suggest that pulsed γ rays are radiated in a wide beam visible to most observers; and (2) X-ray synchrotron nebulae similar to the Crab Nebula have now been found surrounding all three young radio pulsars (Crab[76], Vela[57], and MSH15−52 (ref. 45)), as well as around several older isolated pulsars[77,78]. The soft X-ray luminosities of these nebulae are comparable with or greater than the pulsed radiation and would be easily visible within any of the shell-type remnants out to 5 kpc.

A pulsar similar to Vela would also not have escaped detection in any of the well-studied remnants, although a distance of 5 kpc is about the limit to which the various surveys would have detected such an object. Thus, we can conclude that an active radio pulsar born in any of the 36 closest SNR must be radically different from our two canonical young pulsars (for example, $P > 0.1$ s at birth, and a very low magnetic field strength).

Table 3 Supernova scenarios for binary stars

Case	Primary mass ($M_\odot$)	Companion mass ($M_\odot$)	Resulting system*	Observational consequence
I	>10	>10	(a) SN+>10	Visible: wind-powered binary
			(b) SN+>10	Visible: wind-powered binary
II	>10	4–10	(a) SN+>10	Visible: wind-powered binary
			(b) SN+4–10	Visible: wind-powered binary
III	>10	<4	(a) SN+4–10	Visible: wind-powered binary
			(b) SN+>4	Disrupts
IV	4–10	4–10	(a) SN+>10	Visible: wind-powered binary
			(b) SN+4–10	Visible: wind-powered binary
V	4–10	<4	(a) SN+4–10	Visible: wind-powered binary
			(b) SN+<4	Disrupts
VI	<4	<4	(a, b) WD+<4 → SN+evolved star	Visible: Roch-lobe overflow binary

* Case (a) represents conservative evolution wherein most of the material lost by the primary is transferred to the companion whereas in case (b) most of the mass lost by the primary leaves the system. In more realistic treatments of nonconservative mass transfer, the companion star often still accretes sufficient material to reverse the mass ratio of the system and prevent disruption of the binary in the SN event. Thus, low-mass, Roche-overflow driven binary such as Her X-1 can result from a progenitor system with initial masses greater than in case VI (ref. 131).

An SS433-type object with $L \geq 10^{-2}$ of the X-ray or radio luminosity of the prototype is also unlikely to have escaped detection in any SNR out to at least 5 kpc. Likewise, a less spectacular accretion-powered X-ray source such as that in G109.1−1.0 would also be easily visible to that distance in the X-ray band. In fact, for any compact remnant that remains in a binary system, our X-ray survey luminosity limit of $L_X \leq 2\times10^{33}$ erg s^{-1} allows us to limit the mass loss rate $\dot{M}$ of its companion star as:

$$\dot{M} < 3\times10^{-11}\, M_\odot\, \mathrm{yr}^{-1}\left(\frac{\zeta}{0.1}\right)^{-1}\left(\frac{M_x}{1.4M_\theta}\right)^{-2}\left(\frac{a}{10R_0}\right)^{2} \times\left(\frac{v_w}{500\ \mathrm{km\ s^{-1}}}\right)^{4}\left(\frac{D}{1\ \mathrm{kpc}}\right)^{2}$$

where ζ is the fraction of the rest mass energy of accreted matter that is converted to radiation, v_x is the mass of the collapsed object, a is the orbit semimajor axis and v_w is the velocity of the stellar wind[79]. Thus, the main-sequence companions of any such putative close binaries are rather weakly limited by the X-ray survey to those with mass loss rates typical of spectral types later than A5 ($m_v > 7$) for 1 kpc and later than B5 for 5 kpc ($m_v > 12.5$); optical searches for binaries with unseen companions would clearly be a more sensitive method of searching for such objects. Our limits for an isolated black hole or neutron star accreting interstellar material are similarly weak: for spherical accretion[80],

$$L_X = 7\times10^{31}\left(\frac{\zeta}{0.1}\right)\left(\frac{\rho}{1\ \mathrm{cm^{-3}}}\right)\left(\frac{M}{1.4\ M_\theta}\right) \times\left(\frac{v}{10\ \mathrm{km\ s^{-1}}}\right)^{-3}\ \mathrm{erg\ s^{-1}}$$

where v is the object's velocity with respect to the infalling matter. Thus, an isolated 10 $M_\odot$ black hole might be detectable to 5 kpc, but a neutron star (likely to be moving at a considerably higher velocity) would probably not be observed in even the nearest remnants.

Source classes

We have divided the observed compact objects into three groups: accretion-powered binary sources (SS433 and G109.1−1.0), active pulsars as defined by the presence of pulsed radiation and/or a surrounding synchrotron nebula (Crab, Vela X, MSH15−52 3C58, CTB80), and isolated, noninteracting objects (RCW103, G27.4+0.0, PKS1209−51/52, W28, and G127.1+0.5). Within each category, the sources are listed in Table 1 in order of decreasing likelihood that they are true members of their class; amongst the final group, we feel it is rather unlikely that any of the last three objects are actually associated with the surrounding remnant. Although the survey of galactic SNR in X rays (the preferred band for detection) is incomplete, it is unlikely that any significant new data will be obtained in the near future, so it seems appropriate to consider at this time the statistical implications of the current list of objects.

Binary sources: Although some doubts have been expressed that the nebulae surrounding SS433 and G109.1−0.1 are, in fact, remnants of the SN which created the compact objects we see, the central location and low probability of chance occurrence for these sources is highly suggestive of real association. The sources are radically different from each other in many of their observed properties, but both probably consist of a near main-sequence star and an accreting collapsed companion. While their radio and optical luminosities are very different, both are strong X-ray sources which would have been easily detected (a factor of $>10^2$ above threshold) in any of the 63 shell-type galactic SNR surveyed with the Einstein satellite; SS433 was originally detected by Uhuru and a similar source would have been seen by other all sky surveys out to $\geq$10 kpc, a distance encompassing ~100 of the known galactic SNR. Thus, we can conclude that $\leq$3% of SN leave behind systems which, on a time scale less than the lifetime of the expanding remnant ($3–10\times10^4$ yr), become accreting X-ray binaries.

As most stars occur in binary systems, these results hold important implications for binary disruption probabilities in the SN event. In Table 3 we summarize the possible pre- and post-SN configurations of a binary system. The stars are divided into three mass regions: $M > 10M_\odot$ (earlier than B3) for which there is general agreement that the star will end its life as a SN; $4\,M_\odot < M < 10M_\odot$ (B9–B3), a range in which post-main-sequence evolution is particularly uncertain, but within which it is believed that at least some of the stars explode and leave collapsed remnants; and $M < 4\,M_\odot$ (later than A0), for which there is general agreement that most stars evolve to white dwarfs. Table 3 gives the zero-age main sequence mass range of the primary (more massive) and secondary binary components and lists the post-SN configuration for each of two possible scenarios: first, mass conservation, in which most of the material lost by the primary is transferred to the secondary; and second, extensive mass loss, in which the separation of the stars is sufficiently large that only a small fraction of the primary's mass is accreted by its companion. The final column of Table 3 presents the expected observational consequences of the various scenarios. If the SN does not leave behind a collapsed object (a neutron star or black hole), clearly, no accretion-powered source will be seen. If a collapsed object is produced, we have several possibilities:

(1) The companion is a massive star ($>4\,M_\odot$) and, as such, has a stellar wind strong enough to produce an observable X-ray source (cases Ia and b, IIa and b, IIIa, IVa and b, and Va).

(2) The companion has $M < 4M_\odot$ (cases IIIb and Vb). However, in this case, as greater than one-half the mass of the system is lost in the explosion, the system will essentially always disrupt.
(3) The SN is produced by accretion onto a white dwarf of matter lost from the companion through Roche-lobe overflow. If the SN leaves a collapsed companion, a visible accretion-powered source will result from the continuing mass loss of the post-main-sequence companion (case VI).

The binary source in G109.1−1.0 almost certainly consists of a neutron star fed by Roche-lobe overflow from a low mass ($<1\ M_\odot$) companion[81] and thus probably resulted from case VI. We note that this would require a white dwarf SN to leave behind a neutron star remnant, in conflict with some current theories of Type I explosions[82]. The companion of the collapsed object in the SS433 system is uncertain although the weight of the evidence[83] seems to favour a low mass companion, again suggesting a case VI scenario.

The lack of visible high-mass accretion sources in SNR suggests that nearly all such systems must disrupt when the first star undergoes a SN. The rarity of binary pulsars (~1%) supports this conclusion. There are ~25 massive X-ray binary systems in the Galaxy[84] which, just after the first SN, should have been embedded in a SNR. That at most one such system is currently seen in this early phase (lasting $\lesssim 5\times10^4$ yr) yields a crude estimate of the lifetime L of massive binaries: $\frac{1}{25} \geqslant (5\times10^4)/L$, implying $L \geqslant 10^6$ yr, commensurate with the lifetimes of the massive companions powering these wind-driven sources. The rarity of low mass X-ray binaries within SNR shells suggests that disruption in the SN event is similarly likely or, that collapsed objects are rarely formed in such systems (that is, exploding white dwarfs usually leave no remnant).

An interesting similarity between SS433 and G109.1−1.0 is that both show evidence for the ejection of relativistic beams[85,86]. Of the ~100 galactic X-ray binaries not found in SNR, however, only Sco X-1 evidences this phenomenon[87,88] although most sources have not been studied at comparable sensitivity in either the radio or X-ray regimes. If this lack of detectable beamed emission is not simply an observational selection effect, it suggests that the phenomenon is a relatively short-lived (few per cent) phase of an X-ray binary's evolution. Similar beams are thought to emerge from isolated young neutron stars where they give rise to the synchrotron nebulae discussed below.

Pulsar-driven nebulae: The largest group of detected compact objects associated with SNR are those embedded in centrally peaked radio and X-ray synchrotron nebulae. The Crab Nebula with its central pulsar is the prototype of this class and, in this one case, the total energy budget of the surrounding nebula is successfully accounted for by the rotational kinetic energy loss rate of the neutron star. In other cases, this link has yet to be directly established. Only two of the remaining four objects in this class, Vela and MSH15−52, are radio pulsars for which the spin-down energies have been measured and, in the latter case, the nebula has only been detected at X-ray wavelengths. Another feature which distinguishes the Crab Nebula (and 3C58) from the other members of this class is the lack of a shell of radio and X-ray emission from an expanding SN shock front surrounding the synchrotron nebula. Such shells are present in MSH15−52, Vela and CTB80, as well as in two other remnants in which the presence of a young pulsar may be inferred from the existence of a bright radio and/or X-ray synchrotron nebula: G29.7−0.3 (ref. 59) and G326.3−1.8 (ref. 89). Other cases of pure Crab-like remants also exist: G21.5−0.9 (ref. 58), and G74.9+1.2 (ref. 90). No systematic observational differences are apparent between the synchrotron nebulae found in shell-type remnants and those in which a shell is absent. Similarly, the shell components of the composite remnants do not appear strikingly different from most SNR in which only the shell is seen. All this suggests that, at least on time scales of $\sim 3\times10^2$–3×10^4 yr, the two components evolve independently; that is, the presence or absence of an active neutron star (pulsar) has little effect on the SNR created by the expanding SN blast wave, and the presence or absence of a shell does not affect the formation of a synchrotron nebula.

The implications of such a statement are significant. Consider those remnants which exhibit shells. Only ~10% (6 of ~50 well-studied galactic remnants) contain a Crab-like nebula. Because all young radio pulsars studied by Einstein produce such a nebula (Crab[50], Vela[57], MSH15−52 (ref. 45), PSR1055−52 (ref. 77) and PSR0355+54 (ref. 78)) we must conclude that no active radio pulsar exists in the other 90% of SNR. Thus, either only ~10% of SN which make shell-type remnants produce neutron stars, or most neutron stars turn on as pulsars after the shell has dissipated[20,21] (~30,000 yr). The former possibility suggests that the convergence of the SNR production rate and the pulsar birthrate is fortuitous, and holds important implications for SN theory. The alternative view of the late pulsar turn-on yields potential information on the evolution of neutron star magnetic fields and the pulsar radiation mechanism. We note that, for a pulsar birthrate of 1 per 40 yr, a significant fraction (0.4–1.0) of SN must leave a neutron star, arguing in favour of this latter alternative.

If we adopt the premise from our observation of composite remnants that only ~10% of neutron stars turn on as radio pulsars within $\sim 10^4$ yr of their formation, we are left to speculate on the inactive counterparts to the shell-less Crab-like remnants—those SN which leave neither shells nor active pulsars. As there are roughly equal numbers of Crab-like remnants with and without shells, the number of inactive neutron stars with and without shells should also be similar; that is, there should be 10 SN which leave no visible remnants for each shell-less Crab-like remnant produced. We are thus led to the rather startling conclusion that nearly half of the SN in the Galaxy leave no promptly observable remnant. We find support for this conclusion in the fact that derived radio remnant formation rates in the Galaxy (1 per 40 (ref. 91) to 80 (ref. 92) yr) have consistently been a factor of ~2 below the estimates of the galactic SN rate (1 per 20 to 40 yr)[18]. The first recorded extragalactic SN, SN1885 (S Aud) in M31 is noteworthy in this regard; after 100 yr it is still undetectable as a radio source at a limit only a few per cent of Cas A.

Isolated objects: Sources 8 to 12 in Table 1 are the remaining point sources which have been found within SNR boundaries. The evidence for association is simply positional coincidence. We have argued above that sources 10, 11, and 12, located in remnants of large angular size, are most probably chance superpositions of foreground or background objects. The object at the centre of G127.1+0.5 has already been identified as a radio galaxy[61,62] and work is under way to identify optical and/or radio counterparts to the sources in PKS1209−52 (ref. 93) and W28. For the remaining two sources, the probability of chance superposition is small (Table 1, column (5c)) and decreases even further when their precise central location within the remnants is considered. The remnant G27.4+0.0 is very distant, and little information is yet available on the X-ray properties of the point source at its centre (J. Kriss, personal communication). Either nonthermal pulsed emission from a young pulsar or emission from a synchrotron nebula (as in other composite remnants) are viable possibilities; its X-ray luminosity (although highly uncertain because of the large correction for interstellar absorption in such a distant source) is not dissimilar from the Crab pulsed luminosity. Black-body radiation from a neutron star surface is an unlikely explanation as the required temperature is $\geqslant 9\times10^6$ K, far above that of other neutron stars and the predictions of theoretical cooling calculations. For RCW103, Tuohy *et al.*[94] have recently argued that surface black-body radiation from a neutron star is the most likely explanation for the X rays observed from the central point source. The lack of an optical counterpart brighter than $m_v = 22$ virtually rules out an accretion-powered source, although owing to the steep period dependence of pulsar optical luminosities, a nonthermal magnetospheric emission mechanism is not excluded. The derived surface temperature under the assumption of black-body

emission is $\sim 2 \times 10^6$ K, consistent with standard neutron star cooling calculations[95].

Conclusions

A total of nine point sources of radiation have now been established as the compact remnants of recent SN explosions. They represent a variety of the known observational manifestations of neutron stars and black holes: accretion-powered binary sources, isolated radio pulsars with their associated synchrotron nebulae, and surface thermal emission. The statistics of the observed objects along with the equally important stringent upper limits to similar radiation from sources in most known SNR provide important information on the galactic SN rate and the origin and evolution of neutron stars. Future study of these sources will contribute substantially to our knowledge of these exotic endpoints of stellar evolution.

We acknowledge the support of NASA under contracts NAS 8-30753 and NAG 8-452 and the US Air Force under grant AFOSR 82-0015. This is Columbia Astrophysics contribution no. 245.

1. Baade, W. & Zwicky, F. *Proc. natn Acad. Sci. U.S.A.* **20,** 254–258 (1934).
2. Baade, W. & Zwicky, F. *Proc. natn Acad. Sci. U.S.A.* **20,** 259–263 (1934).
3. Baade, W. *Astrophys. J.* **96,** 188–198 (1942).
4. Minkowski, R. *Astrophys. J.* **96,** 199–207 (1942).
5. Hewish, A., Bell, S. J., Pilkington, J. D. H., Scott, P. F. & Collins, R. A. *Nature* **217,** 709–711 (1968).
6. Tananbaum, H. *et al. Astrophys. J. Lett.* **174,** L143 (1972).
7. Giacconi, R. *et al. Astrophys. J. Lett.* **148,** L129 (1967).
8. Leventhal, M. & McCall, S. L. *Nature* **255,** 690 (1975).
9. Nomoto, K. in *Type I Supernovae* (ed. Wheeler, J. C.) 164–181 (University of Texas, Austin, 1980).
10. Brown, G. E. *Supernovae, A Survey of Current Research* (eds Rees, M. J. & Stoneham, R. J. 13–33 (Reidel, Dordrecht, 1982).
11. Arnett, W. D. *Astrophys. Space Sci.* **5,** 180 (1969).
12. Woosley, S. E. & Weaver, T. A. *Supernovae, A Survey of Current Research* (eds Rees, M. J. & Stoneham, R. J.) 79–122 (Reidel, Dordrecht, 1982).
13. Staelin, D. H. & Reifenstein, E. C. *Science* **162,** 1481 (1968).
14. Comella, J. M., Craft, H. D., Lovelace, R. V. E., Sutton, J. M. & Tyler, G. C. *Nature* **221,** 453–454 (1969).
15. Cocke, W. J., Disney, M. J. & Taylor, D. J. *Nature* **221,** 525–527 (1969).
16. Large, M. I., Vaughan, A. E. & Mills, B. Y. *Nature* **220,** 340 (1968).
17. Lyne, A. G. *Supernovae, A Survey of Current Research* (eds Rees, M. J. & Stoneham, R. J.) 405–417 (Reidel, Dordrecht, 1982).
18. Tamman, G. *Supernovae, A Survey of Current Research* (eds Rees, M. J. & Stoneham, R. J.) 371–403 (Reidel, Dordrecht, 1982).
19. Caswell, J. L. & Lerche, I. *Aust. J. Phys.* **32,** 79–82 (1979).
20. Radhakrishnan, V. *Contemp. Phys.* **23,** 207–231 (1982).
21. Blandford, R. D., Applegate, J. H. & Hernquist, L. Preprint (Cal Tech).
22. Margon, B. *et al. Astrophys. J. Lett.* **230,** L41–L45 (1979).
23. Fahlman, G. G. & Gregory, P. C. *Nature* **293,** 202–203 (1981).
24. Manchester, R. N. & Taylor, J. H. *Pulsars* (Freeman, San Francisco, 1977).
25. Seiradakis, J. H. & Graham, D. A. *Astr. Astrophys.* **85a,** 353–355 (1980).
26. Manchester, R. N., Tuohy, I. R. & D'Amico, N. *Proc. IAU Symp.* No. 101 (in the press).
27. Manchester, R. N., Tuohy, I. R. & D'Amico, N. *Astrophys. J. Lett.* **262,** L31–L33 (1983).
28. Keen, N. J., Wilson, W. E., Haslam, C. G. T., Graham, D. A. & Thomasson, P. *Astr. Astrophys.* **28,** 197–201 (1973).
29. Becker, R. H. & Kundu, M. R. *Nature phys. Sci.* **244,** 138–139 (1973).
30. Margon, B., Downes, R. A. & Gunn, J. E. *Astrophys. J. Lett.* **249,** L1–L4 (1981).
31. Ryle, M., Caswell, J. L., Hine, G. & Shakeshaft, J. *Nature* **276,** 571–573 (1978).
32. van Gorkom, J. H., Goss, W. M., Seaquist, E. R. & Gilmore, W. S. *Mon. Not. R. astr. Soc.* **198,** 757–765 (1982).
33. Hind, J. R. & Plummer, W. E. *Mon. Not. R. astr. Soc.* **34,** 168 (1874).
34. Fesen, R. A., Kirshner, R. P. & Winkler, P. F. *Astrophys. J.* **234,** 174–181 (1979).
35. Horowitz, P., Papaliolios, C. & Carleton, N. P. *Astrophys. J. Lett.* **163,** L5–L10 (1971).
36. Papaliolios, C. & Horowitz, P. *Astrophys. J.* **183,** 233–235 (1973).
37. Pennypacker, C. R. *Astrophys. J.* **244,** 286–289 (1981).
38. Schweizer, F. & Middleditch, J. *Astrophys. J.* **241,** 1039–1044 (1980).
39. Simon, K. P., Hunger, K. & Kudritzki, R. P. *Astr. Astrophys.* **98,** 211–212 (1981).
40. Wu. C. C., Leventhal, M., Saragin, C. L. & Gull, T. R. *Astrophys. J. Lett.* **269,** L5–L9 (1983).
41. Shklovskii, I. S. *Astrophys. J. Lett.* **159,** L77–L80 (1970).
42. Pacini, F. *Astrophys. J. Lett.* **163,** L17–L19 (1971).
43. Giacconi, R. *et al. Astrophys. J. Lett.* **230,** 540–545 (1979).
44. Fritz, G., Henry, R. C., Meelsins, J. F., Chubb, T. A. & Freedman, H. *Science* **164,** 709 (1969).
45. Seward, F. D. & Harnden, F. R. *Astrophys. J. Lett.* **256,** L45–L47 (1982).
46. Fahlman, G. G., Hickson, P., Richer, H. B., Gregory, P. C. & Middleditch, J. *Astrophys. J. Lett.* **261,** L1–L5 (1982).
47. Murray, S. S., Fabbiano, G., Fabian, A. C., Epstein, A. & Giacconi, R. *Astrophys. J. Lett.* **234,** L69–L72 (1979).
48. White, R. L. & Long, K. S. *Astrophys. J.* **264,** 196–205 (1983).
49. Seward, F., Gorenstein, P. & Tucker, N. *Astrophys. J.* **266,** 287–297 (1983).
50. Harnden, F. R. *Proc. IAU Symp.* No. 101 (in the press).
51. Pye, J. P. *et al. Mon. Not. R. astr. Soc.* **194,** 569–582 (1981).
52. Becker, R. H., Helfand, D. J. & Szymkowiak, A. E. *Astrophys. J.* **255,** 557–563 (1982).
53. Pisarski, R. L., Helfand, D. J. & Kahn, S. M. *Astrophys. J.* (in the press).
54. Seward, F. D. & Williams, D. Publ. HEA 81-002 (Center for Astrophysics, 1981).
55. Mathewson, D. S. *et al. Astrophys. J. Suppl.* **51,** 345–355 (1983).
56. Clark, D. H. *et al. Astrophys. J.* **255,** 440–446 (1982).
57. Harnden, F. J. *et al. Bull. Am. astr. Soc.* **11,** 424 (1979).
58. Becker, R. H. & Szymkowiak, A. E. *Astrophys. J. Lett.* **248,** L23–L26 (1981).
59. Becker, R. H., Helfand, D. J. & Szymkowiak, A. E. *Astrophys. J. Lett.* **268,** L93–L97 (1983).
60. Giacconi, R. *et al. Astrophys. J. Lett.* **234,** L1–L7 (1979).
61. Kirshner, R. P. & Chevalier, R. A. *Nature* **276,** 480 (1978).
62. Spinrad, H., Stauffer, J. & Harlan, E. *Publ. astr. Soc. Pacif.* **91,** 619–623 (1979).
63. Geldzahler, B. J. & Shaffer, D. B. *Astrophys. J. Lett.* **260,** L69–L73 (1982).
64. Hillier, R. R., Jackson, W. R., Murray, A., Redfern, R. M. & Sale, R. G. *Astrophys. J. Lett.* **162,** L177 (1970).
65. Thompson, D. J., Fichtel, C. E., Knitten, D. A. & Ogelman, H. B. *Astrophys. J. Lett.* **200,** L79–L82 (1975).
66. Swanenburg, B. N. *et al. Astrophys. J. Lett.* **243,** L69–L73 (1981).
67. Bhat, P. N. *et al. Pulsars* (eds Sieber, W. & Wielebinski, W. R.) 251–254 (Reidel, Dordrecht, 1981).
68. Higdon, J. C. & Lingenfelter, R. E. *Astrophys. J. Lett.* **208,** L107–L110 (1976).
69. Strong, A. W., Wolfendale, A. W. & Dahanayake, C. *Mon. Not. R. astr. Soc.* **179,** 69 (1977).
70. Harding, A. K. *Astrophys. J.* **247,** 639–649 (1981).
71. Harding, A. K. *Astrophys. J.* **245,** 267–273 (1981).
72. Lamb, R. C. *Nature* **274,** 720 (1978).
73. Montmerle, *Astrophys. J.* **231,** 95–110 (1979).
74. Lamb, R. C. & Markert, T. H. *Astrophys.* **244,** 94–101 (1981).
75. Cheng, A., Ruderman, M. & Sutherland, P. *Astrophys. J.* **203,** 209–212 (1976).
76. Bowyer, S., Byram, E., Chubb, T. & Friedmann, H. *Science* **146,** 912 (1964).
77. Cheng, A. & Helfand, D. J. *Astrophys. J.* **271,** 271–282 (1983).
78. Helfand, D. J. *Proc. IAU Symp.* No. 101 (in the press).
79. Helfand, D. J. *Publ. astr. Soc. Pacif.* **92,** 691–695 (1980).
80. Bondi, H. & Hayle, F. *Mon. Not. R. astr. Soc.* **104,** 273–282 (1944).
81. Gregory, P. C., Braun, R. & Fahlman, G. G. *Publ. astr. Soc. Pacif.* **92,** 691–695 (1980).
82. Wheeler, J. C. in *Supernovae, a Survey of Current Research* (eds Rees, M. J. & Stoneham, R. J.) 167–203 (Reidel, Dordrecht, 1982).
83. Crampton, D., Cowley, A. P. & Hutchings, J. B. *Astrophys. J. Lett.* **235,** L131–135 (1980).
84. Bradt, H. V. D. & McClintock, J. E. *Ann. Rev. Astr. Astrophys.* **21,** 13–66 (1983).
85. Seward, F. D., Grindlay, J. E., Seaquist, E. R. & Gilmore, W. S. *Nature* **287,** 806–808 (1980).
86. Gregory, P. C. & Fahlman, G. G. *Vistas Astr.* **25,** 119–125 (1981).
87. Hjellming, R. M. & Wade, C. M. *Astrophys. J. Lett.* **164,** L1–L7 (1971).
88. Geldzahler, B. J. *Astrophys. J. Lett.* **264,** L49–L53 (1983).
89. Weiler, K. W. & Shaver, P. A. *Astr. Astrophys.* **70,** 389 (1978).
90. Wilson, A. S. *Astrophys. J. Lett.* **241,** L19–L22 (1980).
91. Milne, D. K. *Aust. J. Phys.* **32,** 83–92 (1979).
92. Caswell, J. L. & Lerche, I. *Mon. Not. R. astr. Soc.* **187,** 201–216 (1979).
93. Tuohy, I. & Long, K. S. preprint (Mt Stromlo Observatory).
94. Tuohy, I. R., Garmire, G. P., Manchester, R. N. & Dopita, M. A. *Astrophys.* **268,** 778–781 (1983).
95. Nomoto, K. & Tsuruta, S. *Astrophys. J. Lett.* **250,** L19–L23 (1981).
96. Geldzahler, B. J., Pauls, T. & Salter, C. J. *Astr. Astrophys.* **84,** 237–244 (1980).
97. Hjellming, R. M. & Johnston, K. J. *Nature* **290,** 100–107 (1981).
98. Seaquist, E. R., Gilmore, W. S., Johnston, K. J. & Grindlay, J. E. *Astrophys. J.* **260,** 220–232 (1982).
99. Clark, D. H. & Murdin, P. *Nature* **281,** 183–185 (1978).
100. Gregory, P. C. & Falman, G. G. *Nature* **287,** 805–806 (1980).
101. Hughes, V. A., Harten, R. H. & van den Bergh, S. *Astrophys. J. Lett.* **246,** L127–L131 (1980).
102. McNamara, B. J. *Publ. astr. Soc. Pacif.* **83,** 491–492 (1971).
103. Trimble, V. *Astr. J.* **73,** 535–547 (1968).
104. Wilson, A. S. *Mon. Not. R. astr. Soc.* **157,** 229–253 (1972).
105. Manchester, R. N. & Taylor, J. H. *Astr. J.* **86,** 1953–1973 (1981).
106. Manchester, R. N. in *Gravitational Radiation, Collapsed Objects and Exact Solutions* (ed. Edwards, C.) 34 (Springer, Berlin).
107. Buccheri, R. *Proc. IAU Symp.* No. 95, 241 (1981).
108. Manchester, R. N. *et al. Mon. Not. R. astr. Soc.* **184,** 159–170 (1978).
109. Kristian, J. *Astrophys. J. Lett.* **162,** L103–104 (1970).
110. Weiler, K. W. & Panagia, N. *Astr. Astrophys.* **90,** 269–282 (1980).
111. Seward, F. D., Harnden, F. R., Murden, P. & Clark, D. (in preparation).
112. Green, D. A. & Gull, S. F. *Nature* **299,** 606–607 (1982).
113. Wilson, A. S. & Weiler, K. W. *Astr. Astrophys.* **49,** 357–374 (1976).
114. Becker, R. H. & Helfand, D. J. Preprint (VPI+SU).
115. Angerhofer, P. C., Wilson. A. S. & Mould, J. R. *Astrophys. J.* **236,** 143–152 (1980).
116. Margon, B. Preprint (University of Washington).
117. Caswell, J. L. *Mon. Not. R. astr. Soc.* **136,** 11–18 (1967).
118. Goss, W. M. & Shaver, P. A. *Aust. J. Phys. Astrophys. Suppl.* **14,** 1–131 (1970).
119. Tuohy, I. R. & Garmire, G. P. *Astrophys. J. Lett.* **239,** L107–L110 (1980).
120. Angerhofer, P. E., Becker, R. H. & Kundu, M. R. *Astr. Astrophys.* **55,** 11–18 (1977).
121. Milne, D. K. & Dickel, J. R. *Aust. J. Phys.* **28.** 209–230 (1975).
122. Andrews, M. D., Basart, J. P., Lamb, R. C. & Becker, R. H. *Astrophys. J.* **266,** 684–688 (1983).
123. Altenhoff, W. J., Downes, D., Pauls, T. & Schraml, J. *Astr. Astrophys. Suppl.* **35,** 23–54 (1978).
124. Salter, C. J., Pauls, T. & Haslam, C. G. T. *Astr. Astrophys.* **66,** 77–80 (1978).
125. Dickel, J. R., Murray, S. S., Morris, J. & Wells, D. C. *Astrophys. J.* 145–150 (1982).
126. Reynolds, S. & Chevalier, R. Preprint (NRAO).
127. Kamper, K. & van den Bergh, S. *Astrophys. J. Suppl.* **32,** 351–366 (1976).
128. Winkler, P. F. & Seward, F. D. Preprint (Middlebury College).
129. van den Heuvel, E. P. J. *Fundamental Problems in Stellar Evolution* (eds Sugimoto, D., Lamb, D. Q. & Schramm, D. N.) 155–174 (Reidel, Dordrecht, 1981).

Cloud structure on the dark side of Venus

David A. Allen & John W. Crawford*

Anglo-Australian Observatory, PO Box 296, Epping, New South Wales 2121, Australia

Observations of the dark side of the planet Venus at infrared wavelengths between 1.5 and 2.5 μm have shown it to be anomalously bright in portions of this waveband, and to exhibit structured cloud patterns whose rotation period is longer than that of any other clouds.

WHILE it is known[1-3] that the unilluminated hemisphere of the planet Venus radiates at wavelengths near 10 μm with a temperature of 230–240 K, there are no reports of radiation at wavelengths shorter than 3 μm, where thermal radiation should be very weak. We now, however, report observations showing that the unilluminated hemisphere is anomalously bright in this region of the infrared spectrum. We have been able to estimate the rotation period of the features responsible, but cannot offer a definitive explanation of the phenomenon.

We first discovered detectable dark-side radiation at dichotomy (illuminated phase 0.5) in June 1983 using the 3.9 m Anglo-Australian Telescope and IR photometer–spectrometer in its imaging mode. As the planet entered its crescent phase, the structure was easily studied and the observations described here were made on 24 and 25 July (phase 0.23) and 18–23 September (phase 0.19). These observations cover the unilluminated hemisphere near both local sunrise and local sunset, but there are no obvious differences between the cloud patterns in the two cases (Fig. 1). No digital enhancement of the images has been performed so that the contrast is exactly as observed, although our choice of observing wavelength reduced the intensity of the illuminated portion of the disk relative to that of the dark side. At this wavelength, the range of intensity observed on the dark side is 0.03% to 1.0% of the peak intensity.

Observational procedures

The IR photometer–spectrometer is a versatile Cassegrain instrument, and for these observations we used it in its spectroscopy and imaging modes. The InSb detector (0.25 mm diameter) is cooled to 50 K and radiation enters via one of a selection of apertures in the focal plane of the telescope, a filter and a doublet Fabry lens of ZnSe. Samples of the incident data flux are taken over an integration time which may be any integral number of milliseconds from 1 to 10,000.

The filter wheel for the spectroscopic observations contains a circular variable filter with a transmission wavelength which is a function of rotational position. For focal plane apertures less than 3.5 arc s, the bandpass is 0.010 of the wavelength. The spectrum reproduced in Fig. 2 was taken in three portions, corresponding to the three wavebands of atmospheric transmission. In each waveband, the filter was stepped at the end of each integration time, typically 5 seconds being required for a single sweep through the spectrum. The telescope was then driven to a nearby patch of sky (for an account of the d.c. drive electronics, see ref. 4), the spectral sweep repeated and the sky spectrum subtracted from that on the planet. (Since the observations were made at night the sky signal at wavelengths below 2.5 μm was negligible.) The procedure continued for a few minutes in order to derive high signal-to-noise in the faintest portions. The spectra and telluric absorption features were calibrated by observations of a nearby G dwarf star for which accurate photometry is available. The absolute calibration is reliable to 10%, and the detailed shape of the resulting spectrum to significantly greater precision.

When used in imaging mode, the filter remains fixed at a chosen wavelength, the telescope is stepped in a raster pattern in synchrony with the integrations as it moves southwards. Data collection is suspended on its return journey, and irregularities of the telescope's motion at the northern turnaround slightly distort the extreme top of the image, seen here as upward streaking of the northern cusp, an effect enhanced by the high degree of saturation in the illuminated crescent. Successful use of this technique on other planets has been demonstrated[5].

Each image of Venus required about 3.5 min, and usually six consecutive images were combined to produce the final versions, in which irregularities due to seeing are smoothed out. The features of each final image could always be seen on each of its components. All the images were made in daylight, hence the choice of 2.41 μm to maximize contrast against the sky, but images made on 25 July at 1.7 μm and with a broad bandpass of 2.0–2.4 μm were indistinguishable.

The initial pixel size was 1.0 arc s and the focal plane aperture was 2.0 arc s in diameter. Using UK Science and Engineering Research Council's Starlink software, the images were linearly interpolated to 0.5 arc s pixels, and after coaddition, very light gaussian smoothing was applied. The effective resolution was 2.0–2.5 arc s unless poor seeing interfered.

Spectrum of the radiation

Two issues are raised by this discovery. What is the nature of the radiation seen on the dark side? And what causes the intensity modulation?

Figure 2 shows the spectrum of part of the dark side of Venus between 1.5 and 2.5 μm. The spectrum is contaminated to an extent which cannot be accurately determined by radiation from the sunlit portion scattered along the diffraction spikes of the telescope, but in the portions of Fig. 2 where data are shown as a broken line, we believe the signal arises entirely from this cause because of the exact spectral similarity and the credible value of the intensity. An image made at 2.06 μm, in the absorption band of CO_2, shows no structure on the dark side except a gradual fall-off from the sunlit portion. We therefore believe that the dark side radiation is detectable only in the ranges 1.69 to 1.77 μm and 2.18 to 2.50 μm.

We offer the following explanation of the spectral distribution. In the range 2.3–2.4 μm, the prominent double absorption feature is unmistakably that of the first overtone band of CO, at a mixing ratio of 45 p.p.m. convincingly shown to be present by Connes *et al.*[6] who required 600 times greater resolution than ours but who observed the sunlit side of Venus. It is clear that we are viewing radiation that has traversed a much greater path length through the atmosphere, in which circumstances we expect bands of CO_2, the most abundant gas, to be highly saturated. In her important study of the atmosphere of Venus, Young[7] listed all CO_2 bands seen in the data of Connes *et al.* When each one of these is completely opaque, radiation would escape only in the precise wavebands we see, but the peak at 1.74 μm must be further reduced by HCl bands[8]. Apart from very slight changes in the CO band strengths, we find the spectrum of the dark side to be the same in regions differing by a factor of three in intensity.

* Present address: Department of Astronomy, Glasgow University, Glasgow G12 8QQ, UK.

0028-0836/84/030222—03$01.00

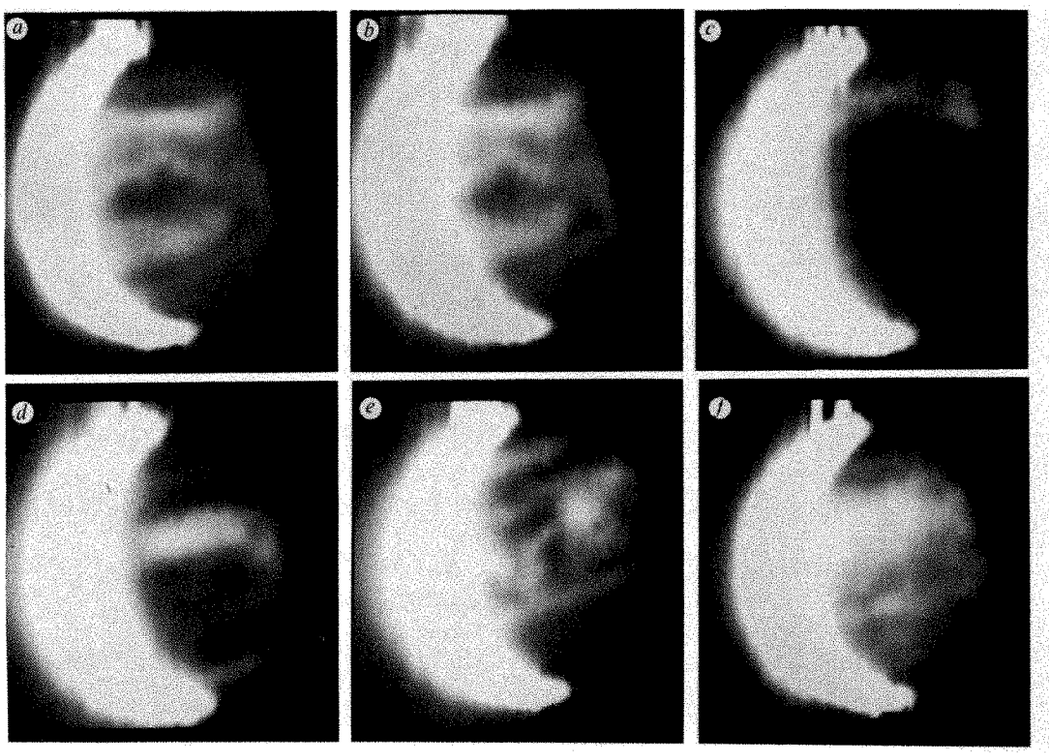


Fig. 1 Images of Venus at a wavelength of 2.41 μm made on several dates in September 1983. The bandpass was 0.025 μm and the spatial resolution better than 2.5 arc s. North is at top and east to the left on all images. Dates and UT times are September 1983 at 1720:54 (*a*), 1723:22 (*b*), 1921:30 (*c*), 2101:13 (*d*), 2120:53 (*e*), and 2220:16 (*f*). Several dark spots in image *b* were caused by electronic faults affecting the raw data.

Using the convenient data of Kuiper[9] we estimate the column absorption of CO to be equivalent to a 10-m path at atmospheric pressure. When allowance is made for the abundance of CO relative to the other components of the atmosphere of Venus, the observed path must be of the order of 200 km atm. This large value may arise in either a vertical or a dominantly horizontal path. The brightness temperature in the peak of the 1.74 μm region is 450 K, a value found only near the base of the major cloud decks[10], which would be consistent with a vertical path of 200 km atm provided that the absorption optical thickness of the clouds permits the passage of radiation. The clouds are known to have very large scattering optical thicknesses at all optical and infrared wavelengths[11–13].

The only other continuum source available is sunlight, which would have to be scattered from the illuminated hemisphere by the upper cloud layers. Since our images show little indication of a fall in brightness away from the terminator, the scattering could not be accompanied by significant absorption. We note that if such a mechanism were operative at visible wavelengths, the unilluminated side of Venus would be far from dark.

Nature of the clouds

The nature of the cloud structure is far from clear. Superficially, the patterns seen in the IR on the unilluminated hemisphere resemble those seen at UV wavelengths on the sunlit side[14]. From observations on successive days during one complete rotation we derive a retrograde rotation period of 5.4 ± 0.1 days based largely on the return of the leading edge of a very extensive dark area seen near the limb. The error does not take account of the considerable changes in the structure of the clouds during one revolution of the planet (compare Fig. 1*a* and *f*). Estimates of the rotation period based on the observing period of less than six hours possible each day range from 5 to 7 days. The rotation period is distinct from that of the UV cloud patterns (3.94 days, though with some suggestion of longer periods)[15], and the infrared dipolar feature (3.0 days)[16], but it is similar to that of low-contrast features at 11–15 μm (5.3 days)[17]. The pole of the rotation is poorly determined from these data.

The origin of the UV clouds is still not definitely known, but it seems most likely that SO_2 and elemental sulphur, lying in a high-altitude haze, absorb sunlight reflected off the uppermost cloud deck[18–20]. Moreover, the same cloud deck reflects sunlight between 1 and 4 μm (ref. 21). But since we see no prominent

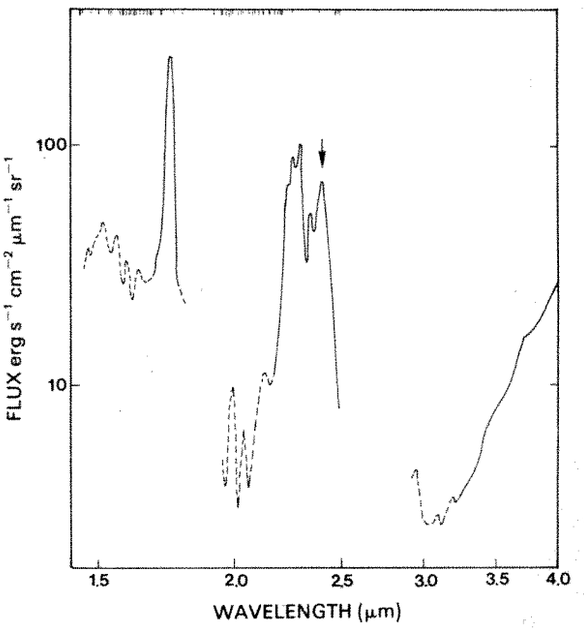


Fig. 2 Spectrum of a small portion of the dark side of Venus, taken on 25 July 1983. Where shown as a broken line the data are dominated by radiation from the sunlit crescent scattered into the beam by the telescopic optics. Beyond 3.3 μm thermal radiation at a brightness temperature of 220 K is seen. Ticks on the upper margin indicate the locations of CO_2 absorption bands. The arrow indicates the observing wavelength for the images in Fig. 1.

cloud structure on the sunlit side at our observing wavelengths, it cannot be the UV absorber that causes the dark-side structure. This conclusion is further strengthened by the fact that neither SO_2 nor elemental sulphur exhibits known absorption in the wavebands of our data except at high overtones.

Markings at very low contrast resembling the UV features, and hence similar to those we now report, have been suspected on the sunlit side at longer IR wavelengths[22,23]. These are of much lower contrast than the patterns we find on the dark side. Hence we infer that the clouds responsible for the dark-side modulation lie below (or perhaps within) the uppermost cloud

layer. They must therefore have an origin distinct from that of the UV features, but a direct correlation would, nonetheless, be of considerable interest.

Received 24 October; accepted 12 December 1983.

1. Westphal, J. A., Wildey, R. L. & Murray, B. C. *Astrophys. J.* **142,** 799–802 (1965).
2. Ksanfomaliti, L. V. *et al. Soviet Astr. J.* **20,** 476–480 (1976).
3. Taylor, F. W. *et al. J. geophys. Res.* **85,** 7963–8006 (1980).
4. Barton, J. R. & Allen, D. A. *Publ. Astr. Soc. Pacific* **92,** 368–374 (1980).
5. Allen, D. A. *Sky & Telescope* **65,** 110–112 (1983).
6. Connes, P., Connes, J., Kaplan, L. D. & Benedict, W. S. *Astrophys. J.* **152,** 731–743 (1968).
7. Young, L. G. *Int. Astr. Un. Symp.* **65,** 77–160 (1974).
8. Connes, P., Connes, J., Benedict, W. S. & Kaplan, L. D. *Astrophys. J.* **147,** 1230–1237 (1967).
9. Kuiper, G. P. *Commun. Lunar Planet. Lab.* **1,** 83–117 (1962).
10. Seiff, A. *et al. Science* **203,** 787–790 (1979).
11. Pollack, J. B. *et al. J. atmos. Sci.* **32,** 1140–1150 (1975).
12. Knollenberg, R. G. & Bunten, D. M. *Science* **203,** 792–795 (1979).

We thank Tony Lacey of Sydney University for discussions, the AAT staff for their assistance, and Don Morton and Harry Hyland for access to the telescope.

13. Taylor, F. W. *et al. Science* **205,** 65–67 (1979).
14. Anderson, J. L., Belton, M. J. S., Danielson, G. E., Evans, N. & Soha, J. M. *Astrophys. J. Suppl.* **36,** 275–284 (1978).
15. del Genio, A. D. & Rossow, W. B. *Icarus* **51,** 391–415 (1982).
16. Schofield, J. T. & Diner, D. J. *Nature* **305,** 116–119 (1983).
17. Apt, J. & Leung, J. *Icarus* **49,** 427–437 (1982).
18. Barker, E. S. *Bull. Am. astr. Soc.* **10,** 732 (1978).
19. Stewart, A. I., Anderson, D. E., Esposito, L. W. & Barth, C. A. *Science* **203,** 777–779 (1979).
20. Toon, O. B., Turco, R. P. & Pollack, J. B. *Icarus* **51,** 358–383 (1982).
21. Mukai, S. & Mukai, T. *Icarus* **38,** 90–99 (1979).
22. Apt, J. & Goody, R. *Science* **203,** 785–787 (1979).
23. Diner, D. J. & Westphal, J. A. *Icarus* **38,** 81–89 (1979).

Trace element patterns at a non-marine Cretaceous–Tertiary boundary

J. S. Gilmore*, J. D. Knight*, C. J. Orth*, C. L. Pillmore† & R. H. Tschudy†

* Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA
† US Geological Survey, Denver, Colorado 80225, USA

At the fossil-pollen-defined Cretaceous–Tertiary boundary in the Raton Basin of New Mexico and Colorado, an iridium abundance anomaly and excess scandium, titanium, and chromium are associated with a thin ash or dust fallout bed (now kaolinitic clay) that was preserved in freshwater coal swamps.

THE Ir abundance anomaly occurring in a thin zone at the planktonic Cretaceous–Tertiary (K–T) boundary in marine sediments, which was discovered by Alvarez *et al.*[1,2] and hypothesized by them to be due to fallout from impact of a large extraterrestrial body, has now been reported at numerous sites around the world[1–11]. Both the evidence and the concept have been challenged, and some opponents have argued that the excess Ir in these sediments can be explained by normal chemical and physical processes in the ocean[12–17].

The possibility of another approach to this problem lay in a search for the anomaly in continental sediments deposited in freshwater conditions. In early 1981 we located an Ir anomaly in a core collected in York Canyon, in the central part of the Raton Basin of northeastern New Mexico and southeastern Colorado[18,19]. The peak Ir concentrations, reaching 5.6×10^{-9} g per g (5.6 ng per g) over a background of 0.004 to 0.020 ng per g, occurred at the base of a thin coal bed within 1 cm of the pollen-defined K–T boundary. Samples taken from above and below the K–T boundary, including the bases of similar coal beds, showed no evidence of Ir enrichment.

Success in locating an Ir anomaly in the York Canyon core led us to search in outcrops and roadcut exposures along the eastern margin of the Raton Basin. Detailed examination of samples from four of six additional Ir anomaly sites that were located, in addition to the original York Canyon core, has provided us with data regarding the distribution of elemental abundance patterns across the boundary zone, as well as the lithology of the zone. We now report elemental abundance patterns across the K–T boundary over a large area with a common palaeoenvironmental setting in the Raton Basin. We include some supplementary observations made recently on another non-marine K–T boundary, near Jordan, Montana (1,200 km north of the Raton Basin). Interpretation of the data provides clues about the source of the excess Ir and the thin kaolinitic clay bed associated with it.

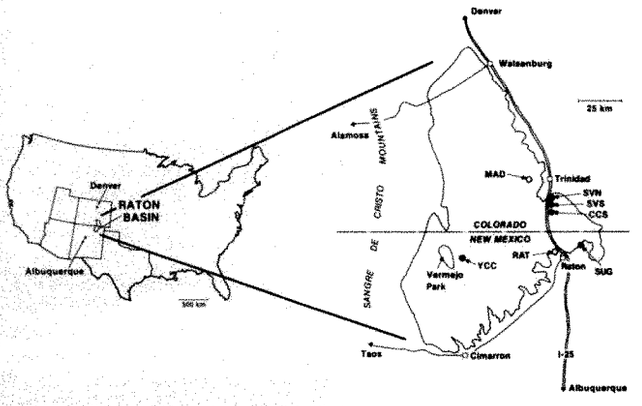


Fig. 1 Outline of present-day Raton Basin and its location in Colorado, New Mexico, and the continental United States. ●, Sites discussed in this work: YCC, York Canyon core; SUG, Sugarite; SVN, Starkville-north; SVS, Starkville-south; CCS, Clear Creek-south. ○, Ir anomaly sites not discussed in this work: MAD, Madrid; RAT, Raton.

Measurements

The five Ir anomaly sites for which elemental abundances are reported are shown in Fig. 1 (also shown are two additional sites, Raton (RAT) and Madrid (MAD), at which the Ir anomaly has been found, but for which sampling was not completed). At the outcrop or roadcut locations, after preliminary sampling and palynological examination to establish the K–T boundary, we collected a continuous sequence of samples across the boundary zone at intervals of 0.5–6 cm and at selected levels both above and below the zone (vertebrate fossils have not been reported here); 10–40 cm of surface material were discarded before sampling. Although some of the results for the York Canyon core have been reported previously[18,19], these and new data are included because they provide a comparison of elemental distributions observed in an unweathered environment (core) with those observed in the more weathered near-surface conditions at the outcrop and roadcut locations.

All of the abundances except for nickel were determined by instrumental neutron activation analysis. Two portions (3–5 g) from each sample were irradiated with thermal neutrons. One portion was placed in an automated system that provides abundances for about 24 elements[20]. The second portion was irradiated

0028–0836/84/030224—05$01.00

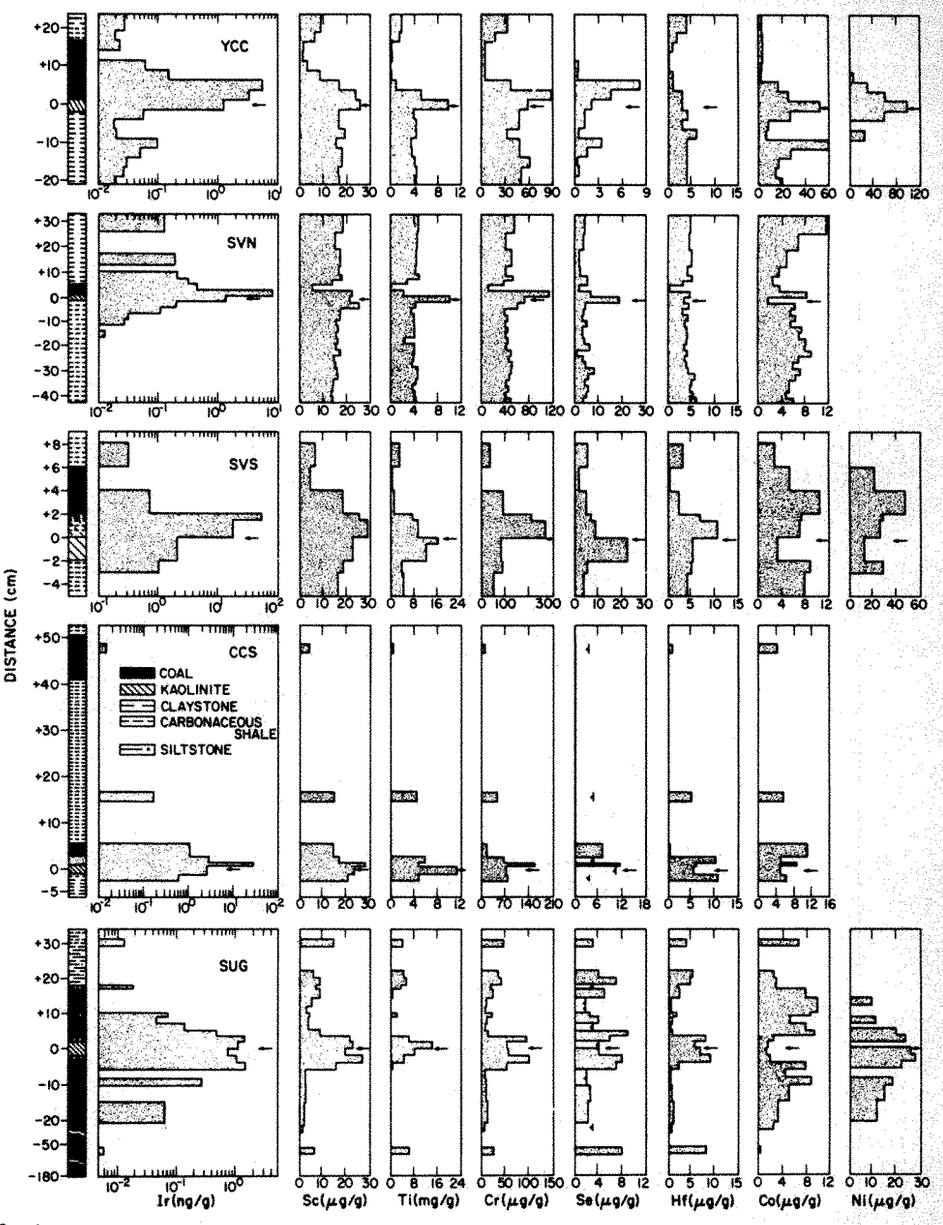


Fig. 2 Elemental abundance diagrams for the sites shown with solid circles in Fig. 1. Note that the Ir abundances are plotted on logarithmic scales. The distances up (+) and down (−) section are referenced to the centre of the K–T kaolinitic clay bed (0); arrows indicate this reference level. Vertical bars with left-pointing arrowheads (Se) represent upper limits. Ni was not measured at the SVN and CCS sites.

for 24 h with 1.4×10^{12} neutrons cm^{-2} s^{-1} in order to build up the radioactivity level of long-lived species such as 74-day ^{192}Ir. For determination of Au/Ir and Pt/Ir ratios a 1-g portion was irradiated for 7 h with 5.7×10^{12} neutrons cm^{-2} s^{-1} and radiochemical separations were performed.

Concentrations of nickel, whose nuclear properties are not well suited for thermal neutron activation techniques, were determined in a few selected samples using a plasma emission spectrometer.

Results

Elemental abundance data for the five sections discussed here are plotted in Fig. 2. At each of these five sites we have found only one Ir anomaly, at the base of the thin coal bed containing the K–T boundary as defined by fossil-pollen changes. This pattern of observations supports the inference that quiet swamp conditions were crucial for the sheltering of Ir-bearing material against dilution by mixing processes. In several closely spaced sections at a site near the city of Raton, the Ir anomaly is rather weak (1 ng per g), and the kaolinite-rich parting, while overlain by a thin coal bed, seems to be contaminated with detrital clay.

In the Raton Basin the palynological K–T boundary interval occurs in a rock sequence deposited in freshwater swamps and flood plains[18,19,21]. These rocks lie in the lower part of the Raton Formation, of late Cretaceous and Palaeocene age.

At York Canyon, the surface density (total deposition per unit area) of the Ir anomaly shown in Fig. 2 is about 40 ng cm^{-2}. The kaolinite-rich bed at the base of the coal is not visibly distinct from the underlying mudstone, owing to darkening from carbonaceous material; the presence of the kaolinitic clay was determined by X-ray analysis and, once its elemental abundance pattern was established from the elemental systematics of the other K–T kaolinite-rich beds in the basin, by that pattern.

At the Starkville–North Section (SVN), in a roadcut south of Trinidad, Colorado, the kaolinitic bed at the K–T boundary is clearly recognizable, because it has weathered to a grey colour and is located between coal and dark brown carbonaceous shale. The Ir surface density is about 50 ng cm^{-2}, and the Ir concentration reaches a maximum of 8 ng per g.

The Starkville–South Section (SVS) was taken from a roadcut about 500 m south of SVN. Here the Ir concentration reaches 56 ng per g, the highest we have observed in the basin, in a thin (5 mm) shaley bed between the base of the coal and the kaolinitic-clay layer. The Ir surface density here is ~120 ng cm^{-2}.

The Clear Creek–South Section (CCS) was taken about 2 km south of SVS. The Ir concentration shown in Fig. 2 reaches a maximum level of 28 ng per g in a thin (2 mm) carbonaceous–kaolinitic shale that lies on the kaolinitic clay stone bed, and the surface density is ~50 ng cm^{-2}.

Table 1 Elemental abundances associated with the Sugarite kaolinitic clay bed compared with various meteorites and terrestrial and lunar rocks

Element*	Sugarite†	Crustal average (ref. 35)	Lunar soil (refs 36–44)	Lunar basalt (refs 36–44)	Achondrite eucrite average (ref. 45)	Chondrite Cl average (ref. 46)
Na(%)	0.3	2.8	0.30–0.37	0.26–0.36	0.35	0.48
Mg(%)	0.3	2.1	3.8–7.2	3.5–4.9	4.3	9.6
Al (%)	17	8.1	6–9	3.8–14.7	6.5	0.86
K (%)	0.35	2.6	0.11–0.14	0.04–0.22	0.03	0.06
Ca (%)	0.4	3.6	8–9.7	7.1–9.3	7.3	0.9
Sc	34	22	54–92	72–96	26	5.8
Ti (%)	1.35	0.44	4.3–5.6	6.0–7.5	0.46	0.044
V	175	135	34–58	63–92	75	57
Cr	100	100	1,850–1,950	2,300	2,300	2,650
Mn	44	950	1,340–1,820	3,900	3,900	1,960
Fe (%)	0.8	5.0	12	14.4	14.4	18.5
Co	7	25	12–37	2	2	510
Ni	50	75	170–200	20	20	11,000
Se	4	0.05	0.4	0.25	0.25	18
Rb	≤15	90	3	5.7	0.3	2.3
Sr	60	375	165	146–210	75	7.9
Cs	0.8	3	0.1	0.2	0.01	0.02
Ba	540	425	150–430	140–450	30	2.2
La	44	30	11–22	16–43	3	0.24
Ce	74	60	46–68	34–84	—	0.62
Yb	2.4	3.4	12–22	11–18	1.8	0.16
Hf	8	3	8–15	11–20	0.4	0.12
Ta	1.3	2	0.9–2.1	1.6–2.7	0.008	0.02
Ir (ng per g)	3.2	≤1	5–11	0.04–0.50	2–20	480
Th	17	7.2	1.4–2.6	0.6–3.7	0.2	0.03
La/Yb	18	8.8	0.9–1.0	1.4–2.3	1.7	1.5

* Concentrations in μg per g unless noted otherwise.
† See text for discussion of this analysis.

The section from the Sugarite site (SUG), located 10 km east of Raton, includes a 2-m-thick coal bed. The Ir surface density is about 32 ng cm^{-2}. This section is of particular interest because the Ir-enriched zone is sandwiched between two layers of coal and appears to be relatively uncontaminated by detrital clay. Figure 2 shows that the Ir concentration reaches its maximum (1.5 ng per g) not in the thin kaolinitic layer, but in the adjacent coal just above and below. A similar displacement is seen at the other four sampling sites: the Ir concentration reaches its maximum in the coal slightly above the clay layer. This distribution is consistent with spreading due to a combination of at least two dispersion mechanisms: (1) mixing with plant matter following deposition of Ir-bearing fallout; and (2) chemical migration in the acid environment associated with the transformation of plant matter to coal. Iridium concentrations in 27 other non-K–T coal beds and 21 other kaolinitic beds examined in the Raton Basin are <0.040 ng per g.

The abundance for each element in the SUG K–T kaolinitic clay bed alone was estimated by integrating its net surface density (coal bed background subtracted from the total measured) over individual sample widths and dividing by the surface density of the clay layer (8.8 g cm^{-2}). These abundances are compared in Table 1 with crustal averages and with lunar soil and basalt, chondrites and achondrites. Figure 2 indicates that at the SUG site boundary, Sc, Ti, Cr, Hf and Ni are enhanced in this layer relative to their values in the strata above and below.

Of the elemental abundances measured, four show strong positive correlation with Ir: Sc, Ti, Cr and Pt. Analysis of all data taken in the K–T kaolinitic clay zone shows the following correlation coefficients: Sc/Ir = 0.93, Ti/Ir = 0.95, Cr/Ir = 0.96, Pt/Ir = 0.98. Two other siderophile elements measured, Co and Ni, exhibit no significant correlation with Ir at any of the outcrop sampling sites. Only in the York Canyon core can a clear positive correlation with Ir be observed for these two elements. All of the anomaly sites in the Raton Basin apparently had a common genesis, and thus we suspect that the lack of correlation for Co and Ni with Ir in the outcrop and roadcut sites is associated with their exposure to oxidizing and leaching conditions.

We have measured Pt and Au in eight samples from the York Canyon core and also some from three of the roadcut sites. In all samples measured the Pt correlated very closely with the Ir, but the Au did not. The concentration of Au peaks near the Ir spike but does not drop to very low levels on either side. Instead the Au background concentration remains at several ng per g throughout the sampled sections.

In Table 2 Pt/Ir and Au/Ir data for the peak Ir samples at four Raton Basin sites are compared with corresponding data for the Stevns Klint, Denmark, and Caravaca, Spain, K–T boundary clay, terrestrial volcanic rocks, lunar soil, and Cl chondrites. Unfortunately, there is a paucity of Pt/Ir data for terrestrial basalts, due probably to the difficulty of measuring Pt at very low concentrations. The Au/Ir ratios for the Raton Basin are higher than observed in the marine K–T sites; however, they are distinctly lower than the Au/Ir ratios reported for terrestrial basalts. Finally, we see that the Pt/Ir ratios at our measured Ir spikes fall around the value for Cl chondrites.

In every Raton Basin sampling site where an Ir anomaly has been found, the anomaly and the pollen break are associated with a 2- to 4-cm kaolinite-rich clay layer overlain by coal. We have performed elemental analyses on this K–T kaolinite-rich clay bed at six sites, and on 21 other thin kaolinitic clay layers found in other coal beds from about 50 m below to about 300 m above the one containing the K–T boundary. The data are summarized in Table 3. The eight trace elements from Sc to Sb, as well as Ir, are higher in the K–T boundary kaolinitic clay bed than in the others. Although thin kaolinitic clay layers of this kind (commonly referred to as tonsteins) are identified as the alteration product of volcanic ash falls[22,23], the distinctively different trace-element character of the K–T layer suggests a different origin.

A similar pattern emerges from results obtained at another group of freshwater K–T boundary exposures, 1,200 km to the north, near Jordan, Montana. From this area, in which the K–T Ir anomaly was first reported by Alvarez[24], we have examined samples collected by Bohor and Triplehorn from Brownie Butte, in the vicinity of Hell Creek, where Tschudy[25] established the palynological K–T boundary at the lowest lignite bed of the Tullock Member of the Fort Union Formation. Here the Ir anomaly is associated with a 0.5- to 1.5-cm kaolinitic clay bed overlain by several centimetres of lignite. Although the Brownie

Table 2 Atom-ratio data for Ir, Pt and Au

Location	Ratio Pt/Ir	Ratio Au/Ir
YCC	3±1	1.1±0.2
SVN	1.7±0.4	0.39±0.06
SVS	2.4±0.5	0.64±0.10
CCS	1.6±0.4	0.64±0.11
Raton Basin		
Stevns Klint, Denmark (ref. 3)	0.41±0.07	0.20±0.02
Stevns Klint, Denmark (ref. 5)	1.0–1.5*	0.12–0.20*
Stevns Klint, beds 3 and 4 (ref. 10)	2.01±0.07	0.281±0.007
Caravaca, Spain (ref. 10)	1.79±0.16	0.303±0.022
Ultramafic nodule, Globe, Arizona	1.6±0.4	0.17±0.03
Terrestrial basalts (refs 47, 48)	—	15–100*
Lunar soil fines (ref. 40)	—	0.23–0.6*
Cl chondrite (ref. 46)	1.75	0.30

* Values shown indicate ranges.

Butte location was in a different floral province during the Cretaceous, the fossil pollen taxon *Proteacidites* occurred in this area and in the Raton Basin. At both locations we have found that this late Cretaceous marker pollen disappears at the top of the clay layer containing the Ir anomaly.

The higher abundance of Ti in the K–T kaolinite-rich clay bed is of some interest. Apart from considerations of its origin, this distinctively higher concentration provides a useful marker for preliminary identification of the boundary bed. In the neutron activation analysis procedure, Ti abundances are determined within minutes, whereas the Ir measurements require long-term irradiation followed by lengthy radiochemical analysis, and thus a high Ti value offers an early alert.

In previous discussions of the Ir enrichment phenomenon, consideration has been given to the possibility that the anomalous material at the K–T boundary may have arisen from volcanism[13,16,17]. The period from the late Cretaceous through the early Tertiary was marked by a high level of volcanic activity in the western interior of North America[26], which could have led to a kaolinitic clay layer of the kind found at the Raton Basin K–T boundary. We have attempted to gain some information on the plausibility of this source by comparing the elemental abundances of the K–T kaolinitic clay bed with those of similar beds above and below it (Table 3), and with those of various other volcanic rocks (Table 4). Table 3 shows that Sc, Ti, Cr and Ir are distinctly more abundant in the K–T bed. The light to heavy rare earth ratios, La/Yb, on the other hand, are about the same and in a range characteristic of crustal rock. The

Table 3 Elemental abundances in thin kaolinitic clay beds in the Raton Basin*

Element†	K–T boundary bed Range	K–T boundary bed Average	Beds above/below K–T boundary Range	Beds above/below K–T boundary Average
Al_2O_3 (%)	24–36	32.9	23–36	31.7
K (%)	0.2–1.1	0.52	0.2–2.5	0.81
Sc	21–26	23.3	3–12	6.0
TiO_2 (%)	1.38–2.67	2.00	0.40–0.95	0.757
V	110–187	137	10–67	27
Cr	46–102	67.3	0.9–5.0	3.3
Co	1.2–53	9.8	0.7–4	2.7
As	1–95	36	0.2–46	4.1
Se	2–19	8	<0.1–6	4.8
Sb	0.3–11.5	6.3	0.1–0.8	0.38
La	16–80	43	9–88	28
Yb	0.7–2.2	1.5	0.8–4.1	2.1
Hf	3.3–6.4	4.5	3.2–10.6	7.6
Ir (ng per g)	0.90–2.7	1.7	0.005–0.020	0.010
Th	5–21	7.8	5–34	9.2
La/Yb	15–57	28.7	5–67	13.3
TiO_2/Al_2O_3	0.056–0.074	0.060	0.016–0.027	0.024
Cr/Al_2O_3‡	1.64–2.83	2.04	0.036–0.147	0.103

* Boundary data from six sampling sites, non-boundary data from 21 sites.
† Concentrations in μg per g unless noted otherwise.
‡ Cr/Al_2O_3 relative (p.p.m./%)

enhanced concentrations of Sc, Ti and Cr in the Raton boundary clay suggest that this bed may represent the alteration product of basalt or the ash from a basic volcano. Spears and Kanaris-Sotiriou[23] have reported that in British and other European Carboniferous-age tonsteins, the ratios TiO_2/Al_2O_3 and Cr/Al_2O_3 remain virtually unchanged as the volcanic ash alters, and basic ash gives high values for these ratios whereas acid ash gives low values. They point out that basic ratios can be lowered by the addition of sedimentary material. On the basis of TiO_2/Al_2O_3 ratios in Table 3, the K–T kaolinitic layer would have to be ascribed to a basic volcano, whereas all the other beds would have to be ascribed to acid volcanoes.

Of the mafic volcanic and plutonic rocks listed in Table 4, only the dunite and the ultramafic nodule contain Ir in concentrations comparable with those found in the K–T clay layer. However, their Cr concentrations are an order of magnitude too high. Thus, we have difficulty constructing a volcanic source

Table 4 Elemental abundances for K–T boundary clay and for various igneous rocks

Element*	K–T clay Raton Basin†	K–T clay Gubbio, Italy‡	BCR-1 Columbia River basalt§‖	BHVO-1 Kilauea basalt§¶	BIR-1 Iceland basalt§‖	East Pacific Rise #	DTS-1 dunite§‖	Ultramafic nodule Globe, Arizona**	Raton diatreme**††	Raton Basin sill**	AGV-1 andesite§‡‡
Al_2O_3 (%)	24–35	17.3	13.3	13.9	14.8	15.6	0.32	1.87	6.42	14.9	17.1
TiO_2 (%)	1.4–2.7	0.78	2.12	2.65	0.93	1.32	0.013	0.28	1.38	2.13	1.05
Sc	21–26	10.0	33	30	45	38	3.5	9.6	27	11.6	12
Cr	46–102	97	17.4	315	350	485	3,920	2,400	1,500	130	12
Co	1.2–53	46	38	46	48	—	139	106	65	19	15
La	16–80	32.4	28	17.5	0.84	2.76	0.029	4.0	110	31.2	38
Yb	0.7–2.2	2.4	3.53	1.6	1.8	2.72	0.015	≤0.4	≤2.5	0.8	1.67
Hf	3–6	2.5	5.0	4	0.66	2.3	0.01	0.70	4	2.8	5.1
Ir (parts per 10^9)	3.2–15	9	0.0035	0.055	0.067	—	0.83	3.0	0.20	0.032	0.011
Th	5–21	7.8	6	0.85	0.60	0.3	0.01	0.7	22	2.5	6.5
TiO_2/Al_2O_3	0.056–0.074	0.045	0.159	0.190	0.063	0.084	0.040	0.151	0.215	0.143	0.062
Cr/Al_2O_3	1.6–2.8	5.6	1.31	23	24	31	12,250	1,283	234	8.7	0.70
Sc/Al_2O_3	0.75–0.80	0.58	2.5	2.2	3.0	2.4	11	5.1	4.2	0.78	0.70
La/Yb	15–57	13.5	7.9	11	0.47	1.01	1.9	≥10	≥44	39	22.8

* Concentrations in μg per g unless noted otherwise. Cr/Al_2O_3 and Sc/Al_2O_3 relative (p.p.m./%).
† This work, the Ir abundance represents the Ir surface density divided by the thickness and density of the K–T clay.
‡ This work, sample provided by A. Montanari and W. Alvarez.
§ US Geological Survey standard rock.
‖ Data from ref. 49, except Ir from this work.
¶ Data from ref. 50, except Ir from this work.
Data from ref. 51.
** This work.
†† This diatreme, located 2 km east of Raton, was discovered by G. T. Scott (USGS Denver) who provided samples.
‡‡ Data from ref. 49.

that yields the elemental abundance patterns we observe in the Raton Basin K–T boundary bed. Also, basic volcanoes are usually less explosive than their acidic counterparts.

The enrichment of Sc, Ti, Cr and Pt-group elements in the K–T kaolinite also suggests a similarity with lunar surface material. Ruderman and Truran[27] have proposed that the K–T fallout resulted from capture by the Earth of lunar surface material ejected by intense photon heating from a nearby supernova burst. The plausibility of this scenario, however, is contradicted by the rare earth measurements shown in Tables 1 and 3. The observed light/heavy rare earth ratios (La/Yb) for our boundary zone samples lie in the range 8–57, characteristic of the Earth's crust. Reported lunar rocks range from 0.9 to about 3, clearly distinct from the former.

Conclusions

If we accept the postulate that the excess Ir is derived from impact of a 10-km diameter asteroid with Type 1 chondritic composition[2] our Ir data can be used to estimate the fraction of the kaolinitic bed contributed by the asteriod itself. The total integrated Ir under the anomaly peak, expressed as a surface density, was divided by the measured surface density of the kaolinitic clay layer to give the concentration of the Ir in the layer (with the assumption that all the Ir came in with this layer), and comparison of this concentration with that for a Type I chondrite yielded the asteroid contribution. This contribution ranged from 0.6 to 2.5% for the five Raton Basin sampling sites. The corresponding contributions for the Gubbio and Stevns Klint K–T anomaly clays have been reported[28] to be 1.6% and 21%, respectively. If we exclude major contributions from nearby volcanic eruptions and aeolian material, the remainder ($\geq$97.5%) of the Raton Basin K–T clay layer can be assigned to ejecta from the impact. The elemental abundances of the lithophiles Sc, Ti and Cr, therefore, reflect the composition of the impact site. Our data are best fitted by ejecta from basalts. The La/Yb ratio is high for oceanic basalt, but could have been made up by contribution from overlying sediments.

From our elemental abundance data we are unable to conclude whether or not the palynological K–T boundary is synchronous with the marine boundary, but there exist palaeomagnetism measurements bearing on this question. The palaeomagnetic polarity sequence for marine rocks has now been established for the late Cretaceous through the early Tertiary[29,30], and during the boundary period the polarity was reversed. However, Payne *et al.*[31], measuring samples taken from the K–T boundary zone in the Raton Basin, have reported normal polarity. More recent measurements on samples from the same sites and others in the Raton Basin by Shoemaker *et al.*[32] lead to assignment of reversed polarity. The latter work emphasizes that the polarity assignment is critically dependent on the removal of the strong normal overprint from the palaeomagnetic signals observed in near-boundary-level rocks in the Raton Basin.

The recent investigations at the K–T boundary in Montana have provided some clues to the synchronism of the two continental K–T boundary sequences. We have already pointed out that the elemental abundance patterns for Raton and Montana are similar and that the Cretaceous pollen *Proteacidites* disappears precisely at the top of the kaolinitic boundary clay. Also, Archibald *et al.*[33] have found that the Montana boundary zone lies in a stratigraphical sequence with reversed palaeomagnetic polarity. The highest dinosaur fossil horizon reported[34] in this region of Montana occurs ~3 m below the palynological K–T boundary and Ir anomaly, but a statistical analysis of the '3-m gap' data shows[24] that one cannot conclude that the dinosaurs disappeared before the Ir layer was deposited.

The body of information we have derived from the elemental abundances, clay mineralogy, stratigraphy and pollen data lead us to the following conclusions:

(1) The Ir-bearing kaolinite-rich clay bed at all the sampling sites resulted from fallout from a common source, and the excess Ir is associated primarily with the fallout and not with chemical concentration of pre-existing amounts in the local medium.

(2) The excess Sc, Ti, V, Cr and possibly Hf appear also to have come with the fallout and not from local enrichment processes. This enrichment pattern is consistent with the patterns observed in basalts.

(3) Although the kaolinite precursor material was deposited on several different local surfaces, in each case it is now overlain by a thin coal bed. This mode of deposition may simply be an indication that quiet swamp conditions preceding coal formation were a favoured environment for the preservation of this fallout material.

(4) The narrowness of the Ir distribution and the relatively small variation in surface density (a factor of four) indicate that the Ir has not undergone any large scale migration, but may have spread locally from the clay layer into the adjacent material.

(5) The uniformity in thickness of the K–T kaolinitic clay bed over a distance of at least 1,200 km indicates that the fallout material came from a common source at a considerable distance.

(6) Although the boundary clay bed may contain some altered volcanic ash or aeolian material, such contribution to the excess Ir does not appear to be significant.

We are indebted to the Los Alamos National Laboratory Reactor Group for performing the neutron irradiations and for deriving chemical abundance data for about 24 trace elements, and to L. R. Quintana for assistance with sample preparation and data reduction. We thank J. J. Connor and J. R. Hatch for critical comments. J.S.G., J.D.K. and C.J.O. thank the US Department of Energy for support.

Received 6 June; accepted 14 September 1983.

1. Alvarez, W., Alvarez, L. W., Asaro, F. & Michel, H. V. *EOS* **60,** 734 (1979).
2. Alvarez, L. W., Alvarez, W., Asaro, F. & Michel, H. V. *Science* **208,** 921–1108 (1980).
3. Ganapathy, R. *Science* **209,** 921–923 (1980).
4. Smit, J. & Hertogen, J. *Nature* **285,** 198–200 (1980).
5. Kyte, F. T., Zhou, Z. & Wasson, J. T. *Nature* **288,** 651–656 (1980).
6. Hsü, K. J. *et al. Science* **216,** 249–256 (1982).
7. Nazarov, M. A. *et al. 13th Lunar Planet. Sci. Conf.*, Houston, abstr., 580 (1982).
8. Ganapathy, R., Gartner, S. & Jiang, M.-J. *Earth planet. Sci. Lett.* **54,** 393–396 (1981).
9. Smit, J. & ten Kate, W. G. H. Z. *Cretaceous Res.* **3,** 307–332 (1982).
10. Alvarez, W., Alvarez, L. W., Asaro, F. & Michel, H. V. *Geol. Soc. Am. Spec. Pap.* **190,** 305–315 (1982).
11. Smit, J. *Geol. Soc. Am. Spec. Pap.* **190,** 329–352 (1982).
12. Kent, D. V. *Science* **211,** 648–650 (1981).
13. McLean, D. M. *Earth planet. Sci. Lett.* **53,** 103–108 (1981).
14. Rampino, M. R. *Geol. Soc. Am. Spec. Pap.* **190,** 455–460 (1982).
15. Keith, M. L. *Geochim. cosmochim. Acta* **46,** 2621–2637 (1982).
16. Rampino, M. R. & Reynolds, R. C. *Science* **219,** 495–498 (1983).
17. Officer, C. B. & Drake, C. L. *Science* **219,** 1383–1390 (1983).
18. Orth, C. J. *et al. Science* **214,** 1341–1343 (1981).
19. Orth, C. J. *et al. Geol. Soc. Am. Spec. Pap.* **190,** 423–433 (1982).
20. Minor, M. M., Hensley, W. K., Denton, M. M. & Garcia, S. R. *J. radioanalyt. Chem.* **70,** 459–471 (1982).
21. Pillmore, C. L., Tschudy, R. H., Orth, C. J. & Gilmore, J. S. Preprint (USGS, Denver, 1983).
22. Bohor, B. F. & Pillmore, C. L. *New Mexico Geol. Soc. Guidebook, 27th Field Conf., Vermejo Park,* 177–183 (1976).
23. Spears, D. A. & Kanaris-Sotiriou, R. *Sedimentology,* **26,** 407–425 (1979).
24. Alvarez, L. W. *Proc. natn. Acad. Sci. U.S.A.* **80,** 327–348 (1983).
25. Tschudy, R. H. *Geol. Soc. Am. Spec. Pap.* **127,** 65–111 (1970).
26. Axelrod, D. I. *Geol. Soc. Am. Spec. Pap.* **185** (1981).
27. Ruderman, M. & Truran, J. W. *Nature* **284,** 328–329 (1980).
28. Kyte, F. T. & Wasson, J. T. *Geol. Soc. Am. Spec. Pap.* **190,** 235–242 (1982).
29. LaBrecque, J. L., Kent, D. V. & Cande, S. C. *Geology* **5,** 330–335 (1977).
30. Lowrie, W. & Alvarez, W. *Geology* **9,** 392–397 (1981).
31. Payne, M. A., Wolberg, D. L. & Hunt, A. A. *Geol. Soc. Am. Abstr. Prog.* **14,** 584 (1982); *New Mexico Geol.* **5,** 41–44 (1983).
32. Shoemaker, E. M., Pillmore, C. L., Tschudy, R. H. & Orth, C. J. *Geol. Soc. Am. Abstr. Prog.* **15,** 309 (1983).
33. Archibald, J. D., Butler, R. F., Lindsay, E. H., Clemens, W. A. & Dingus, L. *Geology* **10,** 153–159 (1982).
34. Archibald, J. D. & Clemens, W. A. *Am. Sci.* **70,** 377–385 (1982).
35. Mason, B. *Principles of Geochemistry* 45, 46 (Wiley, New York, 1966).
36. Haskin, L. A. *et al. Proc. Apollo 11 Lunar Sci. Conf.* 1213–1231 (1970).
37. Turekian, K. K. & Kharkar, D. P. *Proc. Apollo 11 Lunar Sci. Conf.* 1659–1664 (1970).
38. Wakita, H., Schmitt, R. A. & Rey, P. *Proc. Apollo 11 Lunar Sci. Conf.* 1685–1717 (1970).
39. Wänke, H. *et al. Proc. Apollo 11 Lunar Sci. Conf.* 1719–1727 (1970).
40. Wasson, J. T. & Baedecker, P. A. *Proc. Apollo 11 Lunar Sci. Conf.* 1741–1750 (1970).
41. Ganapathy, R., Morgan, J. W., Higuchi, H., Anders, E. & Anderson, A. T. *Geochim. cosmochim. Acta Suppl.* **5,** 1659–1683 (1974).
42. Brunfelt, A. O., Heier, K. S., Nilssen, B., Steinnes, E. & Sundvoll, B. *Geochim. cosmochim. Acta Suppl.* **5,** 981–990 (1974).
43. Wänke, H. *et al. Proc. 8th Lunar Sci. Conf.* 2191–2213 (1977).
44. Wasson, J. T. *et al. Proc. 8th Lunar Sci. Conf.* 2237–2252 (1977).
45. Mason, B., ed., *Handbook of Elemental Abundances in Meteorites* (Gordon and Breach, New York, 1971).
46. Anders, E. & Ebihara, M. *Geochim. cosmochim. Acta* **46,** 2363–2380 (1982).
47. Hertogen, J., Janssens, M.-J. & Palme, H. *Geochim. cosmochim. Acta* **44,** 2125–2143 (1980).
48. Palme, H. *Geol. Soc. Am. Spec. Pap.* **190,** 223–233 (1982).
49. Gladney, E. S. & Burns, C. E. *Geostandards Newslett.* **7,** 3–226 (1983).
50. Flanagan, F. J. *U.S. geol. Surv. Prof. Pap.* **840,** 35–39 (1976).
51. *Basaltic Volcanism on the Terrestrial Planets* (Pergamon, New York, 1981).

Coupled Sm–Nd and U–Pb systematics in late Caledonian granites and the basement under northern Britain

A. N. Halliday

Scottish Universities Research and Reactor Centre, East Kilbride, Glasgow G75 0QU, UK

The British late Caledonian granites display a close correspondence between inherited U–Pb systematics in zircons and initial ε_{Nd} indicating that both were controlled by the age of a major component of continental crust. The data confirm and refine previous models of a basement comprising young crust in the south which includes arc and oceanic crust scrapings and progressively older continental crust northwestwards across Scotland.

MUCH recent research effort and speculation has been concentrated on the origin of granites, and Nd isotope geochemistry has played a key role in this research[1–5]. The Caledonian granites of the British Isles are of particular interest in this respect for several reasons. First, they are classical and as internationally recognized examples it is important that their genesis is properly understood. Second, there is a good background of reconnaissance chemical and isotopic data. Third, the Caledonian granites are not easily related to active subduction and there is no obvious mechanism for their generation. Finally they are by far the largest samples of what the lower crust was like at 400 Myr and their isotope geochemistry can tell us a great deal about the regional deep geology of the British Isles.

Pidgeon and Aftalion[6] discovered a marked Pb isotopic memory in zircons extracted from late Caledonian granites north of the Highland Boundary Fault (Fig. 1) which is absent from zircons from granites to the south. This has been interpreted as reflecting a contrast in basement to the north and south[6]. Pb, Sr, O and Nd isotopic data has confirmed the importance of continental crust in the generation of these granites[8–10]. In particular, O and Sr isotopic compositions plot with a broad correlation between the fields for mantle and crust reservoir types[8,10,11] suggesting overall interaction between mantle-like and crust-like reservoirs during magmatism. The common Pb isotopic compositions generally become more radiogenic southwards implying the southerly diminution of old granulite facies material in the crust[9,12].

Despite the large amount of isotopic data available there has been considerable controversy over the origin of the late Caledonian granites. Some authors have argued that the granites were largely derived from mantle, especially those lacking inherited zircons and with low initial $^{87}Sr/^{86}Sr$ (refs 13–16), others have proposed crustal source regions[4,6,7,17] and still other mixed origins but involving mantle or mafic lower crust[8,10,11,18]. Some of these views have been discussed elsewhere[19].

New Nd isotopic data are now presented which expand the previous data base[4]. From this it is concluded that although a mantle component is important, the lower and middle continen-

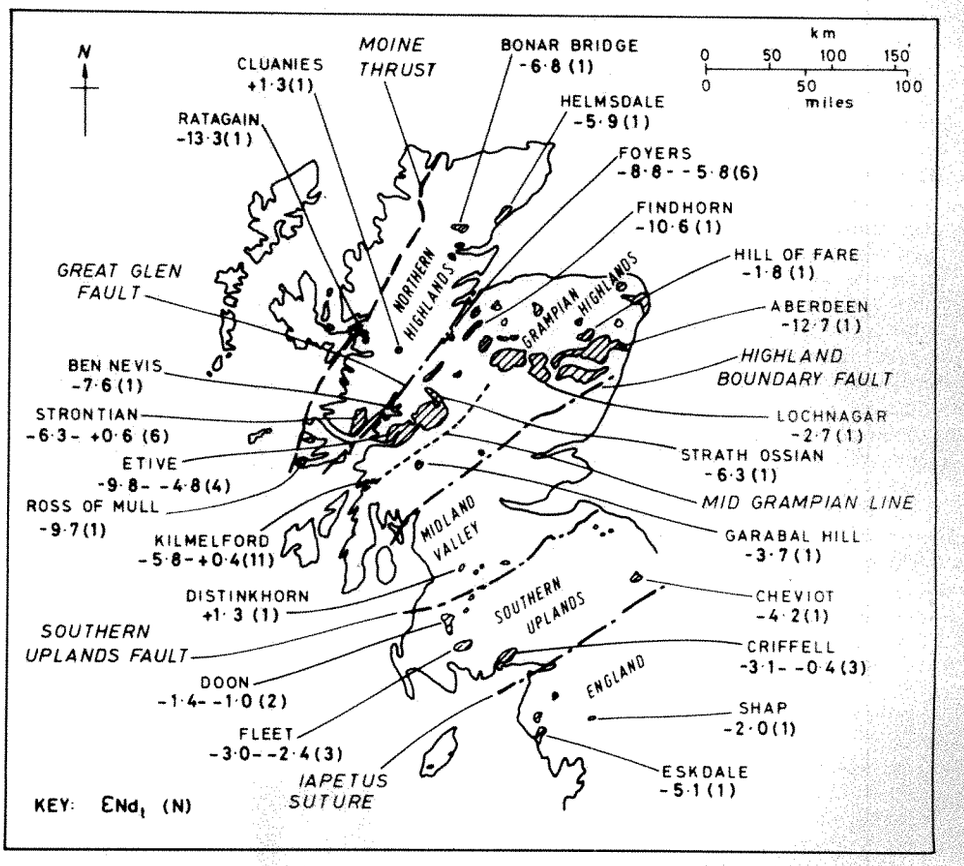


Fig. 1 Map of Scotland and northern England showing distribution of Caledonian granites. The name of the granite is followed by the ε_{Nd_t} value(s) and (in brackets) the number of samples.

© 1984 Macmillan Journals Ltd

Table 1 Sm–Nd and Rb–Sr isotopic data

Sample no.	Locality	Lithology	t (Myr)	Sm (p.p.m.)	Nd (p.p.m.)	$\left(\frac{^{147}Sm}{^{144}Nd}\right)$	$^{143}Nd/^{144}Nd$ $\pm 2\sigma m$	$\left(\frac{^{143}Nd}{^{144}Nd}\right)_t$	ε_{Nd_t}	$\left(\frac{^{87}Rb}{^{86}Sr}\right)$	$^{87}Sr/^{86}Sr$ $\pm 2\sigma m$	$\left(\frac{^{87}Sr}{^{86}Sr}\right)_t$	ε_{Sr_t}
RC 973	Helmsdale	Granite	420	4.804	33.77	0.08597	0.512028 ± 19	0.51179	−5.9	0.8309	0.71118 ± 5	0.70621	+31
RC 720	Ratagain	Monzonite	425	5.119	31.62	0.09784	0.511681 ± 32	0.51141	−13.3	0.08957	0.70575 ± 3	0.70520	+17
RC 721	Cluanies	Granodiorite	417	2.896	12.46	0.1405	0.512548 ± 37	0.51216	+1.3	0.2152	0.70602 ± 4	0.70474	+10
RC 1282	Ross of Mull	Granite	414	4.219	18.20	0.1402	0.511985 ± 18	0.51161	−9.7	0.9525	0.71201 ± 5	0.70639	+34
RC 976	Bonar Bridge	Granite	400	2.430	9.703	0.1514	0.512169 ± 16	0.51177	−6.8	0.4453	0.70847 ± 7	0.70593	+27
RC 872	Hill of Fare	Granite	413	5.105	25.51	0.1210	0.512339 ± 31	0.51201	−1.8			0.7057*	+24
RC 723	Strath Ossian	Granodiorite	400	5.237	23.33	0.1357	0.512151 ± 20	0.51180	−6.3			0.7060*	+28
BC 14	Aberdeen	Granite	440	11.44	57.08	0.1212	0.511766 ± 41	0.51142	−12.7	0.7524	0.71623 ± 5	0.71151	+107
RC 722	Findhorn	Granite	443	2.592	11.14	0.1407	0.511930 ± 23	0.51152	−10.6	1.104	0.72489 ± 4	0.71792	+198
RC 982	Lochnagar	Granite	415	5.055	30.21	0.1012	0.512236 ± 22	0.51196	−2.7	1.082	0.71269 ± 3	0.70630	+33
GH 18	Garabal Hill	Granodiorite	406	3.797	19.63	0.1169	0.512232 ± 30	0.51192	−3.7	0.3081	0.70956 ± 3	0.70778	+54
RC 724	Cruachan	Granodiorite	410	3.844	22.93	0.1013	0.512058 ± 24	0.51179	−6.3	0.5276	0.70783 ± 3	0.70475	+11
80015	Distinkhorn	Granodiorite	390	7.465	37.15	0.1215	0.512512 ± 16	0.51220	+1.3	1.330	0.71219 ± 3	0.70481	+11
70061	Loch Doon	Diorite	408	4.885	25.35	0.1165	0.512372 ± 17	0.51206	−1.0	0.3064	0.70676 ± 2	0.70498	+14
DBL 3	Loch Doon	Granodiorite	408	4.982	26.82	0.1123	0.512338 ± 26	0.51205	−1.4	0.6230	0.70885 ± 3	0.70523	+18
243	Criffell	Granodiorite	397	4.033	28.69	0.08499	0.512326 ± 20	0.51211	−0.4	0.6478	0.70920 ± 3	0.70553	+21
244	Criffell	Granodiorite	397	5.504	38.52	0.08639	0.512317 ± 20	0.51209	−0.6	0.3805	0.70763 ± 3	0.70548	+21
274	Criffell	Granite	397	2.992	16.30	0.1110	0.512255 ± 26	0.51197	−3.1	3.858	0.72866 ± 3	0.70684	+40
55–75	Fleet	Granite	392	7.489	49.77	0.09097	0.512242 ± 22	0.51201	−2.4	2.488	0.72018 ± 3	0.70629	+32
56–72	Fleet	Granite	392	6.013	33.20	0.1095	0.512265 ± 20	0.51198	−2.9	4.139	0.73136 ± 3	0.7083	+60
57–73	Fleet	Granite	392	5.985	32.57	0.1111	0.512261 ± 21	0.51198	−3.0	5.183	0.73638 ± 2	0.7074	+47
RC 1420	S. Uplands	Basic-clast greywacke	400	5.975	31.87	0.1134	0.512473 ± 28	0.51218	+1.1	0.1443	0.70555 ± 6	0.70473	+10
RC 1427	S. Uplands	Acid-clast greywacke	400	5.813	29.54	0.1189	0.512092 ± 19	0.51178	−6.6	1.097	0.71669 ± 2	0.71049	+92
RC 1430	S. Uplands	Acid-clast greywacke	400	4.806	25.29	0.1149	0.512155 ± 26	0.51185	−5.2	0.9055	0.71384 ± 3	0.70872	+67
CH 5	Cheviot	Granite	390	8.469	36.34	0.1409	0.512277 ± 30	0.51192	−4.2	1.380	0.71376 ± 10	0.70610	+29
RC 971	Eskdale	Granite	429	5.688	30.95	0.1111	0.512134 ± 28	0.51182	−5.1			0.7076*	+51

All isotopic analyses were performed using a fully automated V.G. Isomass 54E mass spectrometer except that $(^{87}Sr/^{86}Sr)_t$ marked (*) and all $^{87}Rb/^{86}Sr$ ratios except those of Garabal Hill and Distinkhorn (which are new determinations) are from refs 8 and 11. Ion exchange techniques were similar to those of O'Nions *et al.*[39]. $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ are normalized to $^{146}Nd/^{144}Nd = 0.7219$ and $^{88}Sr/^{86}Sr = 8.37521$ respectively. Bulk-Earth parameters used to calculate ε values are $^{143}Nd/^{144}Nd = 0.512636$, $^{147}Sm/^{144}Nd = 0.1967$, $^{87}Sr/^{86}Sr = 0.7045$, $^{87}Rb/^{86}Sr = 0.0839$. The $^{143}Nd/^{144}Nd$ of BCR-1 is 0.512633 ± 12 and $^{87}Sr/^{86}Sr$ of NBS 987 is 0.710275 ± 7 on the V.G. Isomass 54E at SURRC.

tal crust have affected the isotopic compositions to the extent that a change in the age of the basement is recognizable across the Grampian Highlands.

Nd isotopic data

Sm–Nd isotopic analyses of samples from 18 plutons, together with three Southern Uplands Lower Palaeozoic sediments, are presented in Table 1. Techniques have been presented elsewhere[20] and are summarized in Table 1. Many of the Sr isotopic analyses in Table 1 have been presented before[8,11] but some are new determinations of higher precision. The initial ε_{Nd} data are plotted geographically in Fig. 1 together with those of Hamilton *et al.*[4].

It is immediately apparent that nearly all the Caledonian granitic magmas had negative initial ε_{Nd} values the range being from +1.3 to −13.3. This situation can be compared with that of the coeval I and S type granitoids of the Lachlan fold belt, south-east Australia where the initial ε_{Nd} values have been reported as ranging between +4.4 and −9.8 (refs 5, 21). It appears that the range of initial Nd isotopic compositions in the British Caledonian granitoids is displaced to less radiogenic values by ~3 ε_{Nd} units. The British Caledonian granites can also be contrasted with the younger granitic magmas from convergent plate margins such as the Sierra Nevada[2,3], the Andes[1], and the Transhimalayan Belt[22], all of which are of Mesozoic–Tertiary age and which include substantial associated plutonics with high ε_{Nd}.

The only British intrusions with (just) positive initial ε_{Nd} are Cluanies and Strontian in the Northern Highlands, Kilmelford in the Grampian Highlands and Distinkhorn in the Midland Valley. Very negative initial ε_{Nd} values (<−6) are restricted to the Scottish Highlands (Fig. 1). Thus the Nd isotope systematics display parallels with common Pb and U–Pb zircon systematics in showing differences from north to south across northern Britain[6,9]. In contrast the Sr and O isotope systematics show no such simple geographical zonation[8,10]. The variation in ε_{Nd} could be due to changes in the mantle across Scotland as has been suggested for some of the cogenetic Devonian volcanics[23], greater crustal assimilation in the north or assimilation of different kinds of crust. This is best investigated by combining the Nd data with those of other isotopic systems.

Coupled Sm–Nd and U–Pb systematics

In Fig. 2 the ε_{Nd} values of the Newer granites for which zircon data are available are plotted. The shaded boxes represent granites with marked inherited zircon components and it is clear that an approximate cutoff of −6 in ε_{Nd} generally demarcates the boundary between granites with inherited zircons and those without. Several points arise from this.

(1) As the inherited zircons are unequivocally derived from the continental crust the lower ε_{Nd} values are also a function of crustal contamination or melting.

(2) Granites such as Hill of Fare, Lochnagar, Cruachan which contain little or no inherited zircon, but which lie in the Scottish Highlands, also have high ε_{Nd}. That is, the lack of inherited zircons is not a function of their loss by restite separation, but of a different source region.

(3) The Aberdeen and Findhorn granites are thought to be >~440 Myr in age[8,24] and their low ε_{Nd} values and inherited zircons reflect a dominance of old crustal components expected from magmas which were emplaced relatively soon after the climax of high-temperature metamorphism in that area. This accords with the findings of Hamilton *et al.*[4] for the Strichen and Longmanhill intrusions of similar age and setting.

(4) Excluding Findhorn and Aberdeen, a boundary to the limit of ε_{Nd} values of <−6 can be placed through the middle of the Grampian Highlands (Fig. 1). Hereafter this line is called the Mid-Grampian Line.

(5) The Strontian Complex lacks inherited zircons in its outer more mafic parts but contains zircons with a marked memory in the central biotite granite[6,8]. The ε_{Nd} values correlate with this in that they are close to bulk Earth in the outer parts but −6.3 in the biotite granite[4].

Nd–Sr–O isotopic relationships

In Fig. 3, initial ε_{Nd} is plotted versus initial ε_{Sr} for all the Caledonian granites analysed. There is no correlation between initial ε_{Nd} and initial ε_{Sr}. The >440-Myr granites plot to the

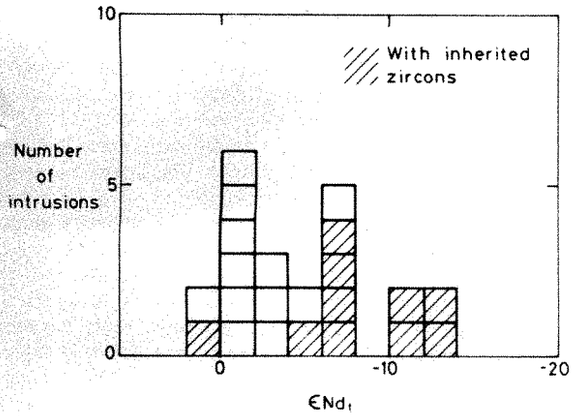


Fig. 2 Histogram of ε_{Nd_t} for all the post-tectonic granite intrusions for which zircon data are available. Shaded boxes are granites with inherited zircons. Data from this study and refs 4, 6, 8.

right with very radiogenic Sr. Moinian or Dalradian assemblages (largely comprising late Proterozoic metasediments) would be suitable crustal components for these magmas[8,25,26]. Some of the granites with inherited zircons plot towards the field of Lewisian crust. Granitic complexes such as Ross of Mull and Ratagain situated very close to the exposed Lewisian and with low ε_{Nd} probably contain a large component of Lewisian crust. However, many of the granites north of the Mid-Grampian Line have exceedingly high Sr contents[8,27] such that assimilation of Moinian or Dalradian assemblages (with low Sr concentrations) would change the ε_{Nd} value towards that of the contaminant more than would be detectable in ε_{Sr}. From this reconnaissance study such a process cannot be ruled out.

The Cluanies granodiorite is anomalous in having low ε_{Sr} and high ε_{Nd} but a marked inherited zircon component. The simplest explanation of this is that it is derived from old light rare-earth elements (LREE)-depleted crust. Some of the more mafic Lewisian granulites might be a suitable source[28].

The granites without inherited zircons plot between mantle-like compositions close to bulk Earth and the field of Southern Uplands Lower Palaeozoic sediments. It has been shown elsewhere that these sediments had Pb and Sr isotopic compositions that were similar to many of the Caledonian granites[8,9,11,41]. They also have the required paucity of Pb isotopic memory in the zircons such that melting or assimilation of these sediments (unlike those of the Moinian or Dalradian) would not greatly affect the zircon U–Pb systematics in the granites[8]. This is important because there is no doubt that these granites have interacted greatly with the continental crust as shown by their Sr and O isotopic signatures[10,11].

In Fig. 4, the initial ε_{Nd} values are plotted against $\delta^{18}O$ values given elsewhere[10,11]. Two samples are ^{18}O-depleted which is thought to be due to high temperature interaction with meteoric waters at their sub-volcanic emplacement level[10]. The Helmsdale granite has high $\delta^{18}O$ which has been attributed to late low-temperature fluid interaction[29]. In contrast the remaining granites have all been regarded as recording magmatic oxygen isotopic compositions, which correlate with initial ε_{Sr} but can be seen to be uncorrelated with initial ε_{Nd}. However, if the granites with no inherited zircons are considered alone, an inverse correlation is evident—the data points lying between values expected for a depleted mantle source ($\delta^{18}O \sim 6.0‰$, $\varepsilon_{Nd} > 0$) and the values for the Southern Uplands acid-clast Lower Palaeozoic sediments. This field of data includes the Strontian granodiorite from the Northern Highlands and samples from Lochnagar, Hill of Fare, Garabal Hill and Distinkhorn—all to the north of the Southern Uplands Lower Palaeozoic sediments. It can be seen that Dalradian metasediments (in which these last granites are situated) are not a major constituent of the source. The field also includes the Southern Uplands granites themselves plus those of the Lake District. The S-type[30] granites, Eskdale, Fleet and central Criffell all have high $\delta^{18}O$ (>10.0‰) and slightly lower ε_{Nd} (<−2). The outer part of Criffell has higher ε_{Nd} consistent with previous hypotheses that the Sr and O isotopic zonation of the pluton is due to interaction with Palaeozoic sediment or its melt derivatives[11,31–33]. The Shap granite has I-type mineralogy but high $\delta^{18}O$ and ε_{Sr} similar to the values for the S-type Eskdale intrusion. The difference in ε_{Nd} is consistent with the hypothesis that crustal metasomatic fluids have affected the Shap granite and modified its Sr and O isotopic compositions.

The granites from the north of the Mid-Grampian Line exhibit a limited range of $\delta^{18}O$ and no correlation with ε_{Nd}. This is hardly surprising as old high-grade metasedimentary rocks such as comprise the local geology commonly have lower $\delta^{18}O$ values than those of lower grade. Therefore , in general terms, it is to be expected that $\delta^{18}O$ will correlate with $^{87}Sr/^{86}Sr$ as observed because high grade metamorphic rocks will be depleted in Rb as well as having relatively low $\delta^{18}O$. In contrast high-grade

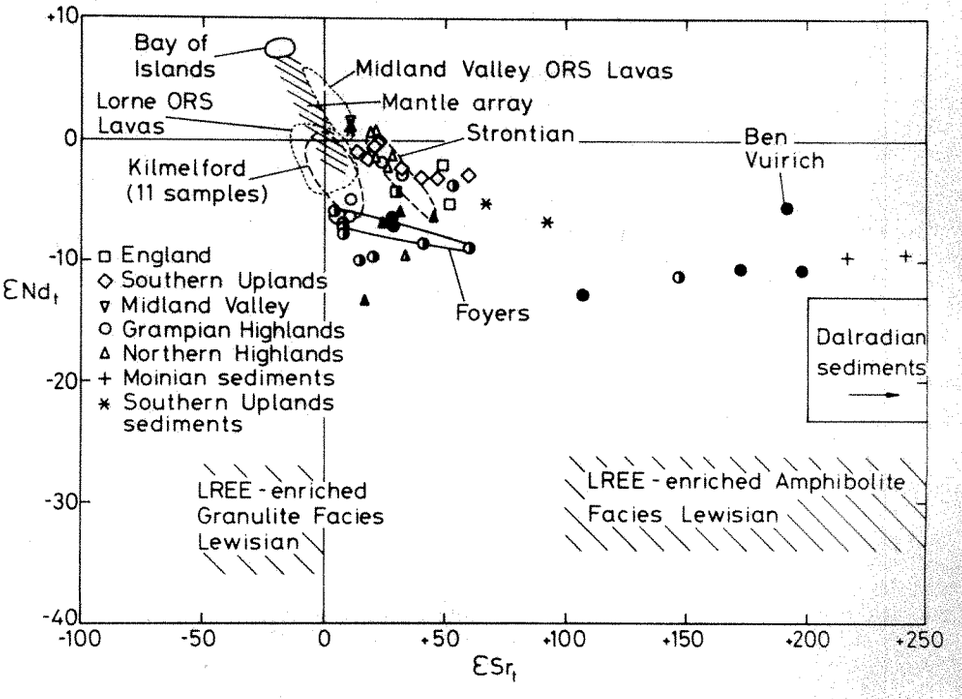


Fig. 3 ε_{Nd_t} versus ε_{Sr_t} plot showing data from the study of Hamilton *et al.*[4] and this study. Open symbols are for intrusions lacking inherited zircons, filled symbols are for intrusions with a marked inherited zircon memory, half-filled symbols are for intrusions with no U–Pb zircon data. Mantle array is constructed using bulk Earth parameters, the Bay of Islands ophiolite data[40] and by analogy with the modern mantle array. Other data sources are: Lewisian (ref. 28), Kilmelford (unpublished data), ORS Lavas (ref. 23), Moinian (ref. 25), Dalradian (ref. 8, 26).

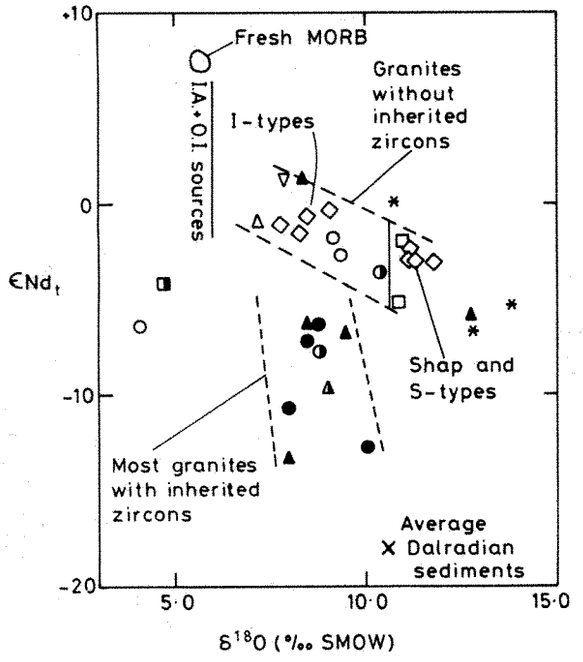


Fig. 4 ε_{Nd_t} versus $\delta^{18}O$. Symbols as in Fig. 3. Data from refs 4, 10, 11 and this study with estimates of fields for mid-ocean ridge basalts (MORB), oceanic islands (OI) and island arcs (IA). Dalradian average is from refs 26, 29.

metamorphism and diagenesis do not readily result in disturbance of U–Pb zircon or Sm–Nd systematics, hence the strong correspondence between those two parameters shown in Fig. 2, but the lack of strong correlation between ε_{Nd} and $\delta^{18}O$ or ε_{Sr} in the Highland granites (Figs 3 and 4).

The fundamentally important feature of Fig. 4 is that it indicates that granites with marked inherited zircon components and low ε_{Nd} have not been more contaminated by the continental crust than granites lacking inherited zircons and with $\varepsilon_{Nd} > -6$. They have instead been contaminated by or melted from a different kind of crust, that is, one with markedly older components.

Following on from this it can be seen that the Lochnagar, Hill of Fare and Garabal Hill granitoids, despite having high ε_{Nd} and no inherited zircons, have interacted to a major degree with continental crust yielding $\delta^{18}O$ values of ~10‰. Hence although situated in the Scottish Highlands their lack of inherited zircons and high ε_{Nd} do not reflect uncontaminated mantle-derived magmas, but magmas contaminated with young continental crust like that with which the Southern Uplands and English granitoids interacted. U–Pb zircon and Sm–Nd studies of Dalradian[8,26] lithologies indicate that melting of Dalradian crust would have lowered the ε_{Nd} drastically and imparted inherited zircons to the magma (Fig. 4). The data are therefore most simply explained if younger continental crust has been thrust under the southern side of the Grampian Highlands and provided the source for these granite magmas. Finally it should be noted that magmas with a large mantle component could easily plot in the area of intersection of the two fields in Fig. 4 whatever the contaminating crust was like.

Magma genesis in northern Britain

The British late Caledonian granites were clearly not generated by melting of mantle or subducted oceanic crust on their own. The combined data indicate that the continental crust has drastically affected the isotopic characteristics of the magmas. There are certain features of the magmas, however, which indicate that the granites are not simply the product of crustal melting but that many contain a mantle-derived component. These include the abundance of cogenetic high Ni dioritic magmas, the high levels of elements such as Sr (most easily achieved by differentiation at mantle depths) and the presence of cogenetic basaltic and andesitic lavas[27]. This, plus the large variation in ε_{Nd} observed in a single complex, such as at Kilmelford, suggests that in some cases mantle-derived magmas have assimilated continental crustal melts during their ascent.

In Fig. 3 the fields of data for Devonian mafic lavas from the Midland Valley and Lorne[23] are plotted. The Lorne Lavas are situated in the south-west Grampian Highlands (Fig. 1) and are thought to be cogenetic with the Kilmelford complex. Note that there is an overall downwards displacement of the plutonic data relative to the volcanics. This partly reflects the fact that the data for the volcanics are for high Ni lavas only, whereas the plutonics are largely granodiorites or granites. *Hence the more* silicic compositions can be (not surprisingly) related to greater interaction with the continental crust. Just as the granites south of the Mid-Grampian Line have higher ε_{Nd} than those to the north so also do the Devonian mafic volcanics but displaced overall to higher values (Fig. 3). This parallel behaviour is not regarded as coincidental but is most simply explained by the same process, that is, assimilation of differing ages of crust north and south of the Mid-Grampian Line. This alternative explanation is totally at variance with the mantle heterogeneity model previously invoked to explain the Nd data for the lavas[23].

The data for the lavas do indicate that magmas with 'depleted mantle' isotopic signatures were intruding the continental crust at the time. There is no way that one can assume that the same such magmas were parental to all the intermediate-acid magmatism in the Highlands. It would not be surprising, for example, if the changes in age of continental basement corresponded to changes in the nature of the underlying mantle. What is surprising is that even the diorites from Loch Doon with quite low $\delta^{18}O$ (Fig. 4) have negative ε_{Nd}. Unlike those of the Midland Valley these magmas yield no evidence of having been derived from depleted mantle or oceanic crust, which suggests that the Iapetus oceanic crust had been completely consumed by subduction under the Midland Valley by late Silurian times. This is contrary to previous suggestions[34]. It further requires that the crust underlying the Southern Uplands accretionary prism is allochthenous as suggested elsewhere[35,36].

Contamination with older continental crust might explain why the ε_{Nd} values of the British granites appear to be displaced by three units relative to the Australian granites. The contaminant clearly varies from Lewisian crust in the far north-west of Scotland to Lower Palaeozoic sediment in the Southern Uplands and Lake District. The exact nature of the basement in between is uncertain. Some of the northerly basement would need to be Lewisian or its sedimentary/magmatic derivatives (Moinian or Dalradian, for example) to produce low ε_{Nd} and marked inherited zircons. The southerly basement would need to be like Palaeozoic upper crust further south. Nd and U–Pb zircon data for granulite facies nodules erupted with Midland Valley Carboniferous Lavas indicate a striking similarity with corresponding data for Lower Palaeozoic sediments of the Southern Uplands[25,37]. A simple way to explain the data for the granites and the granulite facies basement is for the basement to represent Lower Palaeozoic crust thrust northward during Iapetus closure. The view that primary crust of Grenville (~1,000 Myr) age is an important crustal component in the Highlands granites[38] is difficult to reconcile with the U–Pb zircon evidence. In particular, nearly all of the late Caledonian granites analysed which contain inherited zircons yield data which lie about a reverse discordia with an upper intercept of ~1,600 Myr (ref. 6). Furthermore neither U–Pb zircon[8] nor Sm–Nd whole rock[26] studies of Scottish Highlands sediments provide evidence for local Grenville-age primary crust. Underthrusting of the crust during the late Caledonian helps to explain the problem of finding a unique crustal component in the Highlands granites[8,10,29,38] as the crustal structure is likely to have been exceedingly complex. It also provides a partial explanation for the large crustal component in the granites as a whole as juxtaposing crust over hot mantle would be a way of generating granite magma. The simplest interpretation of the data shown

in Fig. 4 is that the southerly crust was largely young accreted material such as arc, trench and oceanic crust fragments.

W. E. Stephens, M. Thirlwall, J. Soper, D. Hutton, B. J. Bluck and B. G. Upton have provided samples and discussion which have benefited the paper. I thank J. Hutchinson for technical assistance. J. Bennet, D. Maclean and J. Hewitt helped prepare the script. The Isotope Geology Unit at SURRC is supported jointly by the Scottish Universities and NERC. Research on the British Caledonian granites is also supported by the NERC grant GR3/4633.

Received 2 June; accepted 19 September 1983.

1. Allègre, C. J. & Othman, D. B. *Nature* **286,** 335–342 (1980).
2. DePaolo, D. J. *Science* **209,** 684–687 (1980).
3. DePaolo, D. J. *J. geophys. Res* **86,** 10470–10488 (1981).
4. Hamilton, P. J., O'Nions, R. K. & Pankhurst, R. J. *Nature* **287,** 279–284 (1980).
5. McCulloch, M. T. & Chappell, B. W. *Earth planet. Sci. Lett.* **58,** 51–64 (1982).
6. Pidgeon, R. T. & Aftalion, M. *Geol. J. Spec. Iss.* **10,** 183–220 (1978).
7. Pitcher, W. S. & Berger, A. R. *The Geology of Donegal: a Study of Granite Emplacement and Unroofing* (Wiley, London, 1972).
8. Halliday, A. N., Aftalion, M., van Breemen, O. & Jocelyn, J. in *The Caledonides of the British Isles—Reviewed* (eds Harris, A. L., Holland, C. H. & Leake, B. E.) 653–661 (Scottish Academic, Edinburgh, 1979).
9. Blaxland, A. B., Aftalion, M. & van Breemen, W. *Scott. J. Geol.* **15,** 139–151 (1979).
10. Harmon, R. S. & Halliday, A. N. *Nature* **283,** 21–25 (1980).
11. Halliday, A. N., Stephens, W. E. & Harmon, R. S. *J. geol. Soc. Lond.* **137,** 329–348 (1980).
12. Halliday, A. N. *Miner Mag.* **44,** 437–442 (1981).
13. Brown, G. C. & Hennessy, J. *Phil. Trans. R. Soc.* A**288,** 631–643 (1978).
14. Brown, G. C. in *The Caledonides of the British Isles—Reviewed* (eds Harris, A. L., Holland, C. H. & Leake, B. E.) 645–651 (Scottish Academic, Edinburgh, 1979).
15. Simpson, P. R., Brown, G. C., Plant, J. & Ostle, D. *Phil. Trans. R. Soc.* A**291,** 385–412 (1979).
16. Brown, G. C. & Locke, C. A. *Earth planet. Sci. Lett.* **45,** 69–79 (1979).
17. Leake, B. E. *Geol. J. Spec. Iss.* **10,** 221–248 (1978).
18. Pankhurst, R. J. in *Origin of Granite Batholites—Geochemical Evidence* (eds Aterton, M. P. & Tarney, J.) 18–33 (Shiva, Orpington, 1979).
19. Leake, B. E., Brown, G. C. & Halliday, A. N. *J. geol. Soc. Lond.* **137,** 93–97 (1980).
20. Halliday, A. N. *et al. Earth planet. Sci. Lett.* **63,** 241–256 (1983).
21. McCulloch, M. T., Chappell, B. W. & Hensel, H. D. *Abstr. 5th int. Conf. Geochron. Cosmochron. Isot. Geol.* 246–247 (1982).
22. Gareipy, C., Xu, R. H. & Allègre, C. J. *Terra Cognita* **3,** 266 (1983).
23. Thirlwall, M. F. *Earth planet. Sci. Lett.* **58,** 27–50 (1982).
24. van Breemen, O. & Piasecki, M. A. J. *J. geol. Soc. Lond.* **140,** 47–62 (1983).
25. van Breeman, O. & Hawkesworth, C. J. *Trans. R. Soc. Edinb. Earth Sci.* **71,** 97–102 (1980).
26. O'Nions, R. K., Hamilton, P. J. & Hooker, P. J. *Earth planet. Sci. Lett.* **63,** 229–240 (1983).
27. Halliday, A. N. & Stephens, W. E. *Phys. Earth planet. Inter.* (in the press).
28. Hamilton, P. J., Evensen, N. M., O'Nions, R. K. & Tarney, J. *Nature* **277,** 25–28 (1979).
29. Harmon, R. S., Halliday, A. N., Stephens, W. E. & Clayburn, J. A. P. *Phil. Trans. R. Soc.* A (in the press).
30. Chappell, B. W. & White, A. J. R. *Pacif. Geol.* **8,** 173–174 (1974).
31. Stephens, W. E. & Halliday, A. N. in *Origin of Granite Batholiths—Geochemical Evidence* (eds Atherton, M. P. & Tarney, J.) 9–17 (Shiva, Orpington, 1979).
32. Stephens, W. E. & Halliday, A. N. *Bull. geol. Soc. Am.* **91,** 165–170 (1979).
33. Halliday, A. N. in *Migmatites and Crustal Melting* (eds Atherton, M. P. & Gribble, C. D.) 54–61 (Shiva, Orpington, 1983).
34. Brown, G. C., Cassiday, J., Tindle, A. G. & Hughes, D. J. *J. geol. Soc. Lond.* **136,** 745–753 (1979).
35. Bluck, B. J. & Halliday, A. N. *Geology* **10,** 331–333 (1982).
36. Leggett, J. K., McKerrow, W. S. & Soper, N. J. *Tectonics* **2,** 187–210 (1983).
37. Halliday, A. N., Aftalion, M., Upton, B. G. J., Aspen, P. & Jocelyn, J. *Trans. R. Soc. Edinb.* (submitted).
38. Clayburn, J. A. P., Harmon, R. S., Pankhurst, R. J. & Brown, J. F. *Nature* **303,** 492–497 (1983).
39. O'Nions, R. K., Hamilton, P. J. & Evensen, N. M. *Earth planet. Sci. Lett.* **34,** 13–22 (1977).
40. Jacobsen, S. B. & Wasserburg, G. J. *J. geophys. Res.* **84,** 7429–7445 (1979).
41. Thirlwall, M. F. *J. Volcanol. Geotherm. Res.* (in the press).

Molecular cloning of cDNA for murine interleukin-3

M. C. Fung, A. J. Hapel,* S. Ymer, D. R. Cohen, R. M. Johnson, H. D. Campbell & I. G. Young

Departments of Biochemistry and *Medicine and Clinical Science, John Curtin School of Medical Research, Australian National University, PO Box 334, Canberra, ACT 2601, Australia

The cDNA sequence for murine interleukin-3, one of the colony stimulating factors that regulate haematopoiesis, codes for a polypeptide of 166 amino acids including a putative signal peptide. The predicted amino acid sequence indicates that formation of mature interleukin-3 involves proteolytic removal of not only the signal peptide but additional amino-terminal amino acids.

INTERLEUKIN-3 (IL-3) is one of a number of colony stimulating factors which are known to regulate haematopoiesis[1–3]. The colony stimulating factors are hormone-like glycoproteins which are biologically active at concentrations of 10^{-12} M. They are produced in minute quantities *in vivo* and because of this have proven to be very difficult to characterize by conventional biochemical methods.

IL-3 is produced by mitogen or antigen-activated T lymphocytes and by a number of continuous cell lines[4,5]. It may also be produced by bone marrow cells. IL-3 is involved in regulating the growth and differentiation of pluripotent stem cells leading to the production of all the major blood cell types. It has a broad range of biological activities and appears to be identical with a number of other factors named on the basis of their biological activities. These include multi-colony stimulating factor CSF, haematopoietic growth factor[6], burst-promoting activity[7], P-cell stimulating factor[8], mast cell growth factor[9], histamine-producing cell stimulating factor[10] and Thy 1-inducing activity[11].

Murine IL-3 derived from the myelomonocytic leukaemia cell line WEHI-3 has recently been purified to homogeneity and a portion of its N-terminal sequence and some of its biochemical and biological properties reported[12,13]. The protein is glycosylated and the molecular weight (MW) of the major glycosylated species was estimated to be 28,000. A second species of IL-3 with the same N-terminus was also purified which had an apparent MW of 32,500 suggesting that the two forms may differ in their degree of glycosylation. In the present work we describe translation of IL-3 mRNA in *Xenopus laevis* oocytes and the cloning and sequence analysis of a cDNA coding for murine IL-3.

Translation of IL-3 mRNA in oocytes

Two different murine cell lines were compared as sources of IL-3 mRNA. The myelomonocytic line WEHI-3 is known to produce IL-3 constitutively[14] whereas the lymphoma line EL-4 produces IL-3 after stimulation with phorbol myristate acetate (PMA)[5]. Messenger RNA was prepared from both cell lines using the guanidine thiocyanate method[15,16]and attempts were made to translate the mRNAs by microinjection into *X. laevis* oocytes[17]. The translates were assayed for biologically active IL-3 using the IL-3 dependent cell line 32D cl-23 (ref. 18). It was found that mRNA from EL-4 could be translated in the oocytes to give readily detectable levels of IL-3 after 48 h incubation (Table 1). Most of the detectable activity was present in the incubation medium indicating that the oocytes were secreting mature IL-3. Assays of oocyte extracts showed that they were inhibitory. mRNA from WEHI-3 cells regularly gave higher levels of IL-3 in the translation assays than preparations from EL-4 (Table 1), in keeping with the higher levels of IL-3

© 1984 Macmillan Journals Ltd

Table 1 Translation of IL-3 mRNA in *Xenopus laevis* oocytes

mRNA	Incubation time	IL-3 activity (c.p.m.) Oocyte	Medium	Total extract
EL-4	24 h	0	3,100	3,700
	48 h	—	20,000	5,600
WEHI-3	48 h	6,000	47,000	28,700

EL-4 cells were grown to a cell density of $\sim 10^6$ per ml in spinner cultures in DMEM medium supplemented with 10% fetal calf serum. The cells were collected by centrifugation, resuspended in serum-free medium containing 100 ng ml^{-1} PMA and subcultured into 150 cm^2 culture flasks at 5×10^8 cells per flask. The cells were incubated for 10 h before collection. WEHI-3 cells were grown to a density of $\sim 10^6$ per ml in RPMI-1640 medium supplemented with 10% fetal calf serum and collected by centrifugation. Afterwards, both cell pellets were resuspended in 0.1 volume of serum-free medium and mRNA was prepared by the guanidine thiocyanate-CsCl method[15,16]. The mRNAs were further purified by chromatography on oligo(dT)-cellulose[32] and after ethanol precipitation, were resuspended in buffer (10 mM Tris-HCl, 0.1 mM EDTA, *p*H 7.5) at a concentration of 1 μg μl^{-1}. The mRNAs were injected into 15 *X. laevis* oocytes (~50 nl per oocyte) according to standard methods[17]. After incubation, the medium was withdrawn and an extract of the oocytes prepared[17]. The incubation medium, the oocyte extract and a mixture of the two were assayed for IL-3 activity using the IL-3 dependent cell line 32D cl-23 (ref. 18) according to the methods described previously, measuring incorporation of ^{3}H-thymidine as an index of cell growth.

Table 2 mRNA hybridization translation assay for detection of IL-3 cDNA clones

DNA sample	IL-3 activity (units per ml)
1	<1
2	<1
3	<1
4	<1
5	<1
6 (pILM1)	121, 106, 139
7	<1
8	<1
9	<1
10	<1

DNA samples (~30 μg) were prepared from each clone by the method of Holmes and Quigley[21]. The hybrid plasmid DNA was converted to a linear form by digestion with *Bam*HI restriction endonuclease, extracted with phenol and ethanol precipitated. The DNA was heated in 0.1 M NaOH for 20 min at 70° C, diluted with 20 vol of 2 M NaCl and applied to 1 cm diameter nitrocellulose filters by slow filtration. The filters were washed with 6×SSC (1×SSC: 0.15 M NaCl, 0.015 M Na citrate, *p*H 7.0) and baked *in vacuo* at 80 °C for 2 h. The 10 membranes were hybridized with 400 μg WEHI-3 mRNA under the conditions described previously[31] and the bound mRNA eluted according to the procedure of Parnes *et al.*[33]. The eluted mRNA was translated in oocytes as described in Table 1. A unit of IL-3 activity is that required to give 50% of the maximum incorporation of ^{3}H-thymidine in the assay.

found in culture supernatants of the WEHI-3 cell line (data not shown).

Hybrid plasmids containing IL-3 cDNA

IL-3 mRNA from WEHI-3 was fractionated on a sucrose density gradient and the fractions assayed by oocyte translation (Fig. 1). The major peak of activity sedimented at ~12S. This procedure resulted in 10–20-fold purification of the IL-3 mRNA. RNA from the peak fractions was used as template to synthesize double-stranded cDNA using the method of Land *et al.*[19]. The resultant cDNA was inserted into pAT153 by G–C tailing[20] and the cDNA library transformed into strain HB101. Approximately 5,000 individual clones were isolated. Of a small sample tested, 92% were ampicillin sensitive indicating the presence of cDNA inserts in the majority of the clones. Since the cDNA was not size fractionated prior to cloning, the sizes of inserts in the cDNA library varied from about 200 to 1,000 base pairs (bp). It was expected that even the short inserts would give satisfactory results in the mRNA hybridization–translation assay (see below).

Clones were grown in pools of 10 and DNA prepared by the method of Holmes and Quigley[21]. The plasmids were converted to a linear form with *Bam*HI restriction endonuclease, contaminating RNA was destroyed by alkaline hydrolysis and the DNA was bound to nitrocellulose filters. The filter-bound DNA was hybridized with WEHI-3 mRNA and bound mRNA eluted and translated in oocytes. One positive pool of 10 clones was detected. The activity observed in translates derived from the positive pool was quite low but was consistently above the background and the values of the other clone pools. DNA was therefore prepared from the individual clones of the pool and the mRNA hybridization translation assay repeated. One of the clones (pILM1) was clearly positive (Table 2). The IL-3 activity detected showed the same titration curve as oocyte translates of WEHI-3 mRNA (Fig. 2).

Nucleotide sequence analysis

The cDNA insert in pILM1 was quite short (139 bp excluding the G-C tails). It was subcloned into the *Sma*I site of M13mp8[22] after shortening the G-C tails with *Bal*31 exonuclease. The DNA sequence was determined on both strands using the chain termination method[23,24]. An oligonucleotide primer corresponding to nucleotides 92–112 of this cDNA insert was synthesized[25,26] and labelled using [γ-^{32}P]dATP and polynucleotide kinase[27]. The labelled probe was used to examine the remaining clones in the cDNA library[28,29]. No other clones containing this sequence were detected.

To isolate a long clone encoding IL-3, a further cDNA library was prepared, but in this case the double-stranded cDNA was size fractionated using Sepharose CL-4B to enrich for cDNA fragments larger than ~500 bp. The resulting cDNA was inserted into pAT153 by G-C tailing and transformed into HB101. The labelled oligonucleotide probe described above was used to identify a longer cDNA clone (pILM3) which was shown by DNA sequencing to carry an insert of 629 bp excluding the G-C tails. The DNA sequence of clone pILM3 was determined on both strands using the chain termination method[23,24] after subcloning into M13mp8 and 9 (ref. 22). The sequencing strategy is given in Fig. 3*a*. The subcloning was complicated by the fact that one of the *Pst*I sites flanking the cDNA insert had been lost due to exonucleolytic removal of one nucleotide during the G tailing of the vector. Clone pILM3 was found to extend beyond pILM1 at the 3′ end only, both clones possessing the same 5′ end. The DNA sequence shows a single large open reading frame (Fig. 3*b*). Assuming that the first ATG from the 5′ end serves as the initiation codon, the reading frame extends from nucleotides 28–525 inclusive and specifies a polypeptide of 166 amino acids. The 5′ untranslated region is therefore quite short (27 bp). The method of Land *et al.*[19], which was used to generate the cDNA clones, is designed to produce a high proportion of cDNA clones with complete 5′ terminii. Since both cDNA clones isolated in the course of this work had the same 5′ end, it is possible that pILM3 carries a close to full length copy of the 5′ untranslated region of IL-3 mRNA. Clone pILM3, however, does not extend to the 3′ end of the IL-3 mRNA and is probably missing a significant portion of the 3′ untranslated region. The presence of the complete coding sequence for IL-3 is the important feature in the context of the present work.

The reading frame specifies a protein of MW 18,540. The strongly hydrophobic stretch of 9 amino acids (residues 13–21) is indicative of the presence of a signal peptide. In the cases studied to date, the last residue of the signal sequence is invariably an amino acid with a small uncharged side chain. Analysis of the signal peptide region suggests a number of potential sites for cleavage by the signal peptidase. Application of the empirical rules proposed by Von Heijne[30] predicts the end of the signal peptide at residue 27 (Ala). This potential cleavage site is shown with an arrow in Fig. 3*b*.

Table 3 Amino acid composition of murine IL-3

	Predicted	Determined
Phe	5	4.2
Leu	15	16.7
Ile	5	4.8
Met	1	1.2
Val	10	8.3
Ser	13	11.3
Pro	10	7.1
Thr	8	7.1
Ala	3	4.2
Tyr	2	1.8
His	2	2.4
Glu, Gln	12	13.7
Asp, Asn	18	16.1
Lys	9	9.5
Cys	4	ND
Trp	0	ND
Arg	11	9.5
Gly	6	8.3

The amino acid composition data are taken from ref. 13. The composition was originally calculated for a MW of 23,722 and has been normalized to the size of mature IL-3 which is predicted from the DNA sequence. The normalization was done using the values for Glu and Asp. ND, not determined.

The published N-terminal amino acid sequence of murine IL-3[12,13] is in complete agreement with the reading frame and extends from residues 33 (Asp) to 47 (Val). It seems most unlikely that the signal peptide would end at residue 32 (Arg) and this suggests that the murine IL-3 isolated from the WEHI-3 cell line has been further processed.

The protein sequence of mature processed IL-3 indicates a MW of 15,102 with four potential *N*-glycosylation sites (Asn-X-Ser or Asn-X-Thr). The four sites are underlined in Fig. 3*b*. One of these is in the region of the published N-terminal sequence and glycosylation at this site would explain the inability to identify the Asn residue during automated Edman degradation. When the amino acid composition figures for murine IL-3 (ref. 13) are normalized to the size predicted by the DNA sequence they give good agreement with the predicted composition (Table 3).

The amino acid sequence of murine IL-3 was compared with the sequences of human interleukin-2 (ref. 31) and human immune interferon[32] both of which are also produced by antigen-activated T lymphocytes. No significant sequence homology was detected. Although human IL-2 is of very similar size to IL-3 it carries no potential sites for *N*-glycosylation and does not appear closely related.

Discussion

The demonstration that IL-3 mRNA can be efficiently translated in oocytes to give biologically active factor provides a very sensitive and useful assay for this mRNA. This assay has enabled the identification of a cDNA clone carrying the entire coding sequence for murine IL-3 using the mRNA hybridization translation method.

Sequence analysis of the cloned cDNA has shown that it codes for a polypeptide of 166 amino acids including a signal peptide of possibly 27 amino acids. Mature IL-3 has been purified to homogeneity[12,13] from the same WEHI-3 cell line as was used as the source of the IL-3 mRNA in the present work. The N-terminal sequence of the mature IL-3 is in complete agreement with the reading frame but starts at residue 33 suggesting an additional proteolytic cleavage has occurred apart from the cleavage of the signal peptide. The proposed proteolytic cleavage could be catalysed by a number of serine-type proteases such as a member of the kallikrein family or plasmin and could provide another level of regulation if processing was required to generate biologically active IL-3. Whether this processing

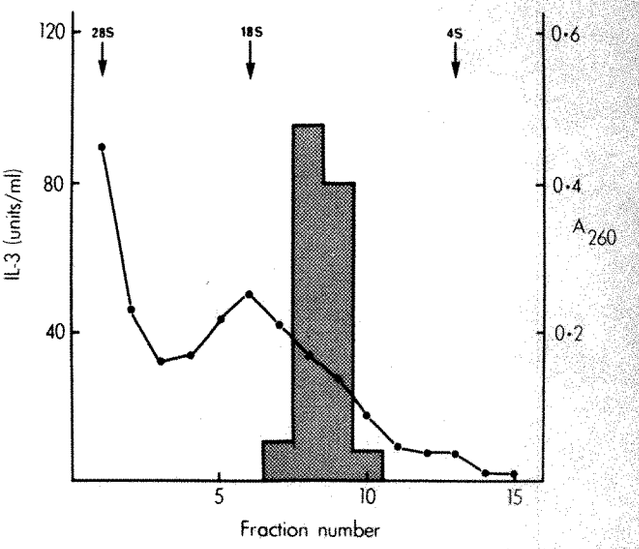


Fig. 1 Sedimentation of IL-3 mRNA through a sucrose density gradient. ●, A_{260}; hatched areas, IL-3 activity. Poly(A)$^+$ mRNA (500 μg) from WEHI-3 was heated at 80° C for 2 min, chilled in ice and applied to a 5–20% sucrose gradient prepared in a Beckman SW41 polyallomer tube. The gradient was centrifuged at 30,000 r.p.m. for 20 h at 4 °C and fractionated into 15 fractions by collection of drops. The fractions were ethanol precipitated and resuspended in 20 μl of buffer (10 mM Tris HCl, 0.1 mM EDTA, *p*H 7.5). A portion of the RNA was electrophoresed on a 1.5% agarose gel. The RNA was also translated in *X. laevis* oocytes as described in Table 1. One unit of IL-3 activity is that required to give 50% of the maximum incorporation of ^{3}H-thymidine in the assay.

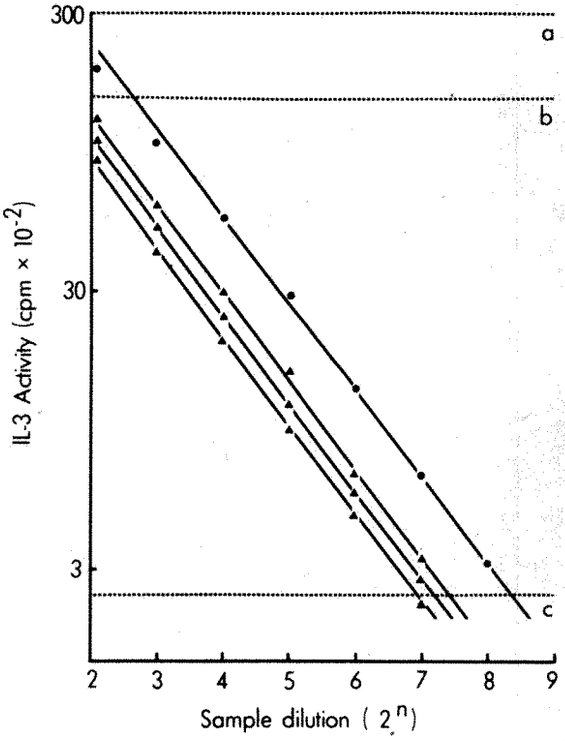


Fig. 2 Titration curves for the IL-3 activity present in oocyte translates. △, Translates of mRNA hybridizing to pILM1 (see Table 2); ●, translates of WEHI-3 mRNA. Twofold dilutions of the oocyte translates were made in microtitre trays and the IL-3 activity present was measured using the IL-3 dependent cell line 32D cl-23 as described previously[18]. The stimulation of growth of the indicator cells is expressed in terms of ^{3}H-thymidine incorporation. The dotted lines designated a, b and c represent incorporations of 100%, 50% and background plus three standard deviations respectively.

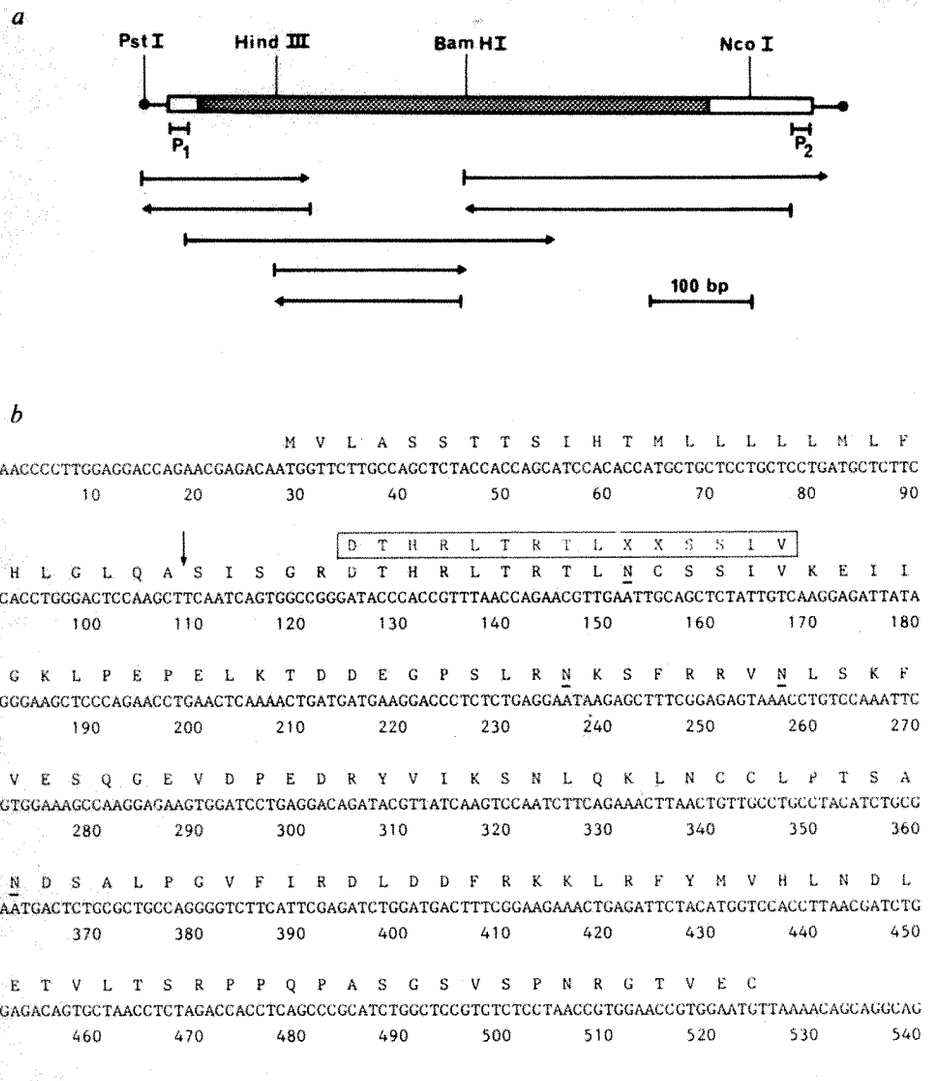


Fig. 3 *a*, The cDNA insert in pILM3 showing the relevant sites of restriction endonuclease cleavage. The reading frame is delineated from the untranslated regions by hatching and the flanking G-C tails are shown as short bars. One of the expected *Pst*I sites was lost during G-tailing of the vector pAT153. The sequencing strategy is summarized beneath the cDNA insert. DNA sequencing was carried out by the chain termination method[23,24] after subcloning fragments of pILM3 into M13mp8 and M13mp9[22]. The computer programs described by Staden[34,35] were used to assemble and analyse the sequence data. To avoid interference from the longer G-C tails, which was encountered using the universal primer of Duckworth *et al.*[36], two internal primers (P_1 and P_2) 17 nucleotides in length were synthesized[25,26] and used with the M13 templates. *b*, Nucleotide sequence of the cDNA insert of pILM-3 and predicted amino acid sequence of murine IL-3. The N-terminal sequence of murine IL-3[12,13] is boxed in above the predicted amino acid sequence. Potential sites for *N*-glycosylation are underlined. The arrow indicates a possible site of cleavage for removal of the signal peptide[30].

step occurs *in vivo* or simply represents adventitious proteolysis during purification is not clear at present.

Apart from the agreement with the N-terminal sequence, the amino acid composition data for mature IL-3 also give good agreement with the predicted sequence. Since it appears that WEHI-3 only produces one member of the group of colony stimulating factors (that is, IL-3), there seems no doubt that the cDNA clone encodes IL-3.

The isolation of a cDNA clone encoding murine IL-3 opens up a number of new molecular approaches to studying the role of this factor in haematopoietic stem cell differentiation. It should prove valuable in studies of proliferative blood disorders such as certain leukaemias and polycythaemias and should enable adequate quantities of murine IL-3 to be produced using suitable expression systems. It should also facilitate isolation of cDNA clones for human IL-3 and allow investigation of potential applications of this factor in clinical medicine in the area of bone marrow transplantation following cancer chemotherapy.

We thank Drs W. Doe and K. Lafferty for discussions, Drs D. Curtis and J. Shelton for assistance with the microinjection methods, Mr G. Mayo, Dr D. Magrath and Dr W. Amarego for their help with the synthesis of oligonucleotides and Ms J. Byatt and Ms J. Osborne for technical assistance.

Note added in proof: The CDNA encoding IL-3 has now been expressed in monkey COS cells using an SV40 expression vector. The expressed factor supports the growth of the IL-3 dependent cell lines; its full range of biological activities is currently being tested in collaboration with Dr D. Metcalf, Walter and Eliza Hall Institute of Medical Research, Melbourne.

Received 31 October; accepted 30 November 1983.

1. Burgess, A. W., Camakaris, J. & Metcalf, D. *J. biol. Chem.* **252,** 1998–2003 (1977).
2. Howard, M., Burgess, A. W., McPhee, D. & Metcalf, D. *Cell* **18,** 993–999 (1980).
3. Burgess, A. W., Metcalf, D., Russell, S. H. M. & Nicola, N. A. *Biochem. J.* **185,** 301–314 (1980).
4. Ihle, J. N., Lee, J. C. & Rebar, L. *J. Immun.* **127,** 2565–2570 (1981).
5. Ihle, J. N., Rebar, L., Keller, J., Lee, J. C. & Hapel, A. J. *Immun. Rev.* **63,** 5–32 (1982).
6. Bazill, C. W., Haynes M., Garland, J. & Dexter, T. M. *Biochem. J.* **210,** 747–759 (1983).
7. Iscove, N. N., Roitsch, C. A., Williams, N. & Guilbert, L. J. *J. cell. Physiol. Suppl.* **1,** 65–78 (1982).
8. Schrader, J. W., Lewis, S. J., Clark-Lewis, I. & Culvenor, J. G. *Proc. natn. Acad. Sci. U.S.A.* **78,** 323–327 (1981).
9. Nabel, G., Galli, S. J., Dvorak, A. M., Dvorak, H. F. & Cantor, H. *Nature* **291,** 332–334 (1981).
10. Dy, M., Lebel, B., Kamoun, P. & Hamburger, J. *J. exp. Med.* **153,** 293–309 (1981).
11. Schrader, J. W., Battye, F. & Scollay, R. *Proc. natn. Acad. Sci. U.S.A.* **79,** 4161–4165 (1982).
12. Ihle, J. N. *et al. J. Immun.* **131,** 282–287 (1983).
13. Ihle, J. N. *Contemp. Topics molec. Immun.* (in the press).
14. Lee, J. C., Hapel, A. J. & Ihle, J. N. *J. Immun.* **128,** 2393–2398 (1982).
15. McCandliss, R., Sloma, A. & Pestka, S. *Meth. Enzym.* **79,** 51–59 (1981).
16. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18,** 5294–5299 (1979).
17. Sloma, A., McCandliss, R. & Pestka, S. *Meth. Enzym.* **79,** 68–71 (1981).
18. Ihle, J. N. *et al. J. Immun.* **129,** 1377–1383 (1982).
19. Land, H., Grez, M., Hauser, H., Lindenmaier, W. & Schutz, G. *Nucleic Acids Res.* **9,** 2251–2266 (1981).
20. Michelson, A. M. & Orkin, S. H. *J. biol. Chem.* **257,** 14773–14782 (1982).
21. Holmes, D. S. & Quigley, M. *Analyt. Biochem.* **114,** 193–197 (1981).
22. Messing, J. & Vieira, J. *Gene* **19,** 269–276 (1982).

23. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
24. Sanger, F., Coulson, A. R., Hong, G. F., Hill, D. F. & Petersen, G. B. *J. molec. Biol.* **162,** 729–773 (1982).
25. Tanaka, T. & Letsinger, R. L. *Nucleic Acids Res.* **10,** 3249–3260 (1982).
26. McBride, L. J. & Caruthers, M. H. *Tetrahedron Lett.* **24,** 245–248 (1983).
27. Goodman, H. M. & MacDonald, R. J. *Meth. Enzym.* **68,** 75–90 (1979).
28. Grunstein, M. & Hogness, D. S. *Proc. natn. Acad. Sci U.S.A.* **72,** 3961–3965 (1975).
29. Wallace, R. B. *et al. Nucleic Acids Res.* **6,** 3543–3557 (1979).
30. Von Heijne, G *Eur. J. Biochem.* **133,** 17–21 (1983).
31. Taniguchi T. *et al. Nature* **302,** 305–310 (1983).
32. Gray, P. W. *et al. Nature* **295,** 503–508 (1982).
33. Parnes, J. R. *et al Proc. natn. Acad. Sci U.S.A.* **78,** 2253–2257 (1981).
34. Staden, R. *Nucleic Acids Res.* **5,** 1013–1015 (1978).
35. Staden, R. *Nucleic Acids Res.* **8,** 3673–3694 (1980).
36. Duckworth, M. L. *et al. Nucleic Acids Res.* **9,** 1681–1706 (1981).

A molecular map of the human major histocompatibility complex class III region linking complement genes C4, C2 and factor B

Michael C. Carroll, R. Duncan Campbell, David R. Bentley & Rodney R. Porter

MRC Immunochemistry Unit, Biochemistry Department, Oxford University, South Parks Road, Oxford OX1 3QU, UK

Four human complement genes, which have previously been mapped between HLA-D and HLA-B on chromosome 6, have now been aligned on a 98-kilobase (kb) section of the chromosome on the basis of four overlapping cosmid clones of genomic DNA. The C2 and factor B genes, less than 2 kb apart, are about 30 kb from two C4 genes separated from each other by about 10 kb.

THE major histocompatibility complex (MHC) in man (HLA) is a highly polymorphic region of approximately 2 centimorgans (2×10^6 base pairs, bp) on the short arm of chromosome 6[1]. It contains three major classes of genes: class I and class II code for cell-surface glycoproteins which are concerned in self–non-self recognition of cells involved in the immune response, and class III code for some proteins of complement, the major effector system of humoral immunity[2]. There is clinical interest in the HLA not only because the products of the class I and class II genes are the major barrier to tissue transplantation but also because susceptibility to many autoimmune diseases is correlated with the presence of certain haplotypes of class I and II[3] and also, because of close linkage[4], of the class III genes. It has been suggested that the polymorphism of the class III proteins may be a factor in susceptibility to autoimmunity[5].

The class III genes code for the complement proteins C2, C4 and factor B. C2 and factor B are novel types of serine proteases which, on activation in association with C4 and C3, respectively, form the C3 convertases of the classical pathway ($\overline{C42}$) and the alternative pathway ($\overline{C3B}$) of complement activation[6]. C2 and factor B are single peptide-chain proteins of approximately 100,000 and 90,000 molecular weight (M_r) while C4 has three disulphide-linked chains, α (95,000 M_r), β (75,000) and γ (30,000), but is synthesized as a single peptide chain of 200,000 M_r (ref. 7).

The structural genes of C4, C2 and factor B have been mapped between HLA-B and HLA-D using protein variants detected mainly by electrophoresis (summarized by Weitkamp and Lamm[8]). C2 and factor B seem to be encoded at single loci but C4 is coded by at least two closely linked loci, C4A and C4B (ref. 9). Four alleles have been recognized in C2 (ref. 10), 11 in factor B[11], 13 at C4A and 22 at C4B[12]. Null (non-functional) alleles have also been found in C2, C4A and C4B but not so far in factor B[8].

The recent availability of cDNA probes specific for factor B[13,14], C4[15–17] and C2[18] has allowed the isolation of these complement genes. The factor B gene has been isolated and characterized by extensive nucleotide sequence analysis[14]. The C4 cDNA probe has been used to characterize partially a C4 gene[15] and to correlate a DNA restriction fragment length polymorphism with the C4 A6 protein phenotype[19].

We have used cDNA probes specific for C4,[15] factor B[14] and C2[18] to map the genes for these three complement proteins in the HLA class III region, by overlapping cloned fragments from a cosmid library of human genomic DNA. The results were confirmed and extended by Southern analysis of whole genomic DNA. There are at least four class III genes in 98 kilobases (kb) of genomic DNA. There are two C4 loci separated by approximately 10 kb and single factor B and C2 loci which are less than 2 kb apart. The genes are arranged in the order C2, factor B and two C4 genes.

Detection of two C4 genes

The HPFH cosmid library (described in Fig. 2 legend) prepared from genomic DNA extracted from blood cells of a 13-yr-old female, complement typed as C4: A3, A2, B1, B2; factor B: SS; (C2 not determined), was screened with a C4-specific cDNA probe, *Alu-7* (ref. 15). Positive clones were identified and the DNA inserts characterized by restriction mapping. Two cosmid clones, 1E3 and 3A3, were shown together to contain a complete C4 coding sequence as determined by hybridization of Southern blots with a full-length cDNA probe, clone TB13 (probe H) (K. T. Belt, M.C.C. and R.R.P., unpublished). The approximate gene size is 21 kb; however, the exact position of the 5′ end of the gene has not been determined. The insert of clone 1E3 also contained 7.5 kb of the 5′ end of a second C4 gene approximately 10 kb downstream from the 3′ end of the *C4z* gene. These two genes have been labelled as *C4y* and *C4z* as it is not clear which gene represents the C4A and C4B loci.

A comparison by restriction mapping of the 5′ ends of the two C4 genes showed that they were similar but that the *C4y* gene was characterized by 3.3-kb *Bam*HI, 3.5-kb *Kpn*I and 12-kb *Bgl*II fragments, whereas the *C4z* gene had 4.6-kb *Bam*HI, 12-kb *Kpn*I and 14.5-kb *Bgl*II fragments in the homologous region.

Southern analysis of genomic DNA from a sample of 72 individuals (data not shown), including the donor (HPFH) for the genomic library, suggests these two different forms of C4 gene are common in the population. Figure 3 shows a southern blot of genomic DNA from the library donor digested with *Kpn*I, *Bam*HI, or both and hybridized with a 400-base pair

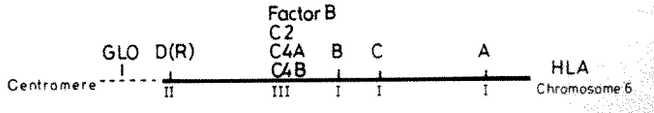


Fig. 1 Map of HLA region on the short arm of human chromosome 6 showing the approximate positions of the class I genes (A, B, C), the class II genes D(R) and the class III genes, factor B, C2, C4A and C4B. The glyoxalase gene (GLO) lies between D(R) and the centromere.

© 1984 Macmillan Journals Ltd

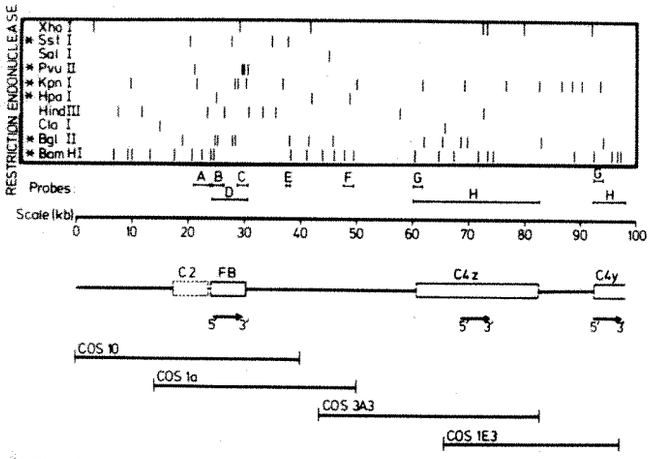


Fig. 2 Molecular map of 98 kb of genomic DNA in the HLA class III region containing four complement genes. Approximately 200,000 colonies from the HPFH cosmid library, prepared by F. Grosveld[26] from genomic DNA extracted from white blood cells of a 13-yr-old female, were screened with C4[15] and factor B[14] cDNA probes. DNA inserts from positive clones were characterized further by restriction mapping and Southern analysis using probes A–H as described below. Four cosmid clones 10, 1a, 3A3 and 1E3 were shown to overlap covering a total distance of 98 kb. Probes used: A, 400-bp C2 cDNA, pC201; B, 660 bp *ClaI*/*Bam*HI fragment from 5′ end of full-length factor B cDNA clone pFB3b; C, 500 bp *Xho*I/*Bam*HI fragment from 3′ end of full-length factor B cDNA clone pFB3b; D, 2.4-kb full-length factor B cDNA clone, pFB3b; E, 600-bp *Sst*I/*Bam*HI fragment from Cos 1a; F, 1.6-kb *Bam*HI fragment from Cos 3A3; G, 400-bp *Bam*HI/*Kpn*I fragment from 5′ end of full-length C4 cDNA clone TB13; H, 5.4-kb full-length C4 cDNA clone.
Methods: Colonies were plated onto 16 nitrocellulose filters (14 cm Millipore HAWP, 0.45 μM) on nutrient agar plates with ampicillin. Replicas were prepared and processed for colony hybridization according to Grosveld *et al.*[27]. Prehybridization and hybridization conditions, solutions and procedure for nick translation of probes are described in Fig. 3 legend. After hybridization, filters were washed with four changes of 0.3 M NaCl/0.03 M sodium citrate at room temperature, four changes of 0.15 M NaCl/0.015 M sodium citrate at 65 °C over 2 h and finally with two changes of 0.03 M NaCl/3 mM sodium citrate at 65 °C for 30 min each. Filters were dried and autoradiographed at −70 °C for 1–2 days. Cosmid DNA was extracted from bacterial colonies by using the alkaline SDS method[28] and the inserts characterized by single and double digestion with various restriction endonucleases using the manufacturer's specified conditions. Separation of restriction fragments by agarose gel electrophoresis and transfer to nitrocellulose filters for Southern analysis was performed as described in Fig. 3 legend. Positioning of the four genes was based on hybridization with the respective cDNA probes. As the probes (all but E and F) were for coding sequence, the precise limits of the genes were only defined for the 3′ end of the factor B gene[14]. Orientation of gene transcription for factor B, *C4z* and *C4y* was determined by hybridization with cDNA probes specific for 5′ and 3′ ends.

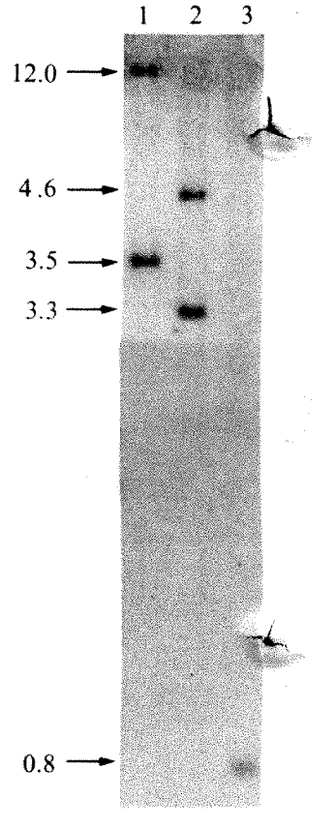


Fig. 3 Two common forms of C4 gene in genomic DNA. Southern[29] analysis of *Kpn*I (track 1), *Bam*HI (track 2) or *Kpn*I/*Bam*HI (track 3) digests of genomic DNA from individual HPFH hybridized with a 400-bp cDNA probe G, specific for 5′ coding sequence of a full-length cDNA TB13, probe H (see Fig. 2 for position of probes). The 3.3-kb *Bam*HI and 3.5-kb *Kpn*I bands are characteristic of *C4y*, whereas the 4.6-kb *Bam*HI and 12-kb *Kpn*I bands are characteristic of the *C4z* gene. The single 0.8-kb *Kpn*I/*Bam*HI band in track 3 is common to both *C4y* and *C4z* genes.
Methods: Genomic DNA was prepared from whole blood by the procedure of B. Sykes (unpublished). Briefly, after lysis of cells in 0.9% NaCl/0.1% Nonidet P40, pellets were broken up in lysis buffer (7 M urea, 0.3 M NaCl, 10 mM EDTA, 10 mM Tris-HCl *p*H 7.5) with a glass rod, and incubated at 37 °C for 10 min after the addition of 2% SDS. DNA was extracted 3× with phenol/isoamyl alcohol/chloroform (45:10:45) followed by precipitation by ethanol and dialysis against 10 mM Tris-HCl *p*H 7.5, 1 mM EDTA. For Southern analysis, 8–10 μg DNA samples were digested for 20 h at 37 °C in a final volume of 50 μl with 18 U of the respective restriction endonuclease according to the manufacturer's specifications (Boehringer Mannheim), electrophoresed in 0.8% agarose and transferred to nitrocellulose filters (Sartorius). Nitrocellulose filters were prehybridized at 42 °C for at least 2 h in 50% formamide, 1 M NaCl, 0.01 M EDTA, 10 × Denhardt's solution, 10% dextran sulphate, 50 mM Tris-HCl *p*H 7.5, 0.1% sodium pyrophosphate and 0.1 mg ml^{-1} of heat-denatured herring sperm DNA[30] and then hybridized with nick-translated (Amersham International)[31], heat-denatured DNA probes with a specific activity of 10^8 c.p.m. μg^{-1} (0.5 μCi ml^{-1}) for 24–48 h. After hybridization, filters were washed six times for 10 min in 2 × SSC, 0.1% SDS (1 × SSC = 0.15 M NaCl, 0.015 Na citrate) and six times for 10 min at 68 °C in 1 × SSC, 0.1% SDS. After drying, filters were exposed to X-ray film with an intensifying screen at −70 °C for 2 days. Sizes of all bands were estimated on the basis of radioactive size markers (not shown).

(bp) C4 cDNA probe, G, a *Bam*HI/*Kpn*I fragment from the 5′ end of full-length cDNA clone TB13 (probe H). Both forms of the C4 gene are seen as characterized by 3.3-kb *Bam*HI and 3.5-kb *Kpn*I for *C4y* and 4.6-kb *Bam*HI and 12-kb *Kpn*I bands for *C4z*. Only a single hybridizing band of 0.8 kb is seen after double digestion with *Kpn*I and *Bam*HI, suggesting that the two genes are similar in this region. It is not known if these restriction site differences are representative of coding sequence differences.

Preliminary characterization of a third cosmid clone which possibly overlaps clone 1E3 but not 3A3 (data not shown) suggests that a third C4 gene may be present and this is being investigated further (M.C.C., unpublished observation).

A single factor B gene

Initial screening of 200,000 clones of the HPFH cosmid library using the 515-bp factor B-specific cDNA probe, FBI[14], resulted in the isolation of one positive clone (cos 1a) with an insert of approximately 35 kb. To generate overlapping clones and extend the region of DNA, a 600-bp *Bam*HI/*Sst*I fragment, probe E (Fig. 2), lying 12 kb from the 3′ end of cos 1a was used as a hybridization probe of the library. A further six positive clones were isolated and characterized. Four appeared to be identical with cos 1a, while two (cos 10 and cos 16) extended cos 1a by 14 kb at the 5′ end (Fig. 2).

Extensive mapping and Southern blot analysis of the cloned genomic DNA using a 2.4-kb full-length cDNA probe, D (ref. 20), showed the presence of only one factor B gene within this region (Fig. 2). The probe hybridized to adjacent 2.7- and 4.4-kb *Hin*dIII fragments, and sequence analysis[14] has shown that the 3′ end of the gene lies approximately 150 bp from the 3′ end of the 4.4-kb fragment (Fig. 2). The 5′ end of the gene was mapped using an 81-bp cDNA probe, generated from the full-length clone by a *Bam*HI/*Sau*3A double digest[20]. The probe, which contained coding information for 9 amino acids at the N-terminus of Ba and 10 amino acids of a putative leader peptide, hybridized to a 600-bp *Bam*HI fragment contained within the 2.7-kb *Hin*dIII fragment (Fig. 2). The 3′ end of the *Bam*HI fragment was shown by sequence analysis to encode the N-terminus of Ba and four amino acids of the leader peptide, before running into an intron. Thus, the region that codes for the mature factor B protein is contained within 5.7 kb of DNA.

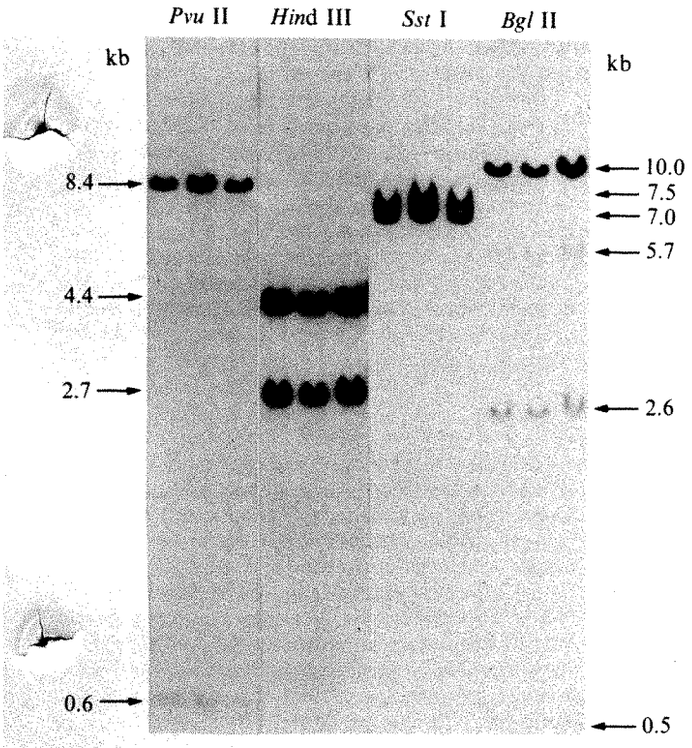


Fig. 4 Southern blot analysis of genomic DNA from three unrelated individuals with the 2.4-kb factor B cDNA probe, pFB3b. 10 μg of DNA was digested to completion with the enzyme indicated, separated on a 0.7% agarose gel and transferred to nitrocellulose filters as described in Fig. 3 legend. The insert of clone pFB3b, probe D (see Fig. 2), was excised using a *Cla*I/*Bam*HI double digest, and the 660-bp and 1.6-kb fragments generated were purified by elution from a polyacrylamide gel. The fragments were mixed in equal ratio and nick translated to a specific activity of $0.4–1 \times 10^8$ c.p.m. per μg DNA such that the hybridization mix contained $\sim 5 \times 10^5$ c.p.m. per ml. Conditions for hybridization, washing and autoradiography were as described in Fig. 3. Unequal intensity of 10-kb and 2.6-kb *Bgl*II fragments is due to unequal labelling of the mixed probes on nick translation.

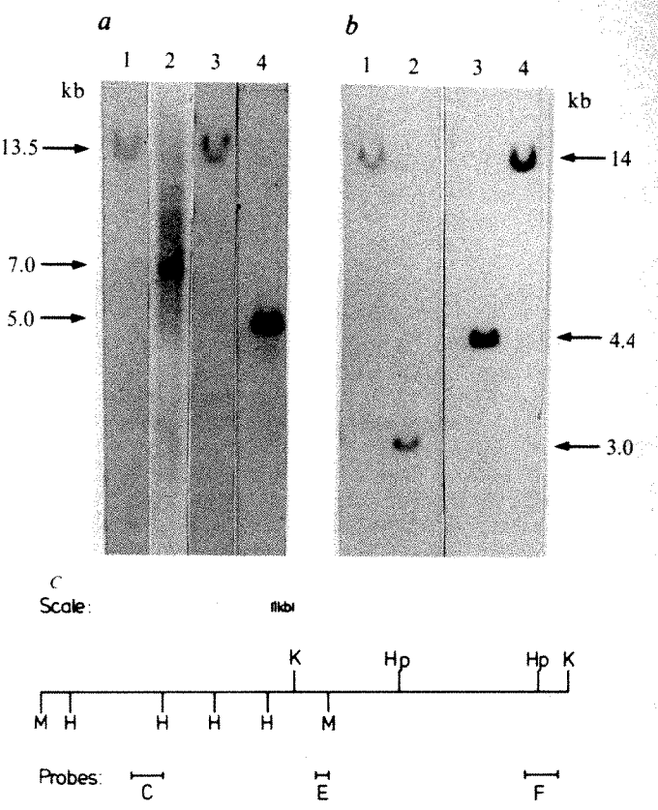


Fig. 5 Genomic probes from cosmid clones containing C4 and factor B genes hybridize to a common genomic restriction fragment. ***a***, Genomic DNA was digested with restriction endonuclease *Kpn*I only (tracks 1, 3) or both *Kpn*I and *Hpa*I (tracks 2, 4) and analysed on Southern blots as described in Fig. 3 legend. After hybridizing first with probe F (tracks 1, 2) from cos 3A3 (containing the C4 gene), the filter was treated with 0.5 M NaOH to remove the first probe, neutralized and prehybridized as described in Fig. 3 legend, then hybridized with probe E (tracks 3, 4) from cos 1a (containing the factor B gene). The 13.5-kb *Kpn*I fragment in tracks 1 and 3 hybridized with both genomic probes. After double digestion with *Kpn*I and *Hpa*I shown in tracks 2 and 4, the 13.5-kb *Kpn*I fragment is split into 7- and 5-kb fragments which hybridize with probes F and E, respectively. The 1.5-kb *Kpn*I/*Hpa*I fragment is not seen, because fragments of that size electrophoresed off the gel. For illustration, tracks 1 and 3 from one filter were combined with tracks 2 and 4 from a second filter. ***b***, Genomic DNA was digested with *Bam*HI (tracks 1, 4) or both *Bam*HI and *Hin*dIII (tracks 2, 3) and analysed on Southern blots (see Fig. 3 legend). Samples 1 and 2 were hybridized with genomic probe E from cos 1a while samples 3 and 4 were hybridized with the cDNA probe C (see Fig. 2 legend). Both probes hybridized to a common 14-kb *Bam*HI fragment (tracks 1, 4). After double digestion with *Bam*HI and *Hin*dIII, probe E hybridized to a 3-kb fragment (track 2) while probe C hybridized to a 4.4-kb fragment (track 3) as predicted from the map in Fig. 2. ***c***, Line diagram of restriction map covering region of overlap between cos 3A3 and Ia and factor B gene. Restriction enzymes used: K, *Kpn*I; Hp, *Hpa*I; M, *Bam*HI; H, *Hin*dIII.

Due to the close linkage of the factor B and C2 genes (see below), it is likely that the regions responsible for the control of factor B expression lie within the 600-bp *Bam*HI fragment, suggesting that the complete gene is about 6 kb long.

Southern blot analysis of various digests of genomic DNA from three unrelated individuals (Fig. 4) showed that in all cases the sizes of the restriction fragments hybridizing to the factor B probe (D) were as predicted from the map in Fig. 2. The results of these and other digests (*Kpn*I, *Bam*HI, *Acc*I, *Eco*RI, data not shown) indicate the presence of a single factor B gene per chromosome.

The C4 and factor B genes are linked

DNA inserts from cosmid clones 3A3 (containing the C4 gene) and 1a (containing the factor B gene) were shown to overlap by approximately 6 kb. Complementary sequence in this region was further proven by preparing a probe from clone 3A3 in the region of overlap. A 1.6-kb *Bam*HI genomic fragment (probe F, see Fig. 2) was nick translated and hybridized to a Southern blot comparing clones 3A3 and 1a. Probe F from clone 3A3 hybridized to the common restriction fragments in clone 1a, proving that the cloned inserts were complementary in the region of restriction site overlap.

To prove that these class III genes exist in the same order in uncloned genomic DNA, the two cosmid probes F and E (see Fig. 2) isolated from clones 3A3 and 1a, respectively, were hybridized to Southern blots of genomic DNA digested with either the single enzyme *Kpn*I or double digested with both *Kpn*I and *Hpa*I. As shown in Fig. 5*a*, both probes hybridized to the 13.5-kb *Kpn*I fragment. The double digest with *Kpn*I and *Hpa*I gave two fragments, a 7-kb band which hybridized only with probe F, and a 5-kb band which hybridized only with probe E. Second, the two probes E and C were hybridized to blots of genomic DNA digested with *Bam*HI only, or *Bam*HI and *Hin*dIII. Both hybridized to a common 14-kb *Bam*HI fragment (Fig. 5*b*, tracks 1, 4). When the DNA was digested with *Bam*HI and *Hin*dIII, probe E hybridized only to a 3-kb fragment while probe C hybridized to a 4.4-kb fragment. Linkage of genomic probe F with probe G, a C4 cDNA probe, was demonstrated by hybridizing to a common 14.5 *Bgl*II fragment (not shown). These results of Southern blots of genomic DNA confirmed the linkage of the *C4z* gene to the factor B gene proposed from the results of the cosmid mapping.

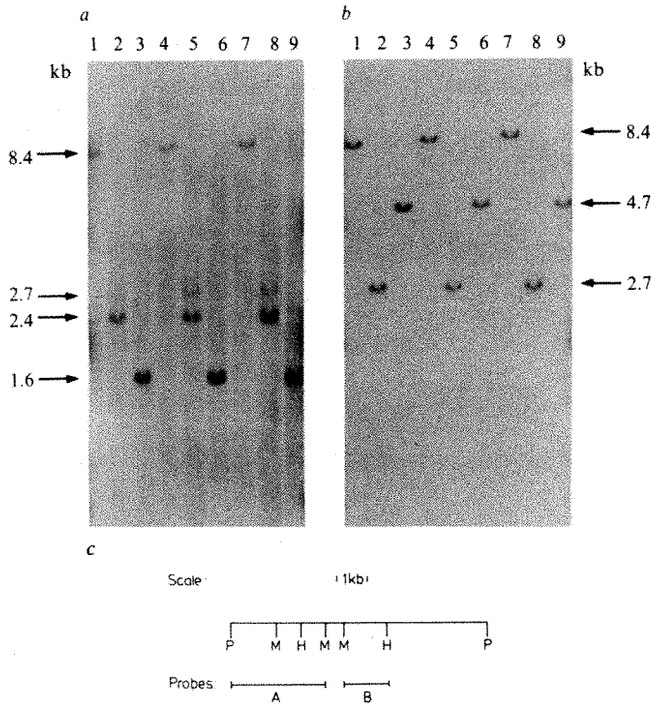


Fig. 6 *a*, *b*, Southern blotting analysis of genomic DNA from three unrelated individuals, KT, PR and AB using the C2 probe A (shown in *a*), or the *Ba* probe B (shown in *b*). *c*, Summary of the restriction map in the region of genomic DNA for which the linkage between the two probes was demonstrated. The restriction enzymes used were as follows: tracks 1, 4, 7: *Pvu*II; tracks 2, 5, 8, *Pvu*II and *Hin*dIII; tracks 3, 6, 9, *Pvu*II and *Bam*HI. The C2 and factor B probes both hybridize to a common 8.4-kb *Pvu*II fragment (tracks 1, 4, 7 in *a* and *b*). When the DNA was simultaneously digested with *Pvu*II and *Hin*dIII, probe A hybridized to two fragments of 2.4 kb and 2.7 kb (tracks 2, 5, 8 in *a*) while probe B hybridized only to the 2.7-kb fragment (tracks 2, 5, 7 in *b*). Both probes hybridized to the 2.7-kb *Hin*dIII fragment as the 3′ end of the C2 gene and the 5′ end of the factor B gene lie within this fragment. Similarly, using DNA digested with *Pvu*II and *Bam*HI, probe A hybridized to fragments of 1.6 kb (tracks 3, 6, 9 in *a*) while probe B hybridized to a 4.7-kb fragment (tracks 3, 6, 9 in *b*) as predicted from the restriction map in Fig. 2.
Methods: 20 μg of DNA was digested to completion with the appropriate one or two restriction enzymes. The reaction mixture was divided into two equal aliquots and one was loaded onto each of two 0.7% 20 cm × 20 cm agarose gels. Each was electrophoresed in parallel and the DNA blotted onto nitrocellulose as described in detail in Fig. 3 legend. DNA fragments used as probes were purified by elution from polyacrylamide thin gels and then nick-translated to a specific activity of $0.2–1.0 \times 10^8$ c.p.m. per μg DNA. Conditions for hybridization, washing and autoradiography were as described in Fig. 3 legend.

Linkage of C2 and factor B genes

A C2-specific 400-bp cDNA probe, pC201, has been isolated that contains sequence coding for a short region of the C2 protein near its C-terminus[18]. The pC201 probe, probe A, was used to identify the position of the C2 locus relative to the genes for C4 and factor B in the cosmid clones already isolated. Probe A hybridized to restriction fragments derived from cos 1a and cos 10, but not 3A3 (results not shown), and the probe site was mapped to within a 2.6-kb region of cos 1a (Fig. 2) that lies approximately 0.5 kb from the 5′ end of the Ba coding sequence. The approximate position of the C2 gene is therefore as indicated by the dotted lines in Fig. 2. Confirmation of the close linkage of the C2 and factor B genes was provided by Southern analysis of genomic DNA of three unrelated individuals using probe A, and a factor B probe that was specific for the 5′ end of Ba (probe B in Fig. 2). The results showed that the C2 (A) and factor B (B) probes hybridize to a common 8.4-kb *Pvu*II fragment (tracks 1, 4, 7, Fig. 6*a*, *b*). When the genomic DNA was simultaneously digested with *Pvu*II and *Hin*dIII, probe A hybridized to a 2.4-kb fragment and more weakly to a 2.7-kb fragment (Fig. 6*a*, tracks 2, 5, 8), while probe B hybridized only to the 2.7-kb fragment (Fig. 6, tracks 2, 5, 8). Similarly, using DNA digested simultaneously with *Pvu*II and *Bam*HI, probe A hybridized to two fragments of 1.6 kb while probe B hybridized to a 4.7-kb fragment (compare Fig. 6*a* and *b*, lanes 3, 6 and 9). These results, which are summarized in Fig. 6*c*, agree with the detailed restriction map already determined for this region of chromosome 6 (see Fig. 2), and therefore confirm that the C2 locus maps within 2 kb of the 5′ end of the factor B gene. These results also indicate that the C2 protein is encoded by a single locus per chromosome.

Discussion

A molecular map of 98 kb of the HLA class III region was prepared by ordering overlapping cloned genomic fragments. Four class III genes were identified in the order C2, factor B, C4z, C4y. These results, combined with results from Southern analysis of genomic DNA, suggests that C2 and factor B are encoded by single loci and C4 is encoded by at least two separate loci. While both forms of C4 gene are common in the population, further analysis will be necessary to determine which genes code for the C4A and C4B serum proteins.

Finding that these four complement genes are located within such a relatively close distance of each other, that is, 30 kb between C4 and factor B and less than 2 kb between factor B and C2, explains the failure to observe cross-overs[21]. It is most likely that the C2 and factor B genes evolved from a common locus as they are very close and the proteins have a similar structure and function. C4 has a function related to but distinct from that of C2 and factor B and although the full amino acid sequence of factor B[20,22] and about a quarter of that of C4 is available[23] there is no obvious similarity. The class I and class II molecules are related in structure to each other (and to the immunoglobulins) and there therefore appears to be at least three distinct types of protein encoded in the MHC, represented by: (1) class I and class II proteins; (2) C2 and factor B; (3) C4A and C4B. The 21-hydroxylase gene has been mapped close to the class III genes in HLA[8] and the neuraminidase gene to class III genes in the H-2 in mice[24] but little information is available on the structures of the corresponding proteins. Their catalytic function seems to be quite unrelated to any of the roles in immunity of the three main classes in the MHC. There is room for more genes between *HLA-D* and *HLA-B*; we hope to identify any coding sequences that may be present and discover whether they are related structurally or functionally to the class I and II or class III genes.

The gene order factor B, C4B, C4A and C2 with C2 nearest the *HLA-B* locus has been proposed by Olaisen *et al.*[25] on the basis of haplotypes existing in the population. Our results corroborate their estimate of the distance between factor B and C4 as 0.04 centimorgans (assuming 1 centimorgan = 10^6 bp), but the order of the genes determined in our analysis places the C2 locus next to factor B, not on the opposite side of the C4 genes.

We thank Dr F. Grosveld for providing the cosmid library of human genomic DNA, also Ms A. Palsdottir, Ms S. Cross and Ms N. Janjua for technical assistance. M.C.C. is recipient of an American Arthritis Investigator Award. D.R.B. is a Beit Memorial Junior Research Fellow.

Received 13 September; accepted 28 October 1983.

1. Francke, U. & Pelligrino, M. A. *Proc. natn. Acad. Sci. U.S.A.* **74,** 1147–1151 (1977).
2. Dorf, M. E. (ed.) *The Role of the Major Histocompatibility Complex in Immunobiology* (Wiley, New York, 1981).
3. Barnstable, C. J., Jones, E. A. & Bodmer, W. F. *Int. Rev. Biochem.* **22,** 151–226 (1979).
4. Aweh, Z. L., Raum, D., Yunis, E. J. & Alper, C. A. *Proc. natn. Acad. Sci. U.S.A.* **80,** 259–263 (1983).
5. Porter, R. R. *Molec. Biol. Med.* **1,** 161–168 (1983).
6. Reid, K. B. M. & Porter, R. R. *A. Rev. Biochem.* **50,** 433–464 (1981).
7. Hall, R. E. & Colten, H. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 1707–1710 (1977).
8. Weitkamp, L. R. & Lamm, L. U. *Cytogenet. Cell Genet.* **32,** 130–143 (1982).

9. O'Neill, G. J., Young, S. & Dupont, B. *Proc. natn. Acad. Sci. U.S.A.* **75,** 5165–5169 (1978).
10. Raum, D. *et al. Am. J. hum. Genet.* **31,** 35–41 (1979).
11. Mauff, G. *et al. Z. Immun. Immunobiol.* **154,** 115–119 (1978).
12. Mauff, G. *et al. Immunobiology* **164,** 184–191 (1983).
13. Woods, D. E., Markham, A. F., Ricker, A. T., Goldberger, G. & Colten, H. R. *Proc. natn. Acad. Sci. U.S.A.* **79,** 5661–5665 (1982).
14. Campbell, R. D. & Porter, R. R. *Proc. natn. Acad. Sci. U.S.A.* **80,** 4464–4468 (1983).
15. Carroll, M. C. & Porter, R. R. *Proc. natn. Acad. Sci. U.S.A.* **80,** 264–267 (1983).
16. Ogata, R. T., Shreffler, D. C., Sepich, D. S. & Lilly, S. P. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5061–5065 (1983).
17. Whitehead, A. S., Goldberger, G., Woods, D. E., Markham, A. F. & Colten, H. R. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5387–5391 (1983).
18. Bentley, D. R. & Porter, R. R. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
19. Palsdottir, A., Cross, S., Edwards, J. & Carroll, M. C. *Nature* **306,** 615–616 (1983).
20. Morley, B. J. & Campbell, R. D. *EMBO J.* (in the press).
21. Alper, C. A., Raum, D., Karp, S., Awdeh, Z. L. & Yunis, E. T. *Vox. Sang.* **45,** 62–67 (1983).
22. Christie, D. L. & Gagnon, J. *Biochem. J.* **209,** 61–70 (1983).
23. Chakravarti, D. N., Campbell, R. D. & Gagnon, J. *FEBS Lett.* **154,** 387–390 (1983).
24. Figueroa, F., Klein, D., Tewarson, S. & Klein, J. *J. Immun.* **129,** 2089–2093 (1982).
25. Olaisen, B., Teisberg, P., Gedde-Dahl, T. Jr, Thorsby, E. & Jonassen, R. *Immunobiology* **164,** 145 (1983).
26. Grosveld, F. G. *et al. Nucleic Acids Res.* **10,** 6715–6732 (1982).
27. Grosveld, F. G., Dahl, H. M., Boer, E. D. & Flavell, R. A. *Gene* **13,** 227–237 (1981).
28. Birnboim, H. C. & Doly, J. *Nucleic Acids Res.* **7,** 1521–1534 (1979).
29. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
30. Maniatis, T., Fritsch, R. F. & Sambrook, J. (eds) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratories, New York, 1982).
31. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113,** 237–251 (1977).

Transcriptional interference in avian retroviruses—implications for the promoter insertion model of leukaemogenesis

Bryan R. Cullen*†, Peter T. Lomedico* & Grace Ju*

* Department of Molecular Genetics, Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110, USA
† Department of Microbiology, University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103, USA

*The downstream (3′) long terminal repeat (LTR) of an avian retroviral provirus is unable to act as an efficient promoter of transcription when a transcriptionally active upstream (5′) LTR is present. This transcriptional interference may explain the observation that only deleted proviruses have been observed inserted adjacent to c-*myc *in avian leukosis virus induced lymphomas of chickens.*

AVIAN leukosis viruses (ALVs) cause neoplastic disease in their hosts only after a long latent period and cannot be shown to cause transformation of cultured cells *in vitro*. This delayed transformation is thus markedly different from that caused by the acutely transforming retroviruses which have been shown to contain oncogenes encoding transforming proteins[1].

Integration of an ALV provirus into the host DNA results in the viral coding sequence being flanked by two long terminal repeats (LTRs)[2,3] (Fig. 1*A*). The viral LTRs contain sequences required for initiation as well as for polyadenylation of viral transcripts and fulfill these roles at the opposite ends of the proviral transcriptional unit[4]. As the proviral LTRs are identical it is possible that the downstream LTR can also act as a transcriptional promoter. The 'promoter insertion' theory (Fig. 1*A*) predicts that ALV causes neoplasms when this downstream LTR transcribes elevated levels of an adjacent *onc* gene analogous to those observed in the acutely transforming viruses[5].

Evidence for promoter insertion oncogenesis has been presented by several workers for lymphomas induced by ALV in chickens[5–7]. Transcripts have been observed containing the U5 region of the ALV LTR and the coding sequence of c-*myc*, the cellular homologue of the transforming gene v-*myc* of the acutely transforming virus MC29. An unexpected result of this research was the finding that the proviruses integrated into the region 5′ of the c-*myc* gene were always defective[5–7]. The observed deletions encompassed different parts of the viral coding region but always appeared to delete the upstream or 5′ LTR. Two theories have been advanced to account for this observed defectiveness[5–7]. One suggestion is that the production of viral gene products by the provirus in a tumour would render it susceptible to detection by the host immune system. However, this appears unlikely as many tumours actively express viral genes encoded by other intact proviruses[5,6]. A second possibility arises from the structure of the LTR (Fig. 2). When the LTR functions as a promoter, RNA polymerase is thought to interact with sequences in the U3 region of the LTR before initiating transcription at the 'CAP' site. ALV mRNAs are polyadenylated at a site 21 bases 3′ to the 'CAP' site[8]. RNA polymerases terminating at this site in the 3′ LTR must transcribe the entire viral U3 region in addition to the 21 bases shared by any initiating transcript (the R region). The observation that

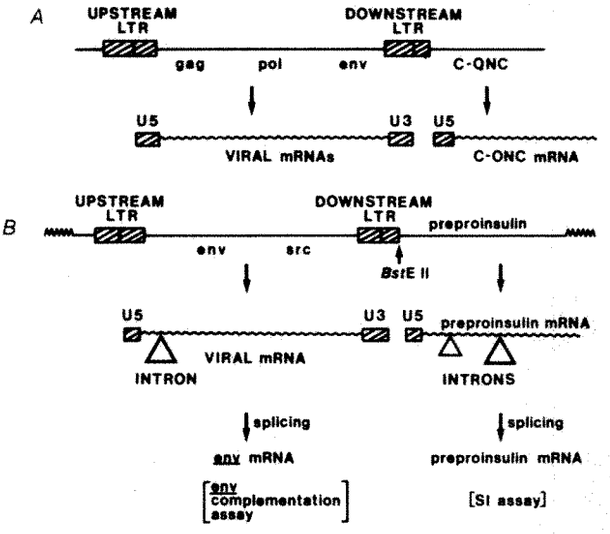


Fig. 1 Experimental strategy for measurement of transcriptional interference. *A*, Expected structure and transcriptional products of an ALV provirus integrated adjacent to a cellular *onc* gene; *B*, Structure of clone pBC8I.
Methods: The proviral region of the clone was constructed as previously described from Schmidt-Ruppin B sequences. The upstream (5′) LTR and *env* gene splice donor site were obtained from clone λSRBtd8[28]. They were joined as previously described[12] to a SR-B DNA fragment from clone pLD6 (D. Stacey, unpublished results) which contains the *env* gene splice acceptor site, the *env* gene, the *src* gene and the downstream LTR. This construction generates a provirus-like structure which is deleted for the majority of the *gag-pol* region of the virus which forms the *env* gene intron. We have previously demonstrated that this deletion does not affect *env* gene transcription and splicing[11]. The rat preproinsulin II gene used contains a *Hin*dIII site inserted in the 5′ non-coding region as previously described[13]. To place the preproinsulin II gene under downstream (3′) LTR control, a *Bst* EII site at the end of the U5 region of the downstream LTR was converted to a *Hin*dIII site. This construction adds an additional 107 bp of 5′ non-coding sequences of retroviral origin to the preproinsulin II transcript.

© 1984 Macmillan Journals Ltd

Table 1 Relative *env* and proinsulin mRNA levels from transfected cells

Lane	Clone	*env* activity		Insulin mRNA synthesis			
		FFU produced*	Per cent	c.p.m. per band	Per cent	Peak area	Per cent
2	pBC8IΔ3	0	0	366	54	237	70
3	pBC8IΔ2	425	0.6	676	100	340	100
4	pBC8IΔ1	52,500	79	225	33	122	36
5	pBC8I	66,000	100	126	19	73	21
9	pBC8I/PA	250	1.3	536	100	306	100
10	pBC8I	18,500	100	149	28	62	20
11	pBC8IΔ3	0	0	287	54	200	66

env activity is shown as the total number of focus forming units produced per transfected culture and as a percentage of the activity of clone pBC8I. Lane nos refer to Fig. 3. Relative insulin mRNA transcription was determined by two methods: peak areas derived by densitometry tracing of the autoradiograph shown using a Beckman DU-8 scanning spectrophotometer, and liquid scintillation counting of actual gel slices from the region of the 170-nucleotide fragment in each lane (values corrected for background counts = 193 c.p.m.). These two measures of insulin mRNA activity are expressed as a percentage of the activity of clone pBC8IΔ2 or pBC8I/PA.

* FFU, focus forming unit.

transcripts extending beyond the 3′ end of the mature mRNA are synthesized during transcription of both retroviral[9] and cellular genes[10] suggests that there is an extensive overlap between the regions involved in initiating and terminating transcription (Fig. 2*A*). This overlap could reduce the ability of the downstream or 3′ LTR to act as an efficient promoter by interfering with initiation of transcription. This inhibition, which we term transcriptional overlap interference, would be relieved by the deletion of the upstream LTR. This deletion may be required if the downstream LTR is to promote sufficient c-*onc* transcription to cause transformation.

Experimental approach

To demonstrate transcriptional overlap interference we have constructed proviral clones in which the upstream and downstream LTRs control the transcription of genes whose levels can be measured independently after transfection into avian cells. The parent plasmid used, pBC8I, is closely analogous to the proposed structure observed after integration of a provirus next to a cellular *onc* gene[5] (Fig. 1*B*). The gene whose transcription is controlled by the upstream LTR is the viral *env* gene whose transcript terminates, as in the proviral case, in the downstream LTR. The gene transcribed under the control of the downstream LTR could, in theory, be any cellular gene. The gene we have chosen to use is the rat preproinsulin II gene. We have previously described sensitive assays for both the viral *env* and the rat preproinsulin II genes[11–13].

The *env* assay is based on complementation of a genetic deficiency in a virus infecting the quail cell line QCl-3. These cells are transformed by the Bryan strain of Rous sarcoma virus [RSV(−)]. The genome of this virus contains *src* but lacks the *env* gene. Virus particles produced by QCl-3 cells are thus not infectious due to the lack of the envelope glycoprotein[11,14]. When QCl-3 cells are transfected with plasmids containing the *env* gene, *env* mRNA may be synthesized leading to production of infectious transforming RSV(−) particles. Stacey *et al.*[14] have shown that the number of infectious virus produced is proportional to the amount of *env* mRNA available in the cell. This

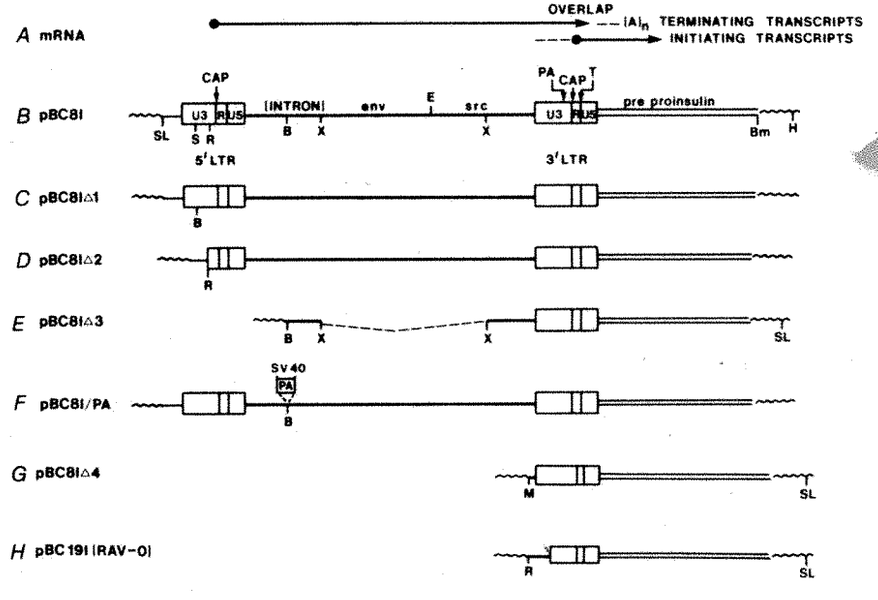


Fig. 2 Structure of the retroviral clones used. *A*, The structure of mRNAs from the *env* and rat preproinsulin II transcriptional units is shown. The region where transcripts initiating in the 5′ LTR would terminate is shown to overlap with the region important for the initiation of transcription in the 3′ LTR. *B*, The parental clone pBC8I is shown. The sequences shown were inserted between the *Sal*I and *Bam*HI sites of pBR322. The approximate location of the *env* gene intron is indicated. E refers to the sequence element noted by Schwartz *et al.*[15] which lies between *env* and *src* in the Schmidt-Ruppin B strain used. (CAP, site of initiation of transcription; PA, polyadenylation signal; T = site of poly(A) addition and termination of viral mRNA). *C*, pBC8IΔ1 was constructed by S_1 exonuclease treatment of the *Sph*I site in the 5′ LTR U3 region followed by insertion of a *Bgl*II linker DNA molecule. This results in a net insertion of 4 bp. *D*, pBC8IΔ2 was constructed by cleavage of the 5′ LTR U3 region with *Pvu*I followed by S_1 exonuclease digestion and religation. A clone retaining the *Eco*RI site was then selected, and nucleotide sequence analysis revealed the deletion of 191 bp of the U3 region 5′ to the *Eco*RI site. *E*, pBC8IΔ3 was created by deletion of the entire 5′ LTR region and viral structural sequences, including element E[15], located between the *Xho*I sites shown. The clone retains ≈1.5 kb of viral gene sequences in addition to the 3′ LTR. *F*, pBC8I/PA contains a 237 bp *Bcl*I to *Bam*HI fragment from SV40 ligated into a *Bgl*II site in the *env* gene intronic region. This fragment has the SV40 late polyadenylation/termination signal inserted in the sense orientation relative to the *env* gene. *G*, pBC8IΔ4 was derived from pBC8IΔ3 by deletion of all viral structural gene sequences 5′ to a *Mst*II site located ≈40 bp from the U3 region of the 3′ LTR. *H*, pBC19I contains the previously described[12] LTR of the endogenous virus RAV-0 ligated to the rat preproinsulin II gene in the same fashion used for the exogeneous virus LTR. It retains ≈130 bp of chicken DNA bounded by an *Eco*RI site. Clones pBC8IΔ3, pBC8IΔ4, and pBC19I were cloned between the pBR322 *Eco*RI and *Bam*HI sites in the opposite orientation to the other clones described relative to pBR322. pBR322 (∿); retroviral sequences (—); preproinsulin sequences (=); S, *Sph*I; X, *Xho*I; *R*, *Eco*RI; H, *Hin*dIII; SL, *Sal*I; B, *Bgl*II; M, *Mst*II; Bm, *Bam*HI.

in turn is a reflection of the ability of the transfected plasmid to synthesize *env* mRNA.

The S_1 nuclease assay used to quantitate preproinsulin mRNA synthesis involves the use of a DNA probe uniquely end-labelled in an exon of the insulin gene[13]. As the probe extends through an intronic sequence of the insulin gene, annealing to correctly spliced preproinsulin mRNA synthesized in the QCl-3 cells followed by S_1 treatment results in the quantitative production of a band of characteristic size on a denaturing acrylamide gel. This S_1 assay does not distinguish between proinsulin transcripts originating in the 3′ LTR and any readthrough transcripts which might initiate in the 5′ LTR. As all the plasmid constructs contain the same preproinsulin transcription unit (Fig. 2), the steady-state levels of insulin mRNA as determined by the S_1 assay should be an accurate measure of the relative promoter activity of each clone tested.

Mutation of the upstream LTR

To determine whether the transcriptional activity of the upstream LTR affects that of the downstream LTR, we introduced mutations into the upstream LTR of plasmid pBC8I and measured the affect of these lesions both on *env* and preproinsulin gene trancription using the two independent assays. The different deletions introduced into pBC8I are outlined in Fig. 2.

In clone pBC8IΔ1 we have introduced a minor change into the U3 region of the upstream LTR by inserting a *Bgl*II linker DNA molecule into the *Sph*I site. This modification has been shown by DNA sequence analysis to result in the net insertion of four base pairs (bp). Clone pBC8IΔ2 has been shown by DNA sequence analysis to contain a deletion of the U3 region of the LTR extending up to the *Eco*RI site, within 57 bp upstream of the CAP site. In clone pBC8IΔ3 the entire upstream LTR and nearly all the viral structural gene sequences, including the *env* gene, are deleted leaving only the downsteam LTR intact.

All modifications of the upstream LTR reduced *env* transcription relative to pBC8I, as determined by the *env* complementation assay (Table 1). In clone pBC8IΔ1 *env* synthesis was reduced only slightly. Clone pBC8IΔ2, although it retains part of the U3 region of the LTR including the 'TATA' box, shows only ≈1% the *env* gene activity of the undeleted clone. This supports the contention that the U3 region of the LTR contains sequences essential for promoting viral mRNA synthesis. As expected, clone pBC8IΔ3, which has a deleted *env* gene, produced no *env* activity. As may be seen in Fig. 3, mutation of the upstream LTR has the opposite effect on transcription of preproinsulin mRNA from the downstream LTR. Clone pBC8IΔ1 (lane 4) shows a small elevation in preproinsulin mRNA synthesis while clone pBC8IΔ2 (lane 3) and pBC8IΔ3 (lane 2) show clearly elevated levels compared to the parental clone pBC8I (lane 5). Clone pBC8IΔ3 shows consistently slightly lower levels of preproinsulin mRNA synthesis than clone pBC8IΔ2. The reason for this is unknown but may be due to the deletion of viral structural gene sequences in pBC8IΔ3 which have been suggested to affect transcription[15]. Alternatively this difference may be explained by the fact that, for technical reasons, pBC8IΔ3 is inserted into the plasmid vector in the opposite orientation compared to the other clones. Superinfection of QCl-3 cells with an avian leukosis virus has no affect on preproinsulin RNA transcription from transfected DNA templates (results not shown), demonstrating that envelope glycoprotein does not directly affect insulin mRNA synthesis.

As noted previously, the S_1 assay for preproinsulin expression would measure transcripts which initiate in the 3′ LTR as well as any readthrough transcripts which initiate in the 5′ LTR. Read through transcripts which fail to terminate using the LTR polyadenylation signal have been observed in avian cells[9]. The contribution of readthrough transcripts in this assay would result in an underestimate of the differences between intact and deleted LTR clones, so that the ≈5-fold difference in proinsulin mRNA transcription between pBC8I and pBC8IΔ2 may represent the minimum effect due to overlap interference.

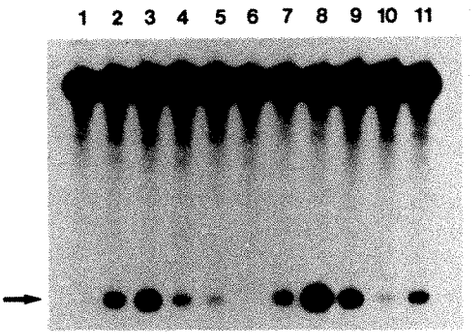


Fig. 3 Effect of 5′ LTR promoted transcription on 3′ LTR activity. Autoradiograph of S_1 assay acrylamide/urea gel. Lane 1, prI2/SV40-46; 2, pBC8IΔ3; 3, pBC8IΔ2; 4, pBC8IΔ1; 5, pBC8I; 6, pBC8; 7, 5 ng of rat insulinoma (RI) poly(A+)-RNA; 8, 25 ng of RI poly(A)$^+$ RNA; 9, pBC81/PA; 10, pBC8I; 11, pBC8IΔ3. Lanes 1–5 and lanes 9–11 contain RNA samples derived from two independent transfection experiments. Relative *env* and proinsulin mRNA levels from transfected cells are given in Table 1.
Methods: QCl-3 cells are seeded at 9×10^6 cells per 100 mm dish 20 h before transfection. 5.8 μg of clone pBC8I DNA or equimolar quantities of other plasmid DNAs were taken up in 2 ml of phosphate buffered saline (PBS) containing 1 mg ml^{-1} of DEAE-dextran. The monolayer of QCl-3 cells was washed once with warm PBS, the DNA solution was added to the cells, and they were incubated at 37 °C for 30 minutes with occasional gentle shaking. At this time, 25 ml of growth media was added to the cells and they were incubated for a further 2.5 h before refeeding with fresh growth media. Media samples for use in the *env* assay were taken at 72 h post-transfection. These were assayed for focus forming unit (FFU) production on chick embryo fibroblasts (CEF) as previously described[11,14]. As the *env* gene used is of the B serotype, the viruses produced are infectious for CEFs but not for quail cells and thus no spread can occur in the transfected culture[29]. After media sampling, RNA was isolated from the QCl-3 cells as described[13]. Total RNA yields were quantitated by UV adsorbance, gel analysis, and dot blots using a *gag*-specific probe as the internal standard. Using 10 μg of total RNA from each transfected culture, preproinsulin mRNA in each sample was quantitated by S_1 mapping[13,30,31]. The probe used was a 750 bp fragment uniquely 3′ end-labelled at a *Bam*HI site in the coding region and extending to a *Hha*I site on the other side of the 499 bp intron in the preproinsulin II gene. Correctly spliced preproinsulin mRNA will anneal to the labelled anti-sense DNA strand, and nuclease S_1 will digest the unhybridized intronic sequences. A 170 nucleotide labelled fragment (indicated by arrow) is protected in proportion to the quantity of preproinsulin mRNA present in the reaction[13].

Clone pBC8, which contains the same retroviral sequences as pBC8I but lacks the insulin gene, is negative in the preproinsulin RNA S_1 assay (Fig. 3, lane 6). If the preproinsulin gene is inserted in the inverted orientation relative to the LTR, no proinsulin mRNA is detectable (results not shown). Interestingly, clone prI2/SV40-46, in which the preproinsulin gene is under Simian virus 40 (SV40) early promoter control, also shows no detectable preproinsulin transcription in avian cells (Fig. 3, lane 1). This contrasts with the situation in monkey cells where this construction is quite active[13]. It is also of interest to note that the hybrid LTR U5 region-preproinsulin mRNA transcribed from the downstream LTR is able to efficiently program the synthesis and secretion of rat proinsulin (results not shown).

These results are consistent with the hypothesis that transcription from the upstream LTR in an integrated provirus inhibits transcription from the downstream LTR due to overlap interference. To prove this point directly, we have constructed clones in which transcription from the upstream LTR was terminated before reaching the downstream LTR.

Insertion of a transcriptional terminator

We have previously presented evidence that modification of the size of the *env* gene intron either by deletion or insertion of exogenous sequences has only limited effect on production of spliced *env* mRNA, given that the inserted sequences do not

Table 2 Relative *env* and proinsulin mRNA levels in the transfected cells

		env activity		Insulin mRNA synthesis	
Lane	Clone	FFU produced	Per cent	Peak area	Per cent
1	PBC8I	131,520	100	81	23
2	pBC8I/PA	260	0.2	346	100
5	pBC8IΔ4	0	0	308	100
6	pBC19I	0	0	46	15

Data are given as in Table 1. Lane nos refer to Fig. 4. Relative insulin mRNA levels were determined by peak area calculation using a DU-8 scanning spectrophotometer. mRNA levels are expressed as a percentage of clone pBC8I/PA (lanes 1 and 2) or pBC8IΔ4 (lanes 5 and 6).

interfere with transcription or contain splice sites[11]. Thus, if we insert a transcriptional terminator into the *env* intron of clone pBC8I, reduced *env* production should be a measure of the premature transcriptional termination produced by this insertion. This is because any transcripts that avoid premature termination would be expected to be spliced in the normal manner, thereby eliminating sequences inserted into the intron and producing normal, functional *env* mRNA.

The transcriptional terminator we have chosen to use is derived from SV40 DNA. A 237 bp fragment containing a polyadenylation site was isolated following cleavage of SV40 DNA at the unique *Bcl*I and *Bam*HI sites. This fragment, which contains no splice sites used by SV40[16], was inserted into the *env* gene intron in clone pBC8I such that the polyadenylation site used in SV40 late gene transcripts was in the sense orientation, thereby yielding clone pBC8I/PA (Fig. 2*F*). Transfection with DNA from clone BC8I/PA results in *env* production reduced by ≈99% relative to clone pBC8I (Table 1) but yields an increase in preproinsulin mRNA synthesis comparable to clone pBC8IΔ2 (Fig. 3). Thus, when transcription from the upstream LTR is prematurely terminated, the inhibition of the promoter activity of the downstream LTR is correspondingly relieved.

Endogenous retroviral LTR

The difference between the levels of transcription promoted by the 3′ downstream LTR in the intact versus deleted clones is only about 5-fold in these experiments. This relatively small difference must be contrasted with the 30- to 100-fold increase in c-*myc* mRNA above basal levels found in the chicken bursal lymphomas[17]. However, small differences in the level of gene expression appear to have biologically significant consequences. We have previously presented evidence that the LTR promoter of the endogenous virus RAV-0 is ≈10-fold less efficient than the exogenous virus LTR in the *env* assay[12]. RAV-0, which also produces 10-fold less mRNA in infected cells *in vivo*, does not cause tumours in chickens[18]. Evidence has been presented by ourselves and others[12,18,19] that this lack of oncogenicity may be due to the lower efficiency of the RAV-0 LTR promoter.

To test the efficiency of the RAV-0 LTR for proinsulin transcription, two additional clones were constructed (Fig. 2,*G*, *H*). pBC8IΔ4 has a deletion of the entire viral transcriptional unit upstream of the 3′ LTR, constructed by the removal of the remaining viral structural gene sequences in pBC8IΔ3. Only ≈40 bp of viral sequences remain in addition to the 3′ LTR. Clone pBC19I contains the RAV-0 LTR in place of the exogenous Schmidt-Ruppin B LTR present in all other constructs, and is in the same orientation as clone pBC8IΔ4.

Data from a transfection experiment using these clones are shown in Fig. 4. In the *env* assay, pBC8I produces the greatest number of focus forming units while, as before, pBC8I/PA gives significantly less activity. pBC8IΔ4 and pBCI9I yield no *env* activity as they lack the *env* gene. Quantitation of the preproinsulin mRNA assay (Table 2) shows that pBC8I/PA (lane 2)

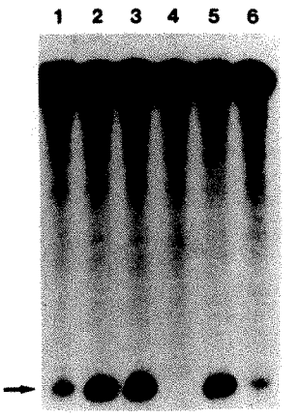


Fig. 4 Comparison to an endogenous retroviral LTR. Lane 1, pBC8I; 2, pBC8I/PA; 3, insulin mRNA positive control; 4, pBC8; 5, pBC8IΔ4; 6, pBC19I. Relative *env* and proinsulin mRNA levels in transfected cells are given in Table 2.
Methods: Transfections were performed as outlined in Fig. 3 with the following minor modifications. Half as much DNA was transfected per culture (2.9 μg of pBC8I and equivalent molar amounts of other clones) and the cultures were treated for 2.5 h with 80 μM chloroquine in media as described by Luthman and Magnusson[32], which results in an increase in transfected DNA uptake. Nine μg of total RNA were analysed per lane.

produces the greatest amount of proinsulin transcripts, about 5-fold more than the pBC8I clone (lane 1). Transcription from pBCI9I yields a low level of insulin mRNA (lane 6), ≈7-fold less than pBC8IΔ4 (lane 5). Thus, the relatively small difference in insulin transcription between the RAV-0 LTR and the exogenous LTR in the deleted clones, which appears sufficient to distinguish a non-oncogenic from an oncogenic LTR promoter, is comparable to the difference caused by transcriptional interference in the downstream LTR of an exogenous retrovirus.

Implications for ALV leukaemogenesis

We have presented evidence that viral mRNA transcription extending into the downstream LTR of a provirus-like construct restricts the ability of this LTR to function as an efficient promoter. The inhibition, which we term transcriptional overlap interference, is removed if the upstream LTR is damaged or deleted or if a transcriptional termination signal is inserted between the LTRs. The increased ability of the downstream LTR of an integrated provirus to act as a promoter following deletion of the upstream LTR provides an explanation for the observation that only deleted proviruses are observed inserted upstream and in the same orientation to c-*myc* in lymphomas of chickens[5–7].

Alternative configurations of the integrated provirus relative to c-*myc* have been described by Payne *et al.*[20]. One bursal lymphoma contained a provirus downstream of c-*myc* in the same orientation, while others had the proviral DNA upstream of c-*myc* in the opposite orientation. In the former case, the provirus was deleted, leaving only the 3′-LTR and adjacent viral DNA. In addition, at least 15 kb of cellular DNA on the 3′ side of c-*myc* was also lost. In the latter cases, deletions of the provirus are also observed. These deletions are, however, internal in most, though not all, cases[6,20]. These two alternative configurations do not allow the direct use of the LTR as the promoter for c-*myc* expression, as predicted by the promoter insertion model. Thus, deletion of LTR sequences would not be required to relieve overlap interference in these cases.

The pathology of lymphoid leukosis in chickens sheds additional light on how neoplastic transformation may proceed[21]. Following infection of newborn chicks by ALV, the earliest histologic evidence of the preneoplastic state is the appearance of transformed follicles in the bursa. A small proportion of these follicles (≈2%) give rise to nodules which latter progress to a disseminated lymphoid leukosis. This multi-step progression to lymphoid tumours may represent discrete events at the

molecular level. Our data are consistent with the hypothesis that malignant transformation may begin by the rare random integration of an ALV provirus adjacent to c-*myc*, which would interrupt the c-*myc* locus. This first event would result in the disruption of normal cellular regulation of the c-*myc* allele, followed by increased expression of c-*myc* promoted by the 3′ LTR of the integrated provirus. A second random event, deletion of the LTR, would cause an additional increase in c-*myc* transcription. It has previously been observed that integrated proviruses may spontaneously delete part of the viral sequence, including an LTR, at a low frequency[22]. These two discrete events may be necessary to allow c-*myc* transcription promoted by the remaining LTR to increase to levels sufficient to cause malignant transformation. Subsequent events, such as the postulated activation of a second oncogene[23], may also be required to produce leukosis.

The control of gene expression

The frequency with which the phenomenon of transcriptional overlap interference occurs remains to be determined. A process analogous to overlap interference has been reported in procaryotes where it has been shown that the upstream P_L promoter of prophage λ inhibits the initiation of transcription at downstream *gal* promoters[24]. Interference between overlapping transcriptional units has also been observed in other prokaryotic operons[25,26]. Therefore, the phenomenon of overlap interference appears not to be unique to the avian provirus but could represent a novel mechanism for the control of gene expression. Transcriptional overlap interference could be used to coordinately regulate two adjacent, overlapping transcriptional units by controlling only the upstream promoter directly. The downstream promoter would become progressively more active as the upstream promoter was repressed and less active as the upstream promoter was induced. A possible example of this phenomenon has been observed in yeast[27]. The 5′ gene in this case is the repressible acid phosphatase gene *PHO*5 while the 3′ gene is the acid phosphatase gene *PHO*3. These genes are closely linked in yeast and were cloned together on a single 8 kb DNA fragment. *PHO*3 is expressed in yeast growing in media containing phosphate while *PHO*5 is repressed. If phosphate is removed *PHO*5 is induced and *PHO*3 is no longer detectable[27]. It has now been demonstrated (R. Kramer and K. Bostian, personal communication) that if the upstream *PHO*5 gene promoter is deleted, the *PHO*3 gene continues to be transcribed in the absence of phosphate. These observations are consistent with the use of transcriptional overlap interference as a mechanism in the coordinate control of differential gene expression.

We thank S. Kahn, S. McAndrew and F. Esch for assistance in DNA sequence analysis, and D. Stacey for the use of clone pLD6. We also thank A. Skalka and R. Kramer for helpful discussions and D. Andriola and S. Sturdivant for typing the manuscript.

Received 22 February; accepted 5 October 1983.

1. Hayward, W. S., Neel, B. G. & Astrin, S. M., *Adv. viral Onc.* **1,** 207–233 (1982).
2. Hughes, S. H. *et al. Cell* **15,** 1397–1410 (1978).
3. Sabran, J. L. *et al. J. Virol.* **29,** 170–178 (1979).
4. Ju, G. & Skalka, A. M. *Cell* **22,** 379–386 (1980).
5. Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. & Astrin, S. M. *Cell* **23,** 323–334 (1981).
6. Payne, G. S. *et al. Cell* **23,** 311–322 (1981).
7. Fung, Y. T., Fadly, A. M., Crittenden, L. B. & Kung, H. J. *Proc. natn. Acad. Sci. U.S.A.* **78,** 3418–3422 (1981).
8. Varmus, H. E. & Swanstrom, R. in *RNA Tumor Viruses* 2nd edn, 369–512 (Cold Spring Harbor Laboratory, New York, 1982).
9. Yamamoto, T., Jay, G. & Pastan, I. *Proc. natn. Acad. Sci. U.S.A.* **77,** 176–180 (1980).
10. Hofer, E. & Darnell, J. E. *Cell* **23,** 585–593 (1981).
11. Cullen, B. R., Kopchick, J. J. & Stacey, D. W. *Nucleic Acids Res.* **10,** 6177–6190 (1982).
12. Cullen, B. R., Skalka, A. M. & Ju, G. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2946–2950 (1983).
13. Lomedico, P. T. *Proc. natn. Acad. Sci. U.S.A.* **79,** 5798–5802 (1982).
14. Stacey, D. W., Allfrey, V. G. & Hanafusa, H. *Proc. natn. Acad. Sci. U.S.A.* **74,** 1614–1618 (1977).
15. Schwartz, D. E., Tizard, R. & Gilbert, W. *Cell* **32,** 853–869 (1983).
16. Griffin, B. E. in *DNA Tumor Viruses* 2nd edn, 61–124 (Cold Spring Harbor Laboratory, New York, 1981).
17. Hayward, W. S., Neel, B. G. & Astrin, S. M. *Nature* **290,** 475–480 (1981).
18. Robinson, H. L., Blais, B. M., Tsichlis, P. N. & Coffin, J. M. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1225–1229 (1982).
19. Tsichlis, P. N. *et al. Molec cell. Biol.* **2,** 1331–1338 (1982).
20. Payne, G. S., Bishop, J. M. & Varmus, H. E. *Nature* **295,** 209–214 (1982).
21. Teich, N., Wyke, J., Mak, T., Bernstein, A. & Hardy, W. in *RNA Tumor Viruses* 2nd edn, 513–648 (Cold Spring Harbor Laboratory, New York, 1982).
22. Varmus, H. E., Quintrell, N. & Ortiz, S. *Cell* **25,** 23–36 (1981).
23. Goubin, G., Goldman, D. S., Luce, J., Neiman, P. E., & Cooper, G. M. *Nature* **302,** 114–119 (1983).
24. Adhya, S. & Gottesman, M. *Cell* **29,** 939–944 (1982).
25. Hausler, B. & Somerville, R. L. *J. molec. Biol.* **127,** 353–356 (1979).
26. Horowitz, H. & Platt, T. *Nucleic Acids Res.* **10,** 5447–5465 (1982).
27. Rogers, D. T., Lemire, J. M. & Bostian, K. A. *Proc. natn. Acad. Sci. U.S.A.* **79,** 2157–2161 (1982).
28. Ju, G., Boone, L. & Skalka, A. M. *J. Virol.* **33,** 1026–1033 (1980).
29. Weiss, R. in *RNA Tumor Viruses* 2nd edn, 209–260 (Cold Spring Harbor Laboratory, New York, 1982).
30. Berk, A. J. & Sharp, P. A. *Proc. natn. Acad. Sci. U.S.A.* **75,** 1274–1278 (1978).
31. Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **3,** 381–391 (1979).
32. Luthman, H. & Magnusson, G. *Nucleic Acids Res.* **11,** 1295–1308 (1983).

LETTERS TO NATURE

A new kind of natural radioactivity

H. J. Rose & G. A. Jones

Department of Nuclear Physics, University of Oxford, Oxford OX1 3RH, UK

From a systematic study of the properties of nuclei heavier than lead, we have concluded that, in one or two cases, radioactive decay by emission of a particle heavier than the α-particle might be observable in competition with the latter. We have observed such decay in ^{223}Ra which occurs in the natural radioactive series emanating from ^{235}U. Two 'by-pass' modes seem likely: emission of ^{14}C, to ^{209}Pb, with a *Q*-value of 32 MeV; and of ^{12}C, to ^{211}Pb, with a *Q*-value of 28 MeV. (Emissions of ^{13}C and ^{15}C from the same nucleus have *a priori* emission rates similar to that of ^{12}C, but the emission of α-like nuclei seems more likely.) In this study we have used a solid-state counter telescope to identify the charge of the particle emitted, and, for greater convenience, a source of ^{227}Ac (half life 21 yr), with which the lower members of the series, including ^{223}Ra, are in secular equilibrium. We have observed particles identifiable as carbon ions and, from their energy and emission rate, they are ^{14}C rather than other carbon isotopes. The branching ratio for emission of ^{14}C nuclei relative to α-particles from ^{223}Ra is $8.5 \pm 2.5 \times 10^{-10}$, corresponding to a reduced width (preformation probability) smaller by a factor of ~10^5 to 10^6.

The obvious problem in the detection of rare modes of decay is to distinguish between these modes and the multiple pile-up of α-particle pulses. Using techniques familiar in nuclear experimentation, we were able to reject events due to pile-up provided that individual α-particles in the event were separated in time by over ~100 ns. Even so, with a detected α-particle rate of ~4,000 s^{-1} we observed, over a counting period of several months, events extending out as far as quadruple pile-up of total energy up to 28 MeV. However, the ratio of ionization in the thin (ΔE) counter of the telescope to that in the thick ($E - \Delta E$) counter for a carbon ion, is quite different from the same ratio arising from α-particle pile-ups of the same or nearly the same total energy, so that all the α-events including multiple pile-up, are clearly segregated from carbon events in a $\Delta E, E$ plot. In any case, the total number of quadruple α-pile-ups to within 5 MeV of our detected events was small; see Fig. 1.

Another problem, which resulted in our doubting the energy measurements of, and consequently rejecting, a run of several months, is that of radiation damage occurring in the semiconductor detectors exposed to a high counting rate over a long period of time. As the ionization density for α-particles is at its highest very close to the end of the range, the $E - \Delta E$ counter responds to damage more quickly than the ΔE counter. Because the α-particles and the carbon ions at the energies of the experiment have much the same range, one cannot reduce the $E - \Delta E$ counter in thickness until it no longer stops the α-particle, but

© 1984 Macmillan Journals Ltd

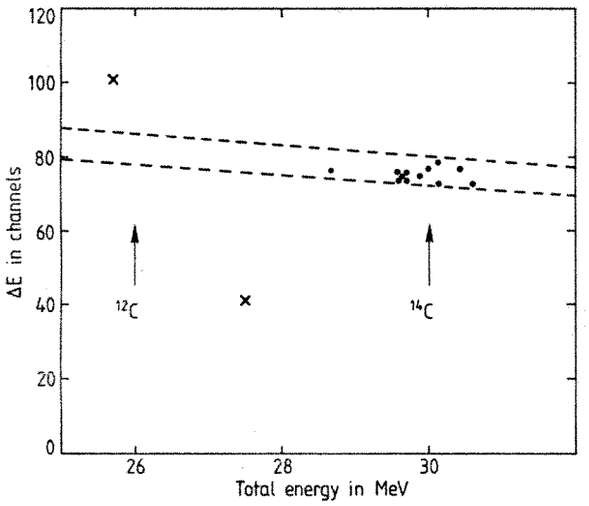


Fig. 1 Contents of the two-dimensional array ΔE versus E_{total} after a run of 189 days. The dotted line indicates the allowed region for carbon ions and the arrows indicate the total energies expected for ^{12}C and ^{14}C emissions in the decay of ^{223}Ra. The lower of the two crosses represents a quadruple pile-up. Below the total energy displayed, large numbers of triple and double α-pile-ups were recorded. Single α-events (and, in part, even double α-pile-ups) were biased out on the analogue side to avoid deadtime problems on the digital side. The upper cross is an event which was recorded during a thunderstorm which affected the mains badly. A run of 194 days was made before this one, yielding 8 events and, in addition, a run of approximately half a year was performed to investigate possible cosmic ray-induced events. Channel 77 in $\Delta E \equiv 6.7$ MeV, which is exactly as expected for 30 MeV ^{14}C. Detector characteristics: The dead layer of the ΔE detector (200 mm^2 active area, 8.2 μm sensitive thickness) was determined to lie between 0.3 and 0.8 μm. In addition a protective layer of gold of thickness 20 $\mu g\,cm^{-2}$ was evaporated on the source and 15 $\mu g\,cm^{-2}$ carbon film inserted between the source and the ΔE detector. An extra 30–40 $\mu g\,cm^{-2}$ of gold is present on the E-detector (300 mm^2 active area). This gives a total of 150–250 $\mu g\,cm^{-2}$ of effective dead layer (Si equivalent) and an energy loss of ^{14}C ions of 0.5–0.8 MeV. The source of strength 3.3 μCi gave a counting rate of $\approx$4,000 s^{-1}, corresponding to an effective solid angle of detection of $\approx 1/3$ sr.

still stops the higher charged particle (a technique used in fission measurements, using gas counters, in order to enhance the fission pulses relative to α-pulses and so overcome the effects of pile-up). After having experienced this effect with a thick detector which had been much used previously, the problem was overcome by using new counters and monitoring closely the single α-pulses in both detectors. While the events detected in the first run were clearly due to carbon ions, the energy measurements were uncertain to ~2.5 MeV, but in the second run the energy measurements were reliable and close to the expected value for ^{14}C emission, that is, 30 MeV, after allowing for the recoil energy of the daughter nucleus (see Fig. 1). The runs were done automatically using on-line LSI-11/Camac-based two-dimensional storage techniques. To mitigate against hardware failure, the results were read out and up-dated every 12 h. In the event of an error occurring during the 12-h period, the computer was programmed to reject that 12-h run and to carry on from the previously stored data.

Before discussing further the energy measurements, the accompanying table is of interest. Of all the exotic emissions possible from the radioactive chain we are using, we have listed those which are most likely to be observed and have given the ratio of their Gamow factors to the appropriate Gamow factors for α-emission for three values of the parameter r_0 in the expression for channel radius, $r = r_0(A^{1/3} + a^{1/3})$, where a represents the mass number of the emitted particle and A that of the daughter nucleus (see Table 1). It is immediately evident that emission of ^{14}C from ^{223}Ra virtually selects itself as the

Table 1 Possible strange decay modes in the ^{235}U natural $(4n+3)$ radioactive series and their relative probabilities for observation

Decay	Q-value (MeV)	Gamow factor for decay/Gamow factor for α-emission $r_0 = 1.15$f	$r_0 = 1.2$f	$r_0 = 1.25$f
$^{227}Th \rightarrow {}^{19}O + {}^{208}Pb$	44.15	4.5×10^{-11}	2.2×10^{-9}	9.7×10^{-8}
$^{227}Th \rightarrow {}^{28}Mg + {}^{199}Pt$	68.25	8×10^{-17}	3.5×10^{-13}	1.0×10^{-10}
$^{227}Th \rightarrow {}^{14}C + {}^{213}Po$	29.4	4.3×10^{-12}	7.7×10^{-11}	1.2×10^{-9}
$^{227}Th \rightarrow {}^{18}O + {}^{209}Pb$	44.2	2.2×10^{-10}	1.1×10^{-8}	4.1×10^{-7}
$^{227}Ac \rightarrow {}^{15}N + {}^{212}Pb$	33.3	2.8×10^{-10}	5.6×10^{-9}	1.2×10^{-7}
$^{223}Ra \rightarrow {}^{15}C + {}^{208}Pb$	29.1	5.3×10^{-12}	1.0×10^{-10}	1.8×10^{-9}
$^{223}Ra \rightarrow {}^{14}C + {}^{209}Pb$	31.8	1.2×10^{-5}	1.7×10^{-4}	2.2×10^{-3}
$^{223}Ra \rightarrow {}^{13}C + {}^{210}Pb$	28.8	1.1×10^{-10}	1.7×10^{-9}	2.1×10^{-8}
$^{223}Ra \rightarrow {}^{12}C + {}^{211}Pb$	27.7	6.7×10^{-12}	8.5×10^{-11}	1.0×10^{-9}
$^{219}Rn \rightarrow {}^{14}C + {}^{205}Hg$	28.03	1.5×10^{-16}	2.7×10^{-15}	4.1×10^{-14}
		Normalizing Gamow factor for α-emission		
$^{227}Th \rightarrow \alpha + {}^{223}Ra$	6.15	4.9×10^{-6}	2.2×10^{-5}	9.8×10^{-5}
$^{227}Ac \rightarrow \alpha + {}^{223}Fr$	5.04	1.3×10^{-11}	6.2×10^{-11}	2.8×10^{-10}
$^{223}Ra \rightarrow \alpha + {}^{219}Rn$	5.98	6×10^{-6}	2.7×10^{-5}	1.2×10^{-4}
$^{219}Rn \rightarrow \alpha + {}^{215}Po$	6.94	5.5×10^{-1}	2.3	9.3

The full Q-values were used in the computations. The major α-modes are known to lead to ground states and/or states nearby (that is, within <300 keV above ground state); l-values can be neglected in the strange decay modes; the considered α-decays for normalization are all leading either to the ground state (or a state nearby) with an $l=0$ or $l=2$ branch, the branching ratio being sufficiently strong for the order-of-magnitude estimates presented.

explanation of our data; emission of ^{12}C, ^{13}C or ^{15}C from the same parent are equally likely (*a priori*) to within a factor of 10, but considerably less so than ^{14}C emission. Since sequences of three α-particles often occur in radioactive chains, one may reasonably expect the preformation probabilities for ^{8}Be, ^{12}C and ^{16}O to be considerably higher than for other configurations; but there seems to be no good reason to assume similar enhancements for ^{13}C and ^{15}C relative to ^{14}C. Considering the data in Table 1, the most likely emission observed is ^{14}C, with ^{12}C (and a more favourable preformation probability) the next most likely. Therefore, we have given the location of the ^{12}C group in Fig. 1.

If we now introduce our measured ratio of $8.5 \pm 2.5 \times 10^{-10}$ carbon nuclei per α-particle and compare it with the Gamow ratio of 1×10^{-5} to 2×10^{-3} (see Table 1) for ^{14}C, 7×10^{-12} to 1×10^{-9} for ^{12}C, 1×10^{-10} to 2×10^{-8} for ^{13}C, and 5×10^{-12} to 2×10^{-9} for ^{15}C, we conclude that the preformation probabilities for ^{12}C, ^{13}C and ^{15}C need to lie somewhere in the range 0.1–10 times that for the α-particle; we consider this to be preposterous for ^{13}C and ^{15}C and unacceptable for ^{12}C. Thus, we conclude that the emissions are of ^{14}C from ^{223}Ra with a preformation probability in the range of 7×10^{-5}–4×10^{-7} times that for α-emission from the same nucleus. These considerations relegate the energy calibration to a somewhat minor role, although important to us as it is a measure of confidence in what we are doing.

The energy calibration was made using ^{241}Am α-particles of 5.48 MeV, and extrapolating up. This may seem rather risky when it is realized that the source was covered by thin gold and carbon foils, to prevent source material sputtering onto the ΔE counter due to daughter recoil on α-decay, and in addition the detectors themselves have unknown dead layers. However, serendipity occasionally comes to the aid of the experimenter; from tables of energy losses of charged particles, it turns out that ^{241}Am α-particles and ~31-MeV ^{14}C ions have almost the same ratio of dE/dx to E thus the fractional energy loss for each particle is the same. Hence the pulse outputs should scale as the total energies independently of the unknown energy losses, provided they are fairly small (extrapolation from energy to energy even for different types of particle is well established for Si semiconductor detectors). As the unknown energy loss is estimated (see Fig. 1 legend) to be of the order of $\leq$1 MeV for carbon ions of these energies, a linear scaling through the origin and the 31-MeV point will produce very little error in the neighbourhood of 30 MeV. It is, therefore, highly gratifying to us that our extrapolated calibration assigns to the closely

grouped events observed almost exactly the energy required for ^{14}C emission from ^{223}Ra.

Finally, we feel that similar by-pass decays must occur in other nuclides in this region, chiefly to the doubly magic nucleus ^{208}Pb and its near isotopes in order to make use of the extra stability of the daughter nucleus. As the neutron excess increases rapidly with Z, the parents will tend to be more neutron-deficient than nuclides in the naturally occurring sequences which are on the neutron-rich side of the valley of stability. The likely candidates for by-pass decay will probably need to be man-made (or arise in series in which the ultimate parent is man-made) rather than occur naturally.

We acknowledge the considerable help and advice of our entire laboratory and particularly thank Mr T. Thacker and Mr P. Dyer. The source was supplied by the Chemistry Division of AERE, Harwell.

Received 7 October; accepted 7 December 1983.

Power spectrum of long-period solar oscillations and 160-min pulsations during 1974–82

A. B. Severny, V. A. Kotov & T. T. Tsap

Crimean Astrophysical Observatory, Nauchny, Crimea, 334413, USSR

We report here the results of solar oscillation observations carried out between 1974 and 1982 using the Crimean solar magnetograph. We have eliminated all peaks of telluric origin from the mean power spectrum; of the remaining 32 dominant ($\geq 2\sigma$) peaks, 19 are of solar origin and 13 are partly blended with the atmospheric peaks. We found that 10 solar peaks are in very good agreement with the modes g_{10}–g_{20} of degree $l = 4$ calculated by Christensen-Dalsgaard *et al.*[1] for the solar model C with the envelope enriched in heavy elements by accretion. Deviations of the observed long-period oscillations from a linearity can be appreciable, leading to the appearance of combination frequencies. The dominant 160-min peak seems to be excited by a strong 'double' resonance of $2g_8$ mode of model C with a combination frequency, and two other combination frequencies of modes $1g_1$ and $2f$ including the unstable mode $l = 1$. As model C is also in good agreement with the neutrino experiment, we suggest that this could be considered as the most appropriate model of the Sun. In contrast to previous detailed studies of solar pulsation with the particular 160.01-min period[2,3], the emphasis here is on the search for other possible long-period solar oscillations of low degree. These oscillations are thought to be bound to the regions beneath the convection zone and penetrate to the Sun's core which makes them a powerful tool for analysing the solar internal structure and the evolution of the Sun.

The Crimean solar magnetograph with a special Doppler shift modification[4] is being used continuously to measure the wavelength difference between two profiles of the same nonmagnetic spectral line λ 5,124 Å—one from the central circular area ($0.66R_\odot$) of the solar disk, and the other from the remaining annular portion of the solar disk[5]. The observations described here were made in 1974–82 during 473 days spanning 2,719 h (for data processing see ref. 5). The double solar magnetograph was used to discriminate real solar peaks in power spectrum from false peaks produced mainly by the Earth's atmosphere (such as fluctuations of transparency and differential extinction). Simultaneous observations over 7 yr of the telluric O_2λ6,879 Å line (1975–82, 905 h in total) were carried out and reduced in a similar way.

The power spectra of the mean 5-min-interval residuals (centre minus limb data) were calculated for both spectral lines in three ways: (1) independent mean power spectra were obtained for each annual data set; (2) the total 1974–82 data set was considered and the coherent spectrum calculated (with a resolution of 1 nHz); (3) nine annual power spectra were normalized and subsequently averaged. The later spectra (with an effective resolution of ~0.07 μHz) in the period range 110–210 min is shown in Fig. 1. Besides the apparent presence of a dominant peak at 160.0 min, there are several other peaks of comparable and lower amplitudes.

To discriminate the real solar peaks of this spectrum we used the similar spectrum of the telluric λ 6,879 Å oxygen line. However, before comparing the solar and atmospheric power spectra we have considered the meaning of the 'coincidence of peaks'. A careful study of the positions of previously selected 93 dominant peaks in the solar and telluric power spectra, based mainly on the comparison of above mentioned three types of spectrum, indicated that the real error in the period's value is $|\Delta T| \leq 0.5$ min for periods ≈160 min. We were able to assign 37 peaks to the telluric power spectrum and they were thus excluded. The 17 peaks observed only at Stanford (1977–80 data) or only at the South Pole were also excluded. Seven peaks that were observed but were not identified with theory[1] were not considered. Of the remaining 32 peaks (listed in Table 1),

Table 1 Real solar peaks

No. of peak	Dominant peaks of solar spectrum (1974–82) T(min)	Identification with the model C $l = 4$ (O–C, min)	$l g_n - l' g_i$
1	200.5		$2g_2$–$2g_4$ (200.6); $2g_1$–$4g_5$ (200.5); atm?
2	200.0 S	$g_{20} = 199.9$ (0.1)	$4g_{20}$
3	196.3		$1g_2$–$4g_{21}$ (196.5)
4	192.6*		$2g_3$–$4g_{14}$ (192.3); atm?
5	192.0*S	$g_{19} = 191.8$ (0.2)	$4g_{19}$
6	188.5*S		$1g_1$–$2g_5$ (188.3)
7	184.7		$3g_4$–$4g_{12}$ (184.9)
8	183.5	$g_{18} = 183.6$ (−0.1)	$4g_{18}$
9	180.2 S		$2g_3$–$4g_{15}$ (180.1)
10	175.6 S	$g_{17} = 175.5$ (0.1)	$4g_{17}$
11	173.9*		$1g_1$–$3g_8$ (173.7)
12	171.0		$3g_4$–$4g_{13}$ (170.8); atm?
13	168.3		$3g_2$–$4g_7$ (168.4); atm?
14	166.9	$g_{16} = 167.2$ (−0.3)	$4g_{16}$
15	160.0 S		$2g_8$(160.80); $1g_1$–$2f$ (160.83); $2g_1$–$4g_6$ (160.24); $3g_4$–$4g_{14}$ (160.21)
16	159.3	$g_{15} = 158.8$ (0.4)	$4g_{15}$
17	157.1		$1g_1$–$2g_6$; (157.2); atm?
18	153.1 S		$1g_1$–$4g_{12}$ (152.85); atm?
19	151.8*		$3g_4$–$4g_{15}$ (151.6); atm?
20	150.0*	$g_{14} = 150.3$ (−0.3)	$4g_{14}$
21	148.4		$3g_3$–$4g_{11}$ (148.1)
22	144.0		$3g_1$–$4g_5$ (144.2); atm?
23	143.2		$1g_1$–$4g_{13}$ (143.0); atm?
24	142.0	$g_{13} = 142.1$ (−0.1)	$4g_{13}$; $2g_3$–$4g_{21}$ (141.7)
25	139.4		$1g_1$–$2g_7$ (139.6); $2g_2$–$4g_{13}$ (139.2); $3g_4$–$4g_{17}$ (139.0)
26	134.3*S	$g_{12} = 133.6$ (0.7)	$4g_{12}$; atm?
27	130.1*S		$3g_3$–$4g_{13}$ (129.8)
28	129.3		$1g_1$–$4g_{15}$ (129.3)
29	122.3*S		$3g_1$–$4g_6$ (122.1); $2g_1$–$4g_8$ (121.9); atm?
30	120.4 S		$2g_1$–$1g_2$ (120.03); $3g_2$–$4g_{10}$ (120.45); $3g_8$ (120.90)
31	119.5		$1g_1$–$3g_{13}$ (119.7); atm?
32	116.4 S	$g_{10} = 116.6$ (−0.2)	$4g_{10}$; atm?

* Peaks not denoted on Fig. 1 (amplitudes ones which are dominant ($>2\sigma$) in the string of 8-year data (1974–81)). Separate peaks 153.3 and 152.9 min have been combined in one peak 153.1 min in accordance with resolution adopted. The same was done with the peaks 170.8 and 171.2 min.

© 1984 Macmillan Journals Ltd

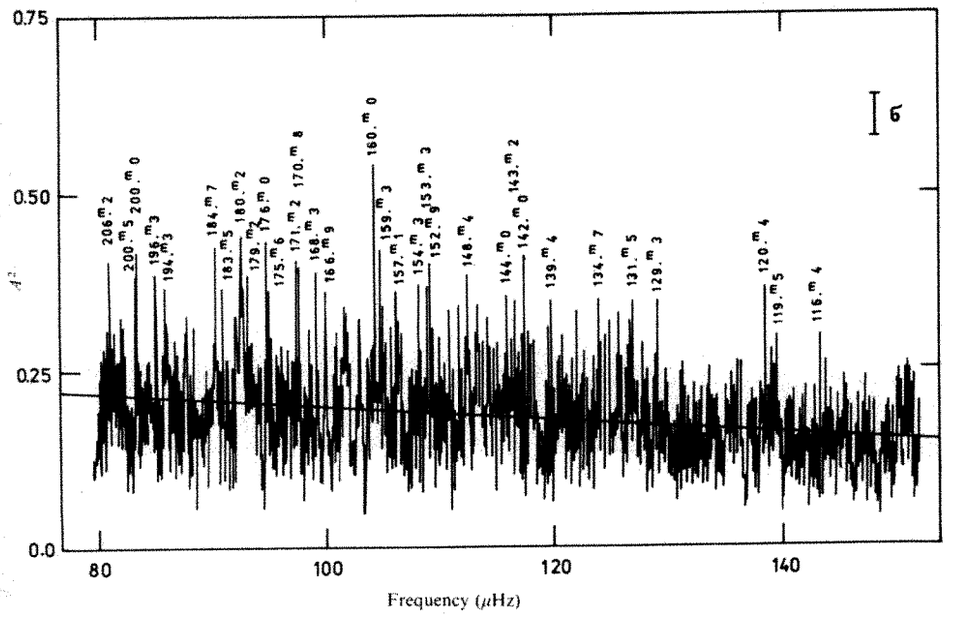


Fig. 1 Power spectrum of solar oscillation, 1974–82. The numbers indicate periods (in min) of dominant peaks, exceeding the 2σ confidence level. Straight line represents the mean level, vertical bar corresponds to 1σ deviation from the mean level.

13 are partly blended with the atmospheric peaks (notified 'atm?') and 19 can be considered as of real solar origin. All these 32 peaks (periods, in minutes) are given in Table 1. Those 12 peaks which coincide with those observed at Stanford (during 1977–80) are denoted (by S). The data obtained at the South Pole are not used here because they are rather scarce and add no essential features either to Table 1 or the echelle-diagram (except one point with the period 204.0 min; the other four points coincide well with our points). Table 1 contains 23 peaks from the 1974–82 data (see Fig. 1) and nine peaks from the 1974–81 data which are dominant for all this time interval.

To estimate the noise level of the power spectrum shown in Fig. 1, the mean of amplitudes and r.m.s. deviation σ_0 associated with it were computed (zero-order approximation). Then all the peaks exceeding the $3\sigma_0$ level were excluded and the new mean amplitude and corresponding r.m.s. (σ_1) were recalculated. Then, all peaks exceeding the $2\sigma_1$ level were excluded, and the final mean amplitude and final r.m.s. noise $\sigma \approx 0.054$ ($m^2\,s^{-2}$) were computed (see Fig. 1 where 32 peaks with amplitudes $A^2 \geq 2\sigma$ are denoted), 23 of these peaks which can be identified are included in Table 1. These 23 peaks are also present in the 1974–81 data which show nine more significant and identified peaks. (Note that the amplitude of 160-min peak is $\approx 6\sigma$).

Having tabulated the periods and considered them as eigenmodes of solar model oscillations, one can immediately find close correspondence of these observed periods with those calculated by Christensen-Dalsgaard *et al.*[1] for their model C of the Sun for degree $l = 4$ and orders ranging from g_{10} to g_{20}. The values 4 g_n calculated in (ref. 1) and taken from their Table 3 are presented in Table 1, as well as on the echelle—diagram (see Fig. 2, where the dashed straight lines connect theoretical values).

There is satisfactory agreement between theoretical 4 g_n values and a string of the observed ones: the mean O–C = 0.05 min for all 10 values. The probability of chance coincidence, within a tolerance ±0.5 min, here $\leq (\frac{1}{2})^{10} \sim 10^{-3}$. The Monte Carlo method, for the determination of probability of the simultaneous chance coincidence, gives the value $\sim 3 \times 10^{-4}$ for 10 g-modes periods (the tolerance window being ±0.33 μHz). We have failed to find a similar significant correspondence for $l = 1$, 2, 3 degrees of C as well as for models A and B of ref. 1.

The mean difference in the observed periods $\overline{\Delta T}$ of successive modes g_n listed in Table 1 is $\overline{\Delta T}_{obs} = 8.36 \pm 0.60$ min. According to the well known asymptotic approximation formula

$$T_{nl} = K\left(n + \frac{l}{2} - \frac{1}{4}\right)(l(l+1))^{1/2} \qquad (1)$$

and $\overline{\Delta T}_{obs}$ we have $K_{obs} = 37.4 \pm 2.7$ min ($l = 4$). On the other hand, Christensen-Dalsgaard *et al.*[1] give $K_{theor} = 37.6$ min for model C, which is in good agreement with observations, and $K_{theor} = 36.2$ min for the standard A-model. If one now compares these values with the $K = 38.6$ found recently by Delache and HScherrer[6] from a very limited set of data (one season during 1979) and for the range of periods mainly $P \geq 200$ min, one comes to the conclusion that their value (38.6 min) is appreciably nearer to our value for the C-model than to K for the standard A-model. The same is true for observations of van der Raay[7] producing the value $K \approx 41.2$ min. As all these authors deal with $l = 1$ or 2 degrees and use a standard model we can conclude that C-model values fit the observed values better than do those of the standard A-model. (However, the disagreement between Delache and Sherrer's K-value and that of van der Raay[7] is quite large, and is ≥ 10 min for periods ≥ 160 min. Difficulties with the standard model and the neccessity of using the lower abundance Z of heavy elements for p-modes ($l < 20$) has recently been pointed out by Rhodes *et al.*[8], see also ref. 9.)

The following evidence favours a deviation of the observed oscillations from linearity. (1) The observed dependence of the amplitude of oscillations on the phase of solar rotation[10] supports the fact that we also observe asymmetric dipole-like ($l = 1$) oscillations contributing to the power spectrum. (2) The recent investigation of Kosovichev and Severny[11] showed that instability really takes place for the $l = 1$ degree in the C-model (for g_1 and g_2-modes; compare with the similar conclusions of Saio[12]). (3) The amplitudes of g-modes increase towards the centre (the core) of the Sun. (4) If one compares the value of harmonic mean of the observed amplitude $\bar{a}_h = 0.67$ with the mean amplitude of bimodal terms $\bar{a}_b = (\overline{a_i a_j})^{1/2} = 0.71$ formed for the dominant peaks, one finds them quite comparable. (S. M. Ruitov, personal communication).

Therefore, because the oscillations are nonlinear, the combination frequencies $\omega_i \pm \omega_k$ can occur (see ref. 13). The corresponding combination frequencies for different peaks were also calculated and are presented in Table 1. The corresponding periods are in fairly good agreement with the observed values.

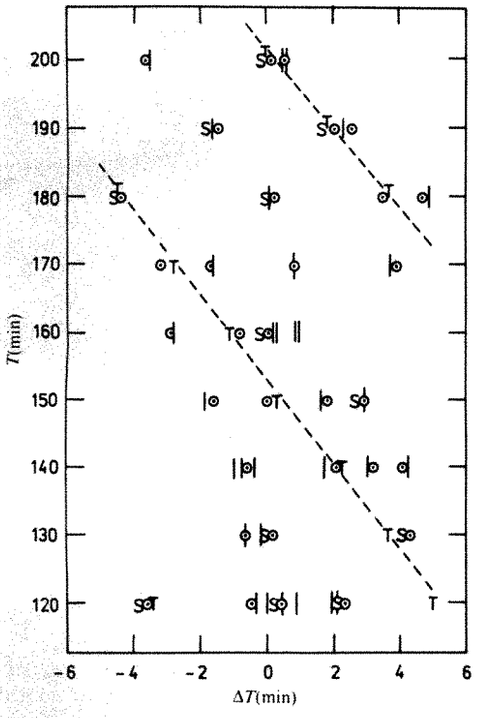


Fig. 2 Echelle-diagram of the positions of all peaks with amplitude $\geq 2\sigma$ for periods between 110 and 210 min constructed with the increment $\Delta T = 10$ min. ⊙, Solar periods; S, peaks in the Stanford spectrum coinciding with ours; T, periods of theoretical[1] 4 g_n-modes for model C and $l = 4$; 1, combination periods (see Table 1). The dashed lines show the sequences of the theoretical 4 g_n-modes.

In conclusion, let us consider 160.0-min oscillation (as well as the 120.4 and 139.4-min peaks). Besides that, at $l = 2$ the g_8 mode does exist for which the calculated value (see Table 3 in ref. 1)

$$T(2g_8) = 160.80 \quad \text{min} \tag{2}$$

and the f- and g_1-modes (the latter can be secularly unstable according to Saio[12]) form the combination mode with the period

$$[T^{-1}(2f) - T^{-1}(1g_1)]^{-1} = 160.83 \quad \text{min} \tag{3}$$

which is almost in resonance with $T(2g_8)$-mode, as $\Delta T_1 = 0.03$ min. Note that $1g_1$ and $2f$ are the lowest modes of model C. There also exists another resonance near the 160-min-period produced from the combination modes

$$\begin{aligned} [T^{-1}(2g_1) - T^{-1}(4g_6)]^{-1} &= 160.24 \quad \text{min} \\ [T^{-1}(3g_4) - T^{-1}(4g_{14})]^{-1} &= 160.21 \quad \text{min} \end{aligned} \tag{4}$$

which also have $\Delta T_2 = 0.03$ min, so that there is a 'higher'-order resonance with $\Delta T_3 = \Delta T_2 - \Delta T_1 \approx 0$ or a 'resonance of two resonances' with $\Delta T = 0.03$ min. If for model C the mode $l = 1$ is unstable (see refs 11, 12) the presence of a resonance mode (3) (and possibly $l = 2$ resonance mode (4)) can lead to a secularly unstable mode near 160.0 min. The possibility of resonance excitation of the 160-min mode was also suggested by Vandakurov[14], but this author had neither proper observational data nor theoretical modes, so that the actual condition for resonance differs from those supposed in ref. 14. The problem of resonance in other cases is now being considered separately, but it is obvious that only a very slight adjustment to model C is needed to make resonance appear precisely at the observed frequency 160.01 min. (For the possible importance of resonance coupling see also ref. 15, and ref. 16 for the instability connected with it).

A similar situation presents itself at $T = 120.4$ min (confirmed by the two Stanford peaks having the mean period 120.1 min). Here we have the secular unstable modes $l = 1$ in two combination frequencies:

$$\begin{aligned} [T^{-1}(2g_1) - T^{-1}(1g_2)]^{-1} &= 120.03 \quad \text{min} \\ [T^{-1}(3g_2) - T^{-1}(4g_{10})]^{-1} &= 120.45 \quad \text{min} \end{aligned} \tag{5}$$

also forming a very close resonance with $\Delta T_1 = 0.42$ min. There also exists a normal mode $l = 3$, g_8 with the period 120.90 min producing the difference $\Delta T_2 = 0.45$ min, so that we again have the condition $\Delta T_3 = \Delta T_2 - \Delta T_1 = 0.03$ min indicating the very close resonance conditions. We think the precise resonance at 120.4 min may mean a very slight change in model C.

We have tried to find out the near-resonance conditions for the standard A-model but have failed so far. Therefore, we have come to the conclusion that model C, which is also in much better agreement with the neutrino experiment than the other two (A and B) models of ref. 1, is a better model of the Sun, and thus the enrichment of solar atmosphere by heavy elements could be connected with the accretion process.

We thank Drs E. Fossat and P. Scherrer for permission to use their power spectra before publication, and Dr Kosovichev for helpful discussions.

Received 24 June; accepted 17 October 1983.

1. Christensen-Dalsgaard, J., Gough, D. O. & Morgan, J. C. *Astr. Astrophys.* **73,** 121–128 (1979).
2. Severny, A. B., Kotov, V. A. & Tsap, T. T. *Nature* **259,** 87–89 (1976).
3. Scherrer, P. H., Wilcox, J. M., Severny, A. B., Kotov, V. A. & Tsap, T. T. *Astrophys. J. Lett.* **237,** L97-L98 (1980).
4. Severny, A. B. *Nature* **224,** 53–54 (1969).
5. Kotov, V. A., Severny, A. B. & Tsap, T. T. *Mon. Not. R. astr. Soc.* **183,** 61–78 (1978).
6. Delache, P. & Scherrer, P. H. *IPR Rep. No 957,* Stanford Univ, (1983).
7. van der Raay, H. B. *EPS Conf., Catania,* 25 (University of Catania, 1983).
8. Rhodes, E. J. Jr, Ulrich R. K. & Brunish, W. M. *EPS Conf.* Catania, 15 (University of Catania, 1983).
9. Ulrich, R. K. & Rhodes, E. J. Jr *Astrophys. J.* **265,** 551–563 (1983).
10. Severny, A. B., Kotov, V. A. & Tsap, T. T. *Proc. 2nd European Meet. on Solar Physics,* 123–134 (CNRS, Toulouse, 1978).
11. Kosovichev, A. G. & Severny, A. B. *EPS Conf.*, Catania, 32 (University of Catania, 1983).
12. Saio, H. *Astrophys. J.* **240,** 685–692 (1980).
13. Landau, L. D. & Lifshitz, E. M. *Mechanics* (Akademie Nauk, Moscow, 1958).
14. Vandakurov, J. V. *Astr. Circ. (USSR),* No 1173, 1–2 (1981).
15. Dziembowski, W. *Solar Phys.* **82,** 259–266 (1983).
16. Perdang, J. *Solar Phys.* **82,** 297–321 (1983).

Early Solar System aqueous activity: Sr isotope evidence from the Orgueil CI meteorite

J. D. Macdougall & G. W. Lugmair

Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA

J. F. Kerridge

Institute of Geophysics and Planetary Physics, University of California, Los Angeles, Los Angeles, California 90024, USA

The CI meteorites are rare but important objects because they may represent our best sample of chemically unfractionated Solar System material (see, for example, ref. 1). Despite the fact that these meteorites apparently retain their original chemical composition, they clearly contain secondary mineral phases, some at least believed to have been produced through the action of liquid water on the parent body[2–6]. The timing of this event, however, was unknown. In an attempt to solve this problem, we have measured the Sr isotopic composition and $^{87}Rb/^{86}Sr$ in carbonates and sulphate separated from the Orgueil meteorite. Both of these phases probably precipitated from aqueous solution. Our first results, reported here, show that carbonate deposition occurred contemporaneously with parent body formation or shortly after it, probably within 100 Myr. On the other hand, at least some of the calcium sulphate seems to have been formed recently.

In the CI meteorites, the Rb/Sr ratio is high, ~0.3 (ref. 7). Thus, in low-Rb phases which contain Sr, the Sr isotopic composition should be a sensitive indicator of the time of formation,

© 1984 Macmillan Journals Ltd

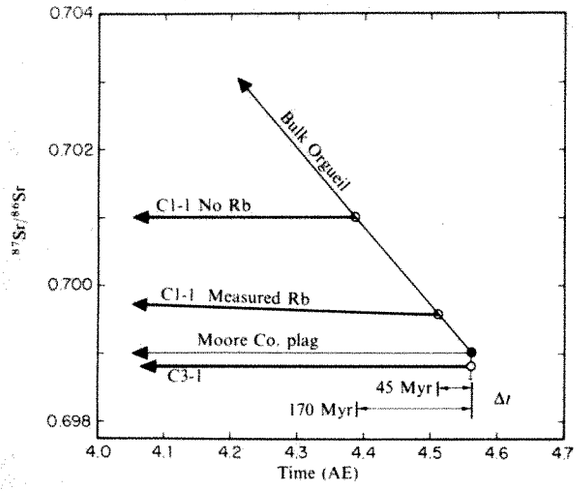


Fig. 1 An illustration of the Sr isotope method for determining the time of formation of low-Rb phases such as carbonates in the CI meteorites, using data for C1-1 (Table 1) as an example. Evolution lines are shown for the bulk meteorite ($^{87}Rb/^{86}Sr \sim 0.8$), the Moore Co. plagioclase, and sample C1-1 assuming as extreme cases that (1) the sample has no indigenous Rb and (2) the measured Rb is the true value. For purposes of calculation, no blank correction was made to the Rb data for case (2), so that $^{87}Rb/^{86}Sr = 0.022$ rather than 0.013 as reported in Table 1. This results in a lower limit for Δt (the time between 4.56 AE and the isolation of C1-1 from bulk Orgueil Sr). Also shown for comparison is the datum for C3-1, which exhibits essentially no change in $^{87}Sr/^{86}Sr$ over 4.56 AE (AE = 10^9 yr).

provided that the Sr was equilibrated with 'bulk meteorite' Sr at that time (Fig. 1). If the CI carbonates and sulphates formed during a period of aqueous activity on the parent body, they would meet this criterion. Indeed, the distribution of Ca in the Orgueil meteorite gives a clue to the behaviour of Sr. Kerridge[8] has shown that the phyllosilicates which make up 60–65% of the meteorite contain about an order of magnitude less Ca than the bulk meteorite. This suggests that most of the Ca—and by implication Sr—is concentrated in Ca-rich carbonates and sulphates.

Carbonates in the CI meteorites span a large compositional range, and sulphates occur primarily as either gypsum or hydrated magnesium sulphate. Sulphates are present as vein minerals clearly deposited along fractures, while the textural relationship between carbonates and the bulk of the meteorite is much less clear[4,6]. Richardson[6], based on a petrographic study of three CI chondrites, claimed that at least three generations of mineralization can be recognized, during which first carbonates, then Ca-sulphates and finally Mg-sulphates were deposited in veins. In the present meteorite, however, only the sulphates exist as true vein minerals; carbonates occur as single crystals or sometimes as aggregates which Richardson[6] interpreted as residual vein fragments. The most abundant carbonates are dolomite and breunnerite, the latter an Fe–Mg-carbonate. Both of these frequently contain considerable Mn[9]. Pure calcium carbonate occurs only rarely.

Sr isotopic data for carbonate and sulphate-rich samples handpicked from Orgueil are given in Table 1. Because of the expected low abundances of the elements of interest, and their susceptibility to leaching and contamination, sample processing was kept to a minimum. Bulk meteorite samples were disaggregated ultrasonically in double-distilled acetone or methanol. Light-coloured grains ≥20 μm were handpicked and grouped according to appearance. Energy-dispersive X-ray analysis using the scanning electron microscope was used to confirm the identity of phases, but because of the small sample amounts, only spot checks could be made and none of the separates is considered to be pure.

Three separate bulk meteorite samples were obtained from P. Pellas (Muséum National d'Histoire Naturelle, Paris) and

Table 1 Sr isotopic data for Orgueil carbonates and sulphates

Sample	$^{87}Sr/^{86}Sr$	Sr (p.p.m.)	$^{87}Rb/^{86}Sr$
ORG-1			
C1-1 (dolomite)	0.701007 ± 38	70	0.013
C1-2 (mixed carbonate)	0.70200 ± 10	68	0.004
ORG-2			
C2-1 (mixed)	0.70202 ± 28	17	0.44
S2-1 (Ca-sulphate)	0.76785 ± 7	87	0.023
ORG-3			
C3-1 (mixed)	0.69882 ± 20	17.1	*
C3-2 (breunnerite + matrix)	0.70522 ± 20	1.06	0.084
Moore Co. plagioclase†	0.69901		
Bulk Orgueil‡	0.75286	7.85	0.805

Sr isotopic data fractionation corrected to $^{86}Sr/^{88}Sr = 0.1194$ and relative to $^{87}Sr/^{86}Sr = 0.71025$ for NBS 987 Sr.
* Rb amount indistinguishable from blank (≤7.4 pg).
† Initial ratio based on measured value for plagioclase, $^{87}Rb/^{86}Sr$ from ref. 15, and 4.56 AE.
‡ After data in ref. 10. The Sr isotopic ratio was calculated using data from Orgueil samples 1 and 3 (ref. 10), our Moore Co. plagioclase initial ratio, and 4.56 AE.

processed as described above. The heterogeneity of the CI meteorites is emphasized by the fact that the populations of light-coloured phases in the three samples varied considerably in mineralogical makeup. In ORG-1, most of the mineral picked as carbonate was dolomite. ORG-2 and ORG-3 both contained considerably more breunnerite than dolomite, and ORG-2 contained a relatively large amount of Ca-sulphate. Each of the mineral separates for which data are reported in Table 1, except for sulphate sample S2-1, was rinsed in double-distilled water in an ultrasonic bath before dissolution; all dissolution was accomplished in 2M HCl and in all cases a small amount of undissolved residue remained. Here we discuss key points of the isotopic data and related sample properties. The details of individual samples will be described more fully elsewhere.

The data in Table 1 show a considerable range in $^{87}Sr/^{86}Sr$ for the carbonates, from 0.6988 to 0.7052. The relatively large errors result from small sample sizes, all ≤1 mg, and therefore extremely small amounts of Sr, ranging from only 32 ng down to 77 pg. The highest measured value is for Ca-sulphate S2-1, with $^{87}Sr/^{86}Sr$ greater than reported values for the bulk meteorite[10] (Table 1). However, the Rb/Sr ratio does not correlate with the Sr isotopic ratios. The lowest measured $^{87}Sr/^{86}Sr$ is for sample C3-1, overlapping within its relatively large error limits our internal initial ratio for basaltic achondrites, determined from measurements of Moore Co. plagioclase (Table 1) and an assumed age of 4.56 AE (AE = 10^9 yr). Sample C3-1 was a small mixed breunnerite + dolomite sample. After dissolution, a small amount of low-Mg breunnerite and a few small enstatite grains remained. The bulk of the dissolved material is assumed to have been dolomite + high-Mg breunnerite. Thus these phases must have been deposited very early in the history of Orgueil, perhaps essentially contemporaneously with parent body formation. The measured isotopic ratio is an upper limit because it is possible that a small amount of high $^{87}Sr/^{86}Sr$, non-carbonate Sr may have been included; nevertheless it provides an upper limit for the initial CI meteorite Sr isotopic ratio. Rb in C3-1 was indistinguishable from blank levels (≤7.4 pg). An important task will be to determine the Sr isotopic ratio more precisely, as other possible indicators of the initial $^{87}Sr/^{86}Sr$ ratio for carbonaceous chondrites come from refractory inclusions in the Allende CV3 meteorite[11,12], and may be representative of neither the bulk Allende meteorite nor carbonaceous chondrites in general. Taking inter-laboratory bias into account, the C3-1 value is consistent with the lowest values reported for Allende[11,12].

As mentioned above, other carbonate separates exhibit considerable variability in $^{87}Sr/^{86}Sr$, and the values of this ratio do not correlate with $^{87}Rb/^{86}Sr$. We do not consider model ages

Table 2 Comparison of water leach and sample data for ORG-3 separates

Sample	$^{87}Sr/^{86}Sr$	Sr (pg)	$^{87}Rb/^{86}Sr$
C3-1	0.69882±20	647	*
C3-1 leach	0.85337±37	680	0.24
C3-2	0.70522±20	77	0.084
C3-2 leach	0.80592±80	175	1.1

* Rb amount indistinguishable from blank (≤7.4 pg).

(see Fig. 1), referred to the bulk meteorite evolution, very meaningful: they range from ~4.5 AE to 9 AE using the values from Table 1. We ascribe this to the mobility of Rb in the meteorite, and the consequent difficulty of making accurate measurements of indigenous Rb in the separates, and also to the problem of 'contamination' of the carbonate separates with non-carbonate (bulk meteorite?) Sr. Even the best characterized of our separates, C3-2, which consisted of two large breunnerite crystals, contained small amounts of what appeared to be matrix material injected along cleavage planes. The isotopic data suggest that some of the less well characterized separates were more pure. It is also possible that some of the observed differences are due to real variability in the initial ratios of the carbonates, which could be caused by local (cm-scale) alteration and carbonate deposition and/or variability in the time scale of deposition. Nevertheless, the timing of deposition would have to be very close to the time of parent body formation. If no non-carbonate Rb or Sr were present in the separates for which data are given in Table 1, they must have formed within ~200 Myr of parent body formation. Even if all Rb is considered to be extraneous to the carbonates, the time scale is still relatively short—ranging from 540 Myr for sample C3-2, which in fact does contain a matrix contaminant, to 170 Myr for C1-1 and zero for C3-1.

A further important result of these initial data concerns samples C3-1 and C3-2. For both of these, Rb and Sr were measured in the 5-min ultrasonic bathwater rinse. In each case the weight loss for this leach treatment was less than several hundredths of a milligram, that is, less than a few per cent of the total sample. However, the amounts of Sr in the leach solution were about the same (C3-1) and greater than (C3-2) those in the samples (Table 2). In addition, the $^{87}Sr/^{86}Sr$ ratio in the leach solutions was very high, considerably greater than that in the bulk meteorite. As in the case of the Ca-sulphate sample, S2-1, the accompanying Rb is insufficient by a large amount to produce this ratio over the lifetime of the meteorite. Thus, there is a mobile, highly radiogenic Sr component in Orgueil not accompanied by its parent Rb. Because of its radiogenic nature, this component must be 'young'. Lacking any knowledge of the Rb–Sr history of the component, it is impossible to quantify this statement, except to say that for Rb/Sr anywhere close to the bulk meteorite, the high $^{87}Sr/^{86}Sr$ implies essentially present-day derivation. Times up to a few tens of millions of years would be permitted. The qualitative similarity of the leach and Ca-sulphate data suggests that Sr in the Ca-sulphate may have been derived recently from such a source. These results also emphasize the problem of determining the true initial ratio of the carbonate phases in the presence of such high $^{87}Sr/^{86}Sr$ components.

The data for sulphate sample S2-1 are intriguing because they suggest that the calcium sulphate was formed very recently. The timing is uncertain but would include the terrestrial age of Orgueil and the presumed breakup time of the parent body, estimated to be ~10 Myr from the cosmic ray exposure age[13,14]. Most previous workers have suggested, implicitly or explicitly, that the vein-filling sulphates and the carbonates were formed at approximately the same time, and certainly were not separated by billions of years[4,6]. Sulphate sample S2-1 was not picked directly from veins so that conceivably it is not composed of vein material; however, the platy or sheet-like habit of most of the sulphate fragments implies rather strongly that they were from fracture linings. Recent dissolution of an early sulphate generation and redeposition incorporating radiogenic Sr could also explain the data. If such an event occurred, it obviously did not greatly affect the carbonates.

These initial experiments show that the Rb–Sr system in Orgueil is complex, but that nevertheless Sr isotopes in the carbonates indicate that they were formed very early, possibly coincident with parent body formation. The lowest measured $^{87}Sr/^{86}Sr$ value among our carbonate separates is consistent with published data for the initial Sr isotopic ratio in refractory inclusions from the carbonaceous chondrite Allende[11,12]. In contrast, the Sr isotopes in a single Ca-sulphate separate suggest that this vein material formed very recently. Further experiments, now underway, will be required to unravel the complicated series of events recorded in the Sr isotopic compositions of the secondary phases of this meteorite.

We thank P. Pellas for supplying the samples for this study and M. Hitchcox for help during manuscript preparation. This work was supported by NASA grants NSG 7121 and NAG 9-49, SIO-IG²L contribution 15.

Received 26 July; accepted 25 October 1983.

1. Ebihara, M., Wolf, R. & Anders, E. *Geochim. cosmochim. Acta* **46,** 1849–1861 (1982).
2. DuFresne, E. R. & Anders, E. *Geochim. cosmochim. Acta* **26,** 1085–1114 (1962).
3. Nagy, B. *Geol. För. Stock. Förh.* **88,** 235–272 (1966).
4. Boström, K. & Fredriksson, K. *Smithson. misc. Collns* **151,** 1–39 (1966).
5. Kerridge, J. F. & Bunch, T. E. in *Asteroids* ed. Gehrels, T., 745–764 (University of Arizona Press, 1979).
6. Richardson, S. M. *Meteoritics* **13,** 141–159 (1978).
7. Anders, E. & Ebihara, M. *Geochim. cosmochim. Acta* **46,** 2363–2380 (1982).
8. Kerridge, J. F. *Earth planet. Sci. Lett.* **29,** 194–200 (1976).
9. Kerridge, J. F., Fredriksson, K., Jarosewich, E., Nelon, J. & Macdougall, J. D. *Meteoritics* **15,** 313–314 (abstr.) (1980).
10. Mittlefehldt, D. W. & Wetherill, G. W. *Geochim. cosmochim. Acta* **43,** 201–206 (1979).
11. Gray, C. M., Papanastassiou, D. A. & Wasserburg, G. J. *Icarus* **20,** 213–239 (1973).
12. Minster, J-F., Birck, J-L. & Allègre, C. J. *Nature* **300,** 414–419 (1982).
13. Jefferey, P. M. & Anders, E. *Geochim. cosmochim. Acta* **34,** 1175–1198 (1970).
14. Kerridge, J. F., Macdougall, J. D. & Marti, K. *Earth planet. Sci. Lett.* **43,** 359–367 (1979).
15. Wasserburg, G. J., Tera, F., Papanastassiou, D. A. & Huneke, J. C. *Earth planet. Sci. Lett.* **35,** 294–316 (1977).

Meteoritic $^{138}Ce/^{142}Ce$ ratio and its evolution

H. Shimizu*, T. Tanaka† & A. Masuda*

* Laboratory for REE Microanalysis, Department of Chemistry, Faculty of Science, The University of Tokyo, Hongo, Tokyo 113, Japan
† Geological Survey of Japan, Higashi 1-1-3, Yatabe, Ibaraki 305, Japan

The nuclide ^{138}La decays to ^{138}Ce and ^{138}Ba by β^- and electron capture, respectively, with a total half life of about 1×10^5 Myr. Rankama[1] suggested the possibility of age determination using this radioactive decay system. Recently, Tanaka and Masuda[2] succeeded in age determination by using the ^{138}La–^{138}Ce geochronometer for a gabbro from the Bushveld complex, South Africa. This La–Ce isotopic system, coupled with the Sm–Nd system, provides a new isotope tracer[2,3] for the geo- and cosmochemical samples, especially for samples having a Ce anomaly in the rare-earth element patterns. Meteoritic primordial composition of cerium isotopes can serve as a basis for La–Ce systematics. Furthermore, Ce isotopic compositions for meteorites will provide information concerning half-life data of the ^{138}La decay system, which remains uncertain. Here, cerium isotope ratios were determined for Juvinas, Pasamonte and ALH-78132 eucrites and the Jilin (H4) chondrite. Neodymium isotope ratios and abundances of lanthanum, cerium, neodymium and samarium were also determined for these meteorites.

The experimental procedures used are almost identical to those of Tanaka and Masuda[2]. About 0.7 to 3 g of each sample was decomposed. Rare-earth elements (REE) were separated

© 1984 Macmillan Journals Ltd

Table 1 Meteoritic $^{138}Ce/^{142}Ce$ and $^{143}Nd/^{144}Nd$ data

	$^{138}La/^{142}Ce$*	$^{138}Ce/^{142}Ce$†	$^{147}Sm/^{144}Nd$*	$^{143}Nd/^{144}Nd$†	Primoridal $^{138}Ce/^{142}Ce$‡
Jilin(1)	0.003046	0.0228538 ± 58	0.1921	0.512587 ± 66	0.0228174 ± 59
Jilin(2)	0.003128	0.0228521 ± 114	0.1915	0.512547 ± 42	0.0228147 ± 115
Pasamonte	0.003082	0.0228518 ± 26	—	—	0.0228149 ± 27
Juvinas	0.003006	0.0228533 ± 26	—	—	0.0228174 ± 27
ALH-78132, 71	0.002341	0.0228489 ± 22	0.1958	0.512543 ± 24	—
ALH-78132, 78	0.003639	0.0228547 ± 118	0.1876	0.512440 ± 30	—
ALH-78132, 79	0.002297	0.0228525 ± 62	0.1971	0.512587 ± 32	—

* Uncertainties are less than 0.3%.

† Errors are 2σ mean and correspond to the last significant figures. The $^{136}Ce/^{142}Ce$ value for Johnson Matthey reagent, JMC 304, was 0.0228559 ± 0.0000011 (2σ) and the $^{143}Nd/^{144}Nd$ value for La Jolla Nd isotope standard was 0.51183 ± 0.00003 (2σ). The average value (±s.d.) of the meteoritic $^{136}Ce/^{142}Ce$ ratio was calculated by the following equation: $\bar{x} \pm \sigma = [\sum(x_i/\sigma_i^2)/\sum(1/\sigma_i^2)] \pm \sqrt{[\sum(x_i - \bar{x})^2/\sigma_i^2]/(n-1)\sum(1/\sigma_i^2)}$.

‡ The values are estimated for 4.56×10^3 Myr ago, by assuming $\lambda_\beta\ ^{138}La = 2.58 \times 10^{-12}\ yr^{-1}$ and $\lambda_{EC}\ ^{138}La = 4.59 \times 10^{-12}\ yr^{-1}$.

from the major elements by an AG50W-X8 resin column in HCl media, while Ce and Nd were separated using an AG50W-X8 resin column with α-hydroxy-isobutyric acid. Ce and Nd isotopic compositions were measured on a semi-automatic Micromass 54R and abundances of La, Ce, Nd and Sm were determined on a JEOL JMS-05RB mass spectrometer. Cerium isotopes (about 5 μg Ce loaded) were measured as CeO^+ using Re triple filaments and the observed $^{138}Ce/^{142}Ce$ were normalized to $^{136}Ce/^{142}Ce = 0.01720$. The neodymium ion was measured as NdO^+ using a Re single filament and the measured isotope ratios were normalized against $^{146}Nd/^{144}Nd = 0.7219$.

Table 1 shows the results of Ce and Nd isotopic ratios as well as $^{138}La/^{142}Ce$ and $^{147}Sm/^{144}Nd$ ratios. The $^{138}Ce/^{142}Ce$ ratios for Juvinas and Pasamonte eucrites and the Jilin chondrite are between 0.0228518 and 0.0228538. An average value of the present $^{138}Ce/^{142}Ce$ ratios for these meteorites is 0.0228527 ± 0.0000009 (2σ). Furthermore, the average value of the $^{138}La/^{142}Ce$ ratios for these meteorites is 0.00306 ± 0.00005 (2σ). This value corresponds to 0.378 of the La/Ce abundance ratio. Here, we evaluate the primordial $^{138}Ce/^{142}Ce$ ratio for these three meteorites by assuming $T = 4.56 \times 10^3$ Myr, $\lambda_\beta\ ^{138}La = 2.58 \times 10^{-12}\ yr^{-1}$ and $\lambda_{EC}\ ^{138}La = 4.59 \times 10^{-12}\ yr^{-1}$ (ref. 2), and by using the present observed values for the La/Ce abundance ratios. The calculated primordial values of $^{138}Ce/^{142}Ce$ are 0.0228174 and 0.0228147 for the Jilin chondrite, 0.0228149 for the Pasamonte eucrite and 0.0228174 for the Juvinas eucrite. The average value of these four calculated ratios for primordial $^{138}Ce/^{142}Ce$ is 0.0228162 ± 0.0000014 (2σ). Tanaka and Masuda[2] estimated primordial $^{138}Ce/^{142}Ce$ to be 0.022816. An error in the calculation of primordial $^{138}Ce/^{142}Ce$ ratio for each meteorite in Table 1 involves the errors in the isotope and concentration determination. In this calculation, the error in the La/Ce abundance ratio is estimated to be 0.3%. In the calculation, a variation of 0.3% in the $^{138}La/^{142}Ce$ value yields only a minor change of 0.0000001 in the primordial $^{138}Ce/^{142}Ce$ value. The primordial value (0.0228162) obtained from the Ce isotope ratios of meteorites will be of great significance as a reference for Ce isotope systematics.

The Antarctic eucrites display a heterogeneous distribution of Ce anomaly[4]. Samples ALH-78132,71 and ALH-78132,79 are from the outermost part of the ALH-78132 Antarctic eucrite and they show a positive Ce anomaly. ALH-78132,78 is a chip from the inner part of the same eucrite (several centimetres inside the ALH-78132,79 sample) and this ALH-78132,78 sample possesses a negative Ce anomaly. The observed $^{138}Ce/^{142}Ce$ ratios are 0.0228489 ± 0.0000022 (2σ) for ALH-78132,71, 0.0228525 ± 0.0000062 for ALH-78132,79 and 0.0228547 ± 0.0000118 for ALH-78132,78. Based on the primordial value for $^{138}Ce/^{142}Ce$, and assuming that the present observed values for the La/Ce abundance ratio are valid for 4.56×10^3 Myr, except for the change due to radioactive decay, one is led to the result that the present $^{138}Ce/^{142}Ce$ ratios should be 0.0228442 ± 0.0000015 for ALH-78132,71, 0.0228437 ± 0.0000015 for ALH-78132,79 and 0.0228597 ± 0.0000015 for ALH-78132,78. Comparing the observed $^{138}Ce/^{142}Ce$ ratios of ALH-78132 with the corresponding values of other meteorites and calculated $^{138}Ce/^{142}Ce$ ratios for ALH-78132, we conclude that the positive Ce anomaly observed in ALH-78132,71 and ALH-78132,79 was not produced 4.56×10^3 Myr ago. This conclusion suggests strongly that the positive Ce anomaly in question was produced by terrestrial weathering. The observed $^{138}Ce/^{142}Ce$ ratio of ALH-78132,78 is somewhat lower than the calculated ratio, but the precision in isotope ratio determination for this sample was relatively poor. Therefore, more precise determination of Ce isotope abundance will be needed before we know whether the negative Ce anomaly for ALH-78132,78 was produced by terrestrial weathering.

We thank Dr H. Mabuchi and Dr B. Mason for offering Juvinas and Pasamonte eucrites, respectively, also the National Institute of Polar Research, Japan for offering the Antarctic eucrite.

Received 27 June; accepted 15 November 1983.

1. Rankama, K. *Isotope Geology* (Pergamon, Oxford, 1954).
2. Tanaka, T. & Masuda, A. *Nature* **300,** 515–518 (1982).
3. Nakamura, N., Tatsumoto, M. & Ludwig, K. R. *Lunar planet. Sci.* **14,** 542–543 (1983).
4. Shimizu, H., Tanaka, T. & Masuda, A. *Mem. natn. Inst. polar Res.* (in the press).

Carbon isotope composition of low molecular weight hydrocarbons and monocarboxylic acids from Murchison meteorite

George Yuen*, Neal Blair†‡, David J. Des Marais† & Sherwood Chang†

* Center for Meteorite Studies, Department of Chemistry, Arizona State University, Tempe, Arizona 85287, USA
† NASA Ames Research Center, Moffett Field, California 94035, USA
‡ Department of Chemistry, Stanford University, Stanford, California 94301, USA

The origin of the organic matter in carbonaceous meteorites remains controversial despite extensive study[1–4] over the past 20 yr. Motivated by the expectation that the patterns of isotopic variation with molecular structure among the organic compounds would contain important clues to their origin, we have measured the carbon isotopic compositions for individual hydrocarbons and monocarboxylic acids from Murchison meteorite, a C2 carbonaceous chondrite which fell in Australia in 1969. With few exceptions, notably benzene, the volatile products are substantially isotopically heavier than their terrestrial counterparts, signifying their extraterrestrial origin. For both classes of compounds, the ratio of ^{13}C to ^{12}C decreases with increasing carbon number in a roughly parallel manner,

0028–0836/84/030252—03$01.00

and each carboxylic acid exhibits a higher isotopic ratio than the hydrocarbon containing the same number of carbon atoms. These trends are consistent with the kinetically controlled synthesis of higher homologues from lower ones[5,6]. The results suggest the possibility that the production mechanisms for hydrocarbons and carboxylic acids may be similar; they also impose constraints on the identity of the reactant species.

Identifications and abundances of individual hydrocarbons[7,8], monocarboxylic acids[9,10], and amino acids[11–20] have been reported, but these data have not identified clearly either the source regions or the production mechanisms for these compounds. Large differences in carbon isotopic composition between carbonate and acid insoluble organic matter have been interpreted as evidence for a Fischer–Tropsch synthesis for all the carbonaceous matter from CO, H_2 and NH_3 in the solar nebula[21,22]. However, recent measurements of the nitrogen[23–25], carbon[3,24–26] and hydrogen[24–27] isotopic compositions of the organic matter question rather than support this simple interpretation. In a summary of the only previous attempt to measure the isotopic composition of individual compounds, a range of values was reported collectively for an amino acid fraction[26].

In a closed, evacuated 700-ml Pyrex apparatus, three interior pieces (23.5 g) of Murchison meteorite were disaggregated by 40 freeze–thaw cycles in water (50 ml) assisted by sonication. After a 20-ml aliquot of the head space gas was removed for analysis of the predominant constituent, carbon dioxide, (Table 1), the remaining head space gases were cryogenically transferred, along with several millilitres of water, to a KOH trap cooled with liquid nitrogen, while the freeze–thaw apparatus was maintained at 40 °C by a water bath. Normally, methane and carbon monoxide are non-condensable at liquid nitrogen temperature, but they become condensable at that temperature in the presence of water, probably by clathrate formation[8]. The gases in the KOH trap were released by warming and introduced into a gas chromatograph–combustion (GCC) system[28,29], which separated and combusted each component to carbon dioxide for isotopic analysis. The ^{13}C abundances of hydrocarbon standards processed through the isolation–combustion procedure differed by no more than 0–0.2‰ from those of unprocessed standards. Ethane was the worst case in that it was 0.2‰ enriched in ^{13}C relative to the unprocessed standard. The recoveries of standards were 94–100% with butene having the poorest at 94%. Apparently, little, if any, sample loss or isotopic fractionation occurred during the overall analytical procedure. Abundances and carbon isotope values for the individual hydrocarbons thus obtained from Murchison meteorite are listed in Table 1.

For the analyses of the carboxylic acids, the solution in the freeze–thaw apparatus was separated from the meteorite solid, neutralized with KOH, and dried by rotary evaporation. The resulting residue was redissolved in water, acidified with H_3PO_4 and distilled. The volatile carboxylic acids in the distillate were separated by ion suppression chromatography and combusted to CO_2 in the GCC system. The isotopic compositions of two known carboxylic acids, acetic and propionic acid, were in good agreement (<0.7‰) before and after processing through the analytical procedure. The abundances and isotopic compositions of the water soluble carboxylic acids in Murchison meteorite are given in Table 1.

The abundances of carboxylic acids and saturated hydrocarbons decrease with increasing carbon number, and acids are more abundant than hydrocarbons of the same carbon number by factors of 10 (C_5) to 100 (C_2). The carbon in the acids is systematically heavier isotopically than that in the hydrocarbons. Generally, for both classes of compounds, increasing carbon number is also associated with decreasing ^{13}C-abundance, and branched isomers are isotopically heavier than their normal (straight-chained) counterparts. The isotopic compositions of the saturated members of each class are plotted against carbon number in Fig. 1, with CO_2 taken as the C_1-acid counterpart to CH_4 and assuming −7.9‰ (Table 1) to be representative of pentane. Except for the deviation by butane, two roughly parallel curves result for the normal isomers.

Table 1 Abundance and isotopic composition of hydrocarbons* and monocarboxylic acids† in Murchison meteorite

Compound	Abundance (nmol g^{-1})	$\delta\,^{13}C_{PDB}$
Carbon dioxide	2.4×10^3	+29.1 ± 0.2
Carbon monoxide	2.1	−32 ± 2
Methane	8.9	+9.2 ± 1.0
Ethane	8.5	+3.7 ± 0.1
Propane	8.9	+1.2 ± 0.1
Isobutane	4.3	+4.4 ± 0.1
Butane	5.5	+2.4 ± 0.1
Pentane	‡	
Ethene	1.3	−0.1 ± 0.4
Butenes	‡	
Benzene	28	−28.7 ± 0.2
Acetic acid	1.70×10^3	+22.7 ± 0.2
Propionic acid	6.2×10^2	+17.4 ± 0.2
Isobutyric acid	2.5×10^2	+16.9 ± 0.2
Butyric acid	1.8×10^2	+11.0 ± 0.3
Isovaleric acid	1.0×10^2	+8.0 ± 0.2
Valeric acid	47	+4.5 ± 0.2

* The gas mixture was transferred to a KOH trap at liquid nitrogen temperature. To separate these volatile gases for GCC analysis, the gases from the KOH trap were passed through a trap at dry ice–acetone temperature (water removal) on the way to the GCC sample loop (liquid nitrogen temperature), where the condensable gases collected and the noncondensable gases (mostly methane and carbon monoxide) flowed through to a collection trap with the aid of a Toepler pump. Except for CH_4 and CO, separation of the hydrocarbons on the GCC system was achieved on a Porasil B column. Chromatographic conditions: thermal conductivity detector, stainless steel column (2.5 m × 0.3 cm internal diameter) packed with Porasil B 100/150 mesh. Initial oven temperature, 28 °C, initial time 16 min; temperature programmed at 6 °C min^{-1} from 28 to 200 °C; flow rate 15 ml min^{-1}. Methane and carbon monoxide were separated on a stainless steel chromatographic column (1 m × 0.3 cm internal diameter) packed with molecular sieve 5A, 100/120 mesh, column temperature, 60 °C, flow rate 15 ml min^{-1}.

† The aqueous acid extract was brought to *p*H 10 with 2.7 M NaOH and dried by rotary evaporation. The sodium salts were dissolved in 5 ml H_2O, acidified to *p*H 2 with H_3PO_4 and distilled cryogenically. The distillate was brought to *p*H 10 and dried at 100 °C. The resulting residue was taken up in 200 μl of 1 M H_2SO_4 and chromatographed on a Hewlett-Packard 1084B liquid chromatograph, equipped with an Alltech RP-8 column (25 cm × 4.6 mm). A gradient of 100% 0.01 M H_2SO_4 going to 85% 0.01 M H_2SO_4 and 15% MeOH was used to separate the acids. The butyric and valeric acids (*n*- and iso-) were collected as two groups. The collected fractions were neutralized with NaOH and dried at 100 °C. Each sample was dissolved in 100 μl of H_2O and 50 μl of H_3PO_4 and cryogenically distilled for GCC. The acids are then separated and combusted to CO_2. Gas chromatographic conditions: stainless steel column (2 m × 0.3 cm internal diameter) packed with 10% Alltech AT 1000 on Gas Chrom A 60–80 mesh, oven temperature 140 °C, flow rate 15 ml min^{-1}.

‡ Analysed together, 1.2×10^{-2} μmol g^{-1}, −7.9 ± 0.05‰.

Although these data alone do not give clear indications of the origin(s) of the hydrocarbons and acids, some general conclusions about the required extraterrestrial processes are warranted. Both classes of compounds are products of kinetically controlled processes. That the abundances of the individual hydrocarbons in the suite are approximately of the same magnitude contradicts relative abundances expected of a thermodynamically controlled process. Calculations carried out for sets of temperature, pressure, and H to C to O elemental ratios that approximate possible solar nebular and planetary conditions[30] yielded relative abundances for methane, ethane, and benzene that vary typically by factors exceeding 10^5. The observed variations shown in Table 1 are even smaller than those reported by Des Marais *et al.*[31] for a similar suite of hydrocarbons in natural gas samples whose kinetically controlled relative abundances differed typically by $<10^3$. A similar conclusion regarding the role of thermodynamic equilibrium is applicable to the carboxylic acid abundances[30,32].

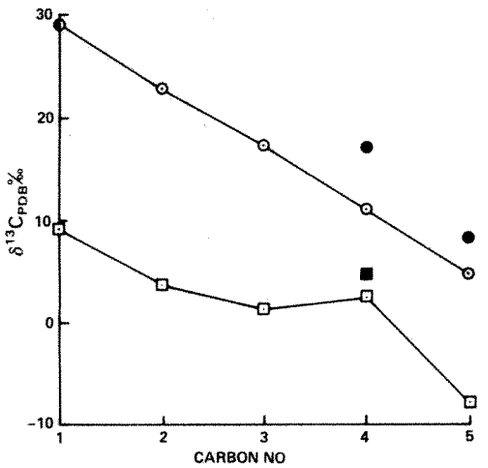


Fig. 1 Plots of $\delta\ ^{13}C_{PDB}$ values of individual saturated hydrocarbons (⊡) and carboxylic acids (⊙) against carbon number. For example, 1 denotes methane or CO_2 (○), 2 ethane or acetic acid and so forth. Values for branched isomers are indicated by filled symbols.

The variation of isotopic composition with carbon number is consistent with formation of higher molecular weight species from lower homologues. Chang *et al.*[6] and Des Marais *et al.*[5] demonstrated that the ratio of ^{13}C to ^{12}C in hydrocarbons synthesized from methane are lower than that of the reactant, decreasing monotonically with increasing carbon number. This pattern follows from the fact that in kinetically controlled synthesis a reactant containing ^{12}C typically reacts more rapidly than its counterpart containing ^{13}C. The trend towards isotopically lighter carbon with increasing carbon number is apparent for both classes of compounds in the data of Table 1. The roughly parallel isotopic variation between both classes implies that some aspects of their histories are similar. The isotopic data also indicate that the carbon monoxide and acid insoluble carbon (both isotopically light, $<-10‰$) are unlikely to have supplied the source carbon for most, if not all, the compounds analysed. No processes have been reported that yield organic compounds in which the carbon is isotopically heavier than that of the starting material. Multiple production mechanisms are not excluded by the data, however. Work in progress is aimed at elucidating the possible connection between CO_2 and the hydrocarbons in the synthesis of the carboxylic acids by determining the intramolecular isotopic composition of the volatile acids.

In principle these compounds in carbonaceous meteorites may have been synthesized in interstellar clouds, the pre-solar nebula or parent bodies (or some combination)[3,4,23–27,32]. This uncertainty in origin may be diminished as more systematic study is directed at determining the variations of C, N, H-isotopic composition with chemical structure in the organic constituents of meteorites, on the one hand, and in the organic compounds produced by model processes, on the other. When the patterns of isotopic variation with chemical structure in the natural and synthetic products are compared, similarities may come to light that are diagnostic of common production mechanisms and are therefore attributable to specific source regions.

We thank the Smithsonian for meteorite specimens 5455. G.Y. was supported by an NRC–NASA fellowship and by NASA NSG-7255.

Received 25 July; accepted 11 October 1983.

1. Hayes, J. M. *Geochim. cosmochim. Acta* **31,** 1395–1440 (1967).
2. Kung, C. C., Hayatsu, R., Studier, M. H. & Clayton, R. N. *Earth planet. Sci. Lett.* **46,** 141–146 (1979).
3. Bunch, T. E. & Chang, S. *Geochim. cosmochim. Acta* **44,** 1543–1577 (1980).
4. Hayatsu, R. & Anders, E. *Topic Curr. Chem.* **99,** 1–37 (1981).
5. Des Marais, D. J., Donchin, J. H., Nehring, N. L. & Truesdell, A. H. *Nature* **292,** 826–828 (1981).
6. Chang, S., Des Marais, D. J., Mack, R., Miller, S. L. & Strathearn, G. *Earth's Earliest Biosphere: Its Origin and Early Evolution* (ed. Schopf, J. W.) (Princeton University Press, 1983).
7. Belsky, T. & Kaplan, T. R. *Geochim. cosmochim. Acta* **34,** 257–278 (1970).
8. Studier, M., Hayatsu, R. & Anders, E. *Geochim. cosmochim Acta* **36,** 189–215 (1972).
9. Yuen, G. & Kvenvolden, K. *Nature* **246,** 301–303 (1973).
10. Lawless, J. & Yuen, G. *Nature* **282,** 396–398 (1979).
11. Kvenvolden, K. *et al. Nature* **228,** 923–926 (1970).
12. Kvenvolden, K., Lawless, J. & Ponnamperuma, C. *Proc. natn. Acad. Sci. U.S.A.* **68,** 486–490 (1971).
13. Lawless, J., Kvenvolden, K., Peterson, E., Ponnamperuma, C. & Moore, C. *Science* **173,** 626–627 (1971).
14. Lawless, J., Kvenvolden, K., Peterson, E., Ponnamperuma, C. & Jarosewich, E. *Nature* **236,** 66–67 (1972).
15. Oro, J., Gibert, J., Lichtenstein, H., Wikstrom, S. & Flory, D. *Nature* **230,** 105–106 (1971).
16. Oro, J., Nakaparksin, S., Lichtenstein, H. & Gil-Av, E. *Nature* **230,** 107–108 (1971).
17. Cronin, J. & Moore, C. *Science* **172,** 1327–1329 (1971).
18. Cronin, J. & Moore, C. *Geochim. cosmochim. Acta* **40,** 853–857 (1976).
19. Cronin, J., Pizzarello, S. & Moore, C. *Science* **206,** 335–337 (1979).
20. Shimoyama, A., Ponnamperuma, C. & Yanai, K. *Nature* **282,** 394–396 (1979).
21. Lancet, M. S. & Anders, E. *Science* **170,** 980–982 (1970).
22. Yoshino, D., Hayatsu, R. & Anders, E. *Geochim. cosmochim. Acta* **35,** 927–938 (1971).
23. Kung, C. C. & Clayton, R. N. *Earth planet. Sci. Lett.* **38,** 421–435 (1978).
24. Robert, F. & Epstein, S. *Geochim. cosmochim. Acta* **46,** 81–95 (1982).
25. Becker, R. & Epstein, S. *Geochim. cosmochim. Acta* **41,** 97–103 (1982).
26. Chang, S., Mack, R. & Lennon, K. *Lunar planet. Sci.* **9,** 157–159 (1978).
27. Kerridge, J. *Lunar planet. Sci.* **14,** 365–366 (1983).
28. Des Marais, D. J. *Analyt. Chem.* **50,** 1405–1406 (1978).
29. Matthews, D. E. & Hayes, J. M. *Analyt. Chem.* **50,** 1465–1473 (1978).
30. Dayhoff, M. O., Lippincott, E. R., Eck, R. V. & Nagarajan, G. *Thermodynamic Equilibrium in Prebiological Atmospheres of C, H, O, N, P, S and Cl* (NASA SP-3040, Office of Technology Utilization, NASA, Washington DC, 1967).
31. Des Marais, D. J., Stallard, M. L., Nehring, N. L. & Truesdell, A. H. *Proc. 4th Symp. on the Cerro Prieto Geothermal Field* (Comision Federal de Electricidad, Baja California, 1982).
32. Hartman, H., Fegley, B., Prinn, R. G. & Lewis, J. S. *Lunar planet. Sci.* **14,** 279–280 (1983).

Stable isotope compositions of gases and vegetation near naturally burning coal

Jim D. Gleason & T. Kurtis Kyser*

US Geological Survey, MS 963, Denver Federal Center, Denver, Colorado 80225, USA

Our measurements of stable isotope compositions of CO_2 issuing from vents produced by naturally burning coal indicate that the coal is oxidized through a kinetic process in which groundwater is the oxidizing agent. The CO_2 produced by the oxidation of the coal is extremely depleted in ^{13}C relative to normal atmospheric CO_2. The change in the $\delta^{13}C$ value of atmospheric CO_2 near the vents resulting from the burning coal was not recorded in tree rings from red cedars, but the $\delta^{13}C$ values of some C_3 and C_4 type plants collected from within the area were greatly affected. Our results indicate that the $\delta^{13}C$ values of some species of plants may be sensitive indicators of changes in the carbon isotopic composition of atmospheric CO_2.

Although the photosynthetic pathway of a plant appears to be the primary factor that determines its carbon isotope ratio[1,2], the range in the carbon isotopic composition of plants having the same pathway must be due to other factors. Mazany *et al.*[3] suggested that variations in the $^{13}C/^{12}C$ ratios of tree rings from the same tree were due to past changes in climate, and that the two species of trees they studied must have had different responses to these climatic changes. Sternberg and DeNiro[4] measured a range of nearly 5‰ in the $^{13}C/^{12}C$ ratios of cellulose of C_3 plants collected from the same area and they ascribed this range to differences among the biological factors of the plants. Inasmuch as the $^{13}C/^{12}C$ ratios measured by Sternberg and DeNiro[4] were different for the cellulose from plants collected from the same area and having the same photosynthetic pathway and physiognomy, the biological factors must be as species-dependent as the climatic factors.

To understand better the response of different species of plants to changes in the $^{13}C/^{12}C$ ratio of atmospheric CO_2 due to the burning of fossil fuels, we have measured the stable isotopic composition of herbaceous plants and trees surrounding vents produced by naturally burning coal as well as of the gases emanating from these vents. Carbon isotope ratios of plants surrounding burning coal vents should be sensitive indicators of

* Present address: Department of Geological Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

0028-0836/84/030254—04$01.00

Author Index for Volume 306

November — December 1983

Issue no.	Issue date	Pagination
5938	3 November	1-100
5939	10 November	101-210
5940	17 November	211-300
5941	24 November	301-410
5942	1 December	411-520
5943	8 December	521-626
5944	15 December	627-718
5945	22/29 December	719-816

Ackley S. F. *see under* Garrison D. L., **306**, 363
Adams J. *see under* Paquin C. E., **306**, 368
Addison F. T. *see under* Incoronato A., **306**, 461
Adelstein R. S. *see under* Naka M., **306**, 490
Adrian T. E., Allen J. M., Bloom S. R., Ghatei M. A., Rossor M. N., Roberts G. W., Crow T. J., Tatemoto K. and Polak J. M. Neuropeptide Y distribution in human brain, **306**, 584
Ager D. *book review of* Great Geological Controversies, (Hallam A.) **306**, 128
Agranovich V. M. and Mills D. L. *Editors*, Surface Polaritons: Electromagnetic Waves at Surfaces and Interfaces, *book review by* Bell R. J. **306**, 95
Aldrich R. W., Corey D. P. and Stevens C. F. A reinterpretation of mammalian sodium channel gating based on single channel recording, **306**, 436
Alerstam T. and Högstedt G. The role of the geomagnetic field in the development of birds' compass sense, **306**, 463
Alexander H., Johnson D. A., Rosen J., Jerabek L., Green N., Weissman I. L. and Lerner R. A. Mimicking the alloantigenicity of proteins with chemically synthesized peptides differing in single amino acids, **306**, 697
Alitalo K., Ramsay G., Bishop J. M., Pfeifer S. O., Colby W. W. and Levinson A. D. Identification of nuclear proteins encoded by viral and cellular *myc* oncogenes, **306**, 274
Allan J. *see under* Kimura T., **306**, 709
Allen J. M. *see under* Adrian T. E., **306**, 584
Almond D. P. and West A. R. Anomalous conductivity prefactors in fast ion conductors, **306**, 456
Alpert J. E. *see under* Bannon M. J., **306**, 791
Anders R. F. *see under* Coppel R. L., **306**, 751
Anderson M. A. *see under* Gusella J. F., **306**, 234
Anderson R. N. World petroleum congress: Hot prospects in the South China Sea, *news and views*, **306**, 227
Andrews J. H. *book review of* A Field Guide to the Birds of Britain and Europe, (Peterson R. T. *editor*) **306**, 513
book review of Birdwatcher's Companion, (Leahy C.) **306**, 514
book review of North American Marsh Birds, (Low G. and Mansell W.) **306**, 513
book review of Owls of Britain and Europe, (Wardhaugh T.) **306**, 513
book review of Owls of Europe, (Mikkola H.) **306**, 513
book review of Seabirds: An Identification Guide, (Harrison P.) **306**, 513
book review of The Birds of the Balearics, (Bannerman D. and Bannerman M.) **306**, 514
book review of The Birdwatcher's Activity Book, **306**, 514
book review of Weather and Bird Behaviour, (Elkins N.) **306**, 514
Aptekar R. L. *see under* Golenetskii S. V., **306**, 451
Arak A. Sexual selection by male–male competition in natterjack toad choruses, **306**, 261
Armstrong D. M. *see under* Vincent S. R., **306**, 688
Arnold F. *see under* Heitmann H., **306**, 747
Aronson J. L., Walter R. C. and Taieb M. Correlation of Tulu Bor Tuff at Koobi Fora with the Sidi Hakoma Tuff at Hadar, *matters arising*, **306**, 210
ar-Rushdi A. *see under* Nowell P., **306**, 494
Artzt K. *see under* Shin H.-S., **306**, 380
Ashby E. *book review of* A Science Policy for Britain, (Dalyell T.) **306**, 136
Ashwal L. D. and Wooden J. L. Isotopic evidence from the eastern Canadian shield for geochemical discontinuity in the Proterozoic mantle, **306**, 679
Asimov I. Exploring the Earth and the Cosmos, *book review by* Wade N. **306**, 133
Popularizing science, *leading article*, **306**, 119
The Roving Mind, *book review by* Press W. H. **306**, 510
Astrin S. M. *see under* Reitsma P. H., **306**, 492
Atiyah M. *book review of* Topology and Geometry for Physicists, (Nash C. and Sen S.) **306**, 296
Attenborough R. *book review of* Ever-Expanding Horizons: The Dual Informational Sources of Human Evolution, (Swanson C. P.) **306**, 624
book review of Human Culture: A Moment in Evolution, (Dobzhansky T. and Boesiger E., Wallace B. *editor*) **306**, 624
Audley-Charles M. G. Reconstruction of eastern Gondwanaland, **306**, 48
see under Price N. J., **306**, 572
Azuma H. *see under* Stevenson H. W., **306**, 291
Baath L. *see under* Moore R. L., **306**, 44
Baer R. *see under* Rabbitts T. H., **306**, 760
see under Rabbitts T. H., **306**, 806
Baldo B. A. and Fisher M. M. Substituted ammonium ions as allergenic determinants in drug allergy, **306**, 262
Ballard D. H., Hinton G. E. and Sejnowski T. J. Parallel visual computation, *review article*, **306**, 21
Balonek T. J. *see under* Dent W. A., **306**, 41
Bannerman D. and Bannerman M. The Birds of the Balearics, *book review by* Andrews J. H. **306**, 514
Bannerman M. *see under* Bannerman D., **306**, 514
Bannon M. J., Elliott P. J., Alpert J. E., Goedert M., Iversen S. D. and Iversen L. L. Role of endogenous substance P in stress-induced activation of mesocortical dopamine neurones, **306**, 791
Barbacid M. *see under* Sukumar S., **306**, 658
Barinaga M., Yamonoto G., Rivier C., Vale W., Evans R. and Rosenfeld M. G. Transcriptional regulation of growth hormone gene expression by growth hormone-releasing factor, **306**, 84
Barlow D. *see under* blundell T., **306**, 281
Barnes R. D. Phylogenetics: Origins of the lower invertebrates, *news and views*, **306**, 224
Barnstable C., Jessel T., Sanes J., Stevens C. and Robertson M. Neurosciences: How molecular is neurobiology?, *news and views*, **306**, 14
Barrow J. D. *book review of* Frame of the Universe: A History of Physical Cosmology, (Durham F. and Purrington R. O.) **306**, 123
Barry J. C. *see under* Raza S. M., **306**, 52
Barry J. D. *see under* Crowe J. S., **306**, 389
Barry J. D., Crowe J. S. and Vickerman K. Instability of the *Trypanosoma brucei rhodesiense* metacyclic variable antigen repertoire, **306**, 699
Bar-Shavit Z. *see under* Reitsma P. H., **306**, 492
Barton N. and Jones J. S. Evolutionary biology: Mitochondrial DNA: new clues about evolution, *news and views*, **306**, 317
Bartram C. R. *see under* Heisterkamp N., **306**, 239
Bath G. T. *book review of* Cosmology and Astrophysics: Essays in Honor of Thomas Gold, (Terzian Y. and Bilson E. M. *editors*) **306**, 127
Baxter R. M. and Carey J. H. Evidence for photochemical generation of superoxide ion in humic waters, **306**, 575
Bazin H. *see under* Sümegi J., **306**, 497
Beck-Engeser G. B. *see under* Burrows P. D., **306**, 243
Becker Y. *see under* Gilden D. H., **306**, 478
Bell B. R. Significance of ferrodioritic liquids in magma mixing processes, **306**, 323
Bell R. J. *book review of* Surface Polaritons: Electromagnetic Waves at Surfaces and Interfaces, (Agranovich V. M. and Mills D. L. *editors*) **306**, 95
Belluardo N. *see under* Forni G., **306**, 181
Benedict R. Race and Racism, *book review by* Bynum W. F. **306**, 138
Bennett D. *see under* Shin H.-S., **306**, 380
Bennett P. M. *see under* Harvey P. H., **306**, 314
Benton M. J. *book review of* Collins Guide to Dinosaurs, (Lambert D.) **306**, 515
book review of Fossils: the Key to the Past, (Fortey R.) **306**, 515
Berlin N. I. AIDS funding, *correspondence*, **306**, 310
Bernstein A. *see under* Rubin J. S., **306**, 206
Berridge M. J. *see under* Streb H., **306**, 67
Bienz B. *see under* Zakut-Houri R., **306**, 594
Billard T. C. and Burns G. Time-delayed photoelectric effect, **306**, 247
Bilson E. M. *see under* Terzian Y., **306**, 127
Bindoni M. *see under* Forni G., **306**, 181
Bird A. *see under* Macleod D., **306**, 200
Biretta J. A., Cohen M. H., Unwin S. C. and Pauliny-Toth I. I. K. Non-radial ejection in 3C345, **306**, 42
Bishop J. M. *see under* Alitalo K., **306**, 274
see under Ralston R., **306**, 803
Bittle J. L. *see under* Rowlands D. J., **306**, 694
Blake C. Molecular biology: Exons — present from the beginning? *news and views*, **306**, 535
Blattner F. R. *see under* Richards J. E., **306**, 483
Bleil U. and Petersen N. Variations in magnetization intensity of ocean floor basalts—Reply, *matters arising*, **306**, 92
Blinkhorn S. *book review of* The Throwing Madonna: Essays on the Brain, (Calvin W. H.) **306**, 139
Bloom B. R. *see under* Nagata Y., **306**, 597
Bloom S. R. *see under* Adrian T. E., **306**, 584
blundell T., Barlow D., Borkakoti N. and Thornton J. Solvent-induced distortions and the curvature of α-helices, **306**, 281
Boden M. *book review of* In-Depth Understanding: A Computer Model of Integrated Processing for Narrative Comprehension, (Dyer M. G.) **306**, 130
Boesiger E. *see under* Dobzhansky T., **306**, 624
Bohren C. F. and Huffman D. R. Absorption and Scattering of Light by Small Particles, *book review by* Kam Z. **306**, 625
Bond D. J. and Chandley A. C. Aneuploidy, *book review by* Tease C. **306**, 816
Bond G. C., Kominz M. A. and Devlin W. J. Thermal subsidence and eustasy in the Lower Palaeozoic miogeocline of western North America, **306**, 775
Bonilla E. *see under* Gusella J. F., **306**, 234
Bonner W. A. *see under* Rubenstein E., **306**, 118
Boraschi D. *see under* Tagliabue A., **306**, 184
Borkakoti N. *see under* blundell T., **306**, 281
Borst P. *see under* Heymans H. S. A., **306**, 69
Boulet A. M. *see under* Walker M. D., **306**, 557
Bowler P. J. The Eclipse of Darwinism: Anti-Darwinian Evolution Theories in the Decades around 1900, *book review by* Hull D. L. **306**, 714
Boyce A. J., Coleman M. L. and Russell M. J. Formation of fossil hydrothermal chimneys and mounds from Silvermines, Ireland, **306**, 545
Boyle E. *see under* Wong C. S., **306**, 623
Bradley J. P. and Buseck P. R. Catalytically grown carbon filaments from a smelter aerosol, **306**, 770
Bradley R. *book review of* The Farming of Prehistoric Britain, (Fowler P. J.) **306**, 716
Brennan M. The Stars and the Stones: Ancient Art and Astronomy in Ireland, *book review by* Daniel G. **306**, 516
Brenner D. The macromolecular states of solvent-swollen and dry coal, **306**, 772
Brewer J. *see under* King G., **306**, 147
Brewer P. G. *Editor*, Oceanography: The Present and Future, *book review by* Holligan P. M. **306**, 815
Brickell P. M., Latchman D. S., Murphy D., Willison K. and Rigby P. W. J. Activation of a *Qa/Tla* class I major histocompatibility antigen gene is a general feature of oncogenesis in the mouse, **306**, 756
Brinster R. L., Ritchie K. A., Hammer R. E., O'Brien R. L., Arp B. and Storb U. Expression of a microinjected immunoglobulin gene in the spleen of transgenic mice, **306**, 332
Brittain T. *see under* Wells R. M. G., **306**, 500
Broda E. Ludwig Boltzmann. Man. Physicist. Philosopher, *book review by* Roche J. J. **306**, 128
Broda P. and Paterson A. Applied molecular biology: Lignin biodegradation becomes biochemistry, *news and views*, **306**, 737
Brothers A. J. *see under* Ezzell R. M., **306**, 620
Brown F. *see under* Rowlands D. J., **306**, 694
Brown F. H. Correlation of Tulu Bor Tuff at Koobi Fora with the Sidi Hakoma Tuff at Hadar—Reply, *matters arising*, **306**, 209
Brown G. S. *see under* Rubenstein E., **306**, 118
Brown G. V. *see under* Coppel R. L., **306**, 751
Bruland K. W. *see under* Wong C. S., **306**, 623
Buck K. R. *see under* Garrison D. L., **306**, 363
Bullock B. *see under* Jansen M., **306**, 609
Bunn H. F. and McDonald M. J. Electrostatic interactions in the assembly of haemoglobin, **306**, 498
Burger K. and Vértes A. Capillary Mössbauer spectroscopy for solution chemistry, **306**, 353
Burke J. F. *see under* Sinclair J. H., **306**, 198
Burley N. *see under* Willson M. F., **306**, 405
Burns G. *see under* Billard T. C., **306**, 247
Burrows P. D., Beck-Engeser G. B. and Wabl M. R. Immunoglobulin heavy-chain class switching in a pre-B cell line is accompanied by DNA rearrangement, **306**, 243
Burton J. D. *see under* Wong C. S., **306**, 623
Buseck P. R. *see under* Bradley J. P., **306**, 770
see under Tomeoka K., **306**, 354
Butler R. P-wave travel time and amplitude in western North America, **306**, 677
Bynum W. F. *book review of* Race and Racism, (Benedict R.) **306**, 138
Cain A. J. *book review of* Dear Lord Rothschild: Birds, Butterflies & History, (Rothschild M.) **306**, 506
Cairns J. and Logan J. Cancer and cell senescence, *correspondence*, **306**, 742
Calder N. *book review of* Quarks: The Stuff of Matter, (Fritzsch H.) **306**, 508
Calvert P. *book review of* An Introduction to Speciality Poly-

mers, (Ise N. and Tabushi I., *editors*) **306,** 295
Calvin W. H. The Throwing Madonna: Essays on the Brain, *book review by* Blinkhorn S. **306,** 139
Camerino G., Mattei M. G., Mattei J. F., Jaye M. and Mandel J. L. Close linkage of fragile X-mental retardation syndrome to haemophilia B and transmission through a normal male, **306,** 701
Camerman A., Mastropaolo D., Karle I., Karle J. and Camerman N. Crystal structure of leucine-enkephalin, **306,** 447
Camerman N. *see under* Camerman A., **306,** 447
Canaani E. *see under* Cohen J. B., **306,** 797
Cande W. Z. *see under* Ezzell R. M., **306,** 620
Cannon F. and Postgate J. Expression of *Klebsiella* nitrogen fixation genes in *Azotobacter* — a caution, **306,** 290
Caplan A. L. Animals do have rights, *correspondence,* **306,** 110
Carey J. H. *see under* Baxter R. M., **306,** 575
Carlin C. R., Phillips P. D., Knowles B. B. and Cristofalo V. J. Diminished *in vitro* tyrosine kinase activity of the EGF receptor of senescent human fibroblasts, **306,** 617
Carney D. N. *see under* Little C. D., **306,** 194
Caron M. G. *see under* Cerione R. A., **306,** 562
Carr B. J., McDowell J. and Sato H. Can pregalactic stars or black holes generate an IR background? **306,** 666
Carroll A. R. *see under* Rowlands D. J., **306,** 694
Carroll M. C. *see under* Palsdottir A., **306,** 615
Cartwright D. E. IOS closures, *correspondence,* **306,** 530
Cattermole P. *see under* Moore P., **306,** 507
Cerione R. A., Strulovici B., Benovic J. L., Lefkowitz R. J. and Caron M. G. Pure β-adrenergic receptor: the single polypeptide confers catecholamine responsiveness to adenylate cyclase, **306,** 562
Chameides W. L. *book review of* Atmospheric Chemistry, (Goldberg E. D. *editor*) **306,** 714
Chandley A. C. *see under* Bond D. J., **306,** 816
Chandra T. *see under* Woo S. L. C., **306,** 151
Chandrasekhar S. The Mathematical Theory of Black Holes, *book review by* Penrose R. **306,** 404
Chapman R. G. and Duval C. T. *Editors,* Charles Darwin: A Centennial Commemorative, *book review by* Jones J. S. **306,** 512
Chapman V. M. *see under* Lindahl K. F., **306,** 383
Charlesworth D. *book review of* Mate Choice in Plants: Tactics, Mechanisms and Consequences, (Willson M. F. and Burley N.) **306,** 405
Chen C.-L. C. *see under* Gee C. E., **306,** 374
Childs G. *see under* Liebermann, D., **306,** 342
Chini R. *see under* Middleditch J., **306,** 163
Ciejek E. M., Tsai M.-J. and O'Malley B. W. Actively transcribed genes are associated with the nuclear matrix, **306,** 607
Cifelli R. L. *see under* Novacek M. J., **306,** 683
Ciochon R. L. and Corruccini R. S. *Editors,* New Interpretations of Ape and Human Ancestry, *book review by* Wood B. **306,** 140
Clarke B. E. *see under* Rowlands D. J., **306,** 694
Clarke C. J. S. *book review of* The Enigma of Time, (Landsberg P. T.) **306,** 131
book review of The Genesis and Evolution of Time: A Critique of Interpretation in Physics, (Fraser J. T.) **306,** 131
Claussen M. J. *see under* Lo K. Y., **306,** 647
Clegg J. B. *see under* Higgs D. R., **306,** 398
Clements M. A. *book review of* Wild Orchids of Britain and Europe, (Davies P. and J. and Huxley A.) **306,** 626
Close F. Particle physics: On the scent of glue, *news and views,* **306,** 312
book review of The Cosmic Onion: Quarks and the Nature of the Universe, **306,** 508
Clutton-Brock T. *book review of* Social Behavior of Female Vertebrates, (Wasser S. *editor*) **306,** 716
Coe M. *book review of* The Ecology of a Tropical Forest: Seasonal Rhythms and Long Term Changes, (Leigh E. G., Rand A. S., Windsor D. M. *editors*) **306,** 625
Cohen D. *book review of* Landscapes of the Night: How and Why We Dream, (Evans C. *editor*) **306,** 511
Cohen J. B., Unger T., Rechavi G., Canaani E. and Givol D. Rearrangement of the oncogene c-*mos* in mouse nyeloma NSI and hybridomas, **306,** 797
Cohen M. H. *see under* Biretta J. A., **306,** 42
Cohen S. N. *see under* Liebermann, D., **306,** 342
Cohn Z. A. *see under* Young, J. D.-E., **306,** 186
Colby W. W. *see under* Alitalo K., **306,** 274
Cole M. and Smither R. *book review of* Biochemistry and Genetic Regulation of Commercially Important Antibiotics, (Vining L. *editor*) **306,** 94
book review of Legal Protection for Microbiological and Genetic Engineering Inventions, (Saliwanchik R.) **306,** 94
Coleman M. L. *see under* Boyce A. J., **306,** 545
Coles P. Psychology: Widening vistas for eye movement research,Vision *news and views,* **306,** 116
Coll J. *see under* Leprince D., **306,** 395
Connealy P. M. *see under* Gusella J. F., **306,** 234
Conrad P. J. *see under* Janeway Jr, C. A., **306,** 80
Conrath B. J. and Gierasch P. J. Evidence for disequilibrium of ortho and para hydrogen on Jupiter from Voyager IRIS measurements, **306,** 571
Conroy G. C. *see under* Falk D., **306,** 779
Cook D. J. Relief in microscopy, *correspondence,* **306,** 428
Cooke J. *see under* Gimlich R. L., **306,** 471
Cooke J. and Webber J. A. Developmental biology: Vertebrate embryos: diversity in developmental strategies, *news and views,* **306,** 423
Coppel R. L., Cowman A. F., Lingelbach K. R., Brown G. V., Saint R. B., Kemp D. J. and Anders R. F. Isolate-specific S-antigen of *Plasmodium falciparum* contains a repeated sequence of eleven amino acids, **306,** 751
Cordes J. M. *see under* Stinebring D. R., **306,** 349
Corey D. P. *see under* Aldrich R. W., **306,** 436
Corruccini R. S. *see under* Ciochon R. L., **306,** 140
Cotman C. W. *see under* Monaghan D. T., **306,** 176
Cowman A. F. *see under* Coppel R. L., **306,** 751
Cox F. E. G. Parasitology: Malaria and babesiosis, *news and views,* **306,** 114
Craddock V. M. Toxicology: Nitrosamines and human cancer: proof of an association? *news and views,* **306,** 638
Craig R. Cell biology: Muscle *au naturel, news and views,* **306,** 112
Crawley M. J. Herbivory: The Dynamics of Animal–Plant Interactions, *book review by* Whittaker J. B. **306,** 816
Cresse I. *see under* Leff S. E., **306,** 586
Cristiani S. *see under* Pedersen H., **306,** 568
Cristofalo V. J. *see under* Carlin C. R., **306,** 617
Croce C. M. *see under* Nowell P., **306,** 494
Cross S. J. *see under* Palsdottir A., **306,** 615
Crow T. J. *see under* Adrian T. E., **306,** 584
Crowe J. S. *see under* Barry J. D., **306,** 699
Crowe J. S., Barry J. D., Luckins A. G., Ross C. A. and Vickerman K. All metacyclic variable antigen types of *Trypanosoma congolense* identified using monoclonal antibodies, **306,** 389
Cudaback D. *see under* Middleditch J., **306,** 163
Curry W. B. and Lohmann G. P. Reduced advection into Atlantic Ocean deep eastern basins during last glaciation maximum, **306,** 577
Dainton F. *book review of* How the PhD came to Britain: A Century of Struggle for Postgraduate Education, (SimpsonR.) **306,** 93
Dalla Favera R. *see under* Gelmann E. P., **306,** 799
see under Nowell P., **306,** 494
Dalyell T. A Science Policy for Britain, *book review by* Ashby E. **306,** 136
Daniel G. *book review of* The Stars and the Stones: Ancient Art and Astronomy in Ireland, (Brennan M.) **306,** 516
Darius J. *book review of* The Atlas of the Solar System, (Moore P., Hunt G., Nicolson I., Cattermole P.) **306,** 507
Datta N. and Hughes V. M. Plasmids of the same Inc groups in enterobacteria before and after the medical use of antibiotics, **306,** 616
Davidson J. P. Lesser Antilles isotopic evidence of the role of subducted sediment in island arc magma genesis, **306,** 253
Davies A. M. *see under* Lumsden A. G. S., **306,** 786
Davies P. and J. and Huxley A. Wild Orchids of Britain and Europe, *book review by* Clements M. A. **306,** 626
Davies R. D. *see under* van der Hulst J. M., **306,** 566
Day R. A. How to Write and Publish a Scientific Paper, 2nd edn, *book review by* Kletz T. A. **306,** 133
de Klein A. *see under* Heisterkamp N., **306,** 239
de Taisne C. *see under* Leprince D., **306,** 395
de Wilde M. *see under* Mekalanos J. J., **306,** 551
Dearborn D. *see under* Middleditch J., **306,** 163
Deeter W. B. *see under* Gracely R. H., **306,** 264
DeFeo-Jones D., Scolnick E. M., Koller R. and Dhar R. *ras*-Related gene sequences identified and isolated from *Saccharomyces cerevisiae,* **306,** 707
Degueurce A. *see under* Edvinsson L., **306,** 55
Deken J. Computer Images: State of the Art, *book review by* Richards W. G. **306,** 509
del Pino E. M. and Elinson R. P. A novel development pattern for frogs: gastrulation produces an embryonic disk, **306,** 589
Delache P. Making news, *correspondence,* **306,** 310
Delache P. and Scherrer P. H. Detection of solar gravity mode oscillations, **306,** 651
deLange T. *see under* Kioussis D., **306,** 662
Dent W. A., O'Dea C. P., Balonek T. J., Hobbs R. W. and Howard R. J. A rapid millimetre wave outburst in the nucleus of NGC1275, **306,** 41
Devlin M. *see under* Gilden D. H., **306,** 478
Devlin W. J. *see under* Bond G. C., **306,** 775
Dhar R. *see under* DeFeo-Jones D., **306,** 707
Diamond B. *see under* Nagata Y., **306,** 597
Dirckx J. H. The Language of Medicine: Its Evolution, Structure, and Dynamics, 2nd Edn., *book review by* Gratzer W. **306,** 814
Diringer H., Gelderblom H., Hilmert H., Özel M., Edelbluth C. and Kimberlin R. H. Scrapie infectivity, fibrils and low molecular weight protein, **306,** 476
Ditfurth H. v. Heinegg P. (*Trans.*) The Origins of Life: Evolution as Creation, *book review by* Marsden G. M. **306,** 403
Djeu J. Y., Lanza E., Pastore S. and Hapel A. J. Selective growth of natural cytotoxic but not natural killer effector cells in interleukin-3, **306,** 788
Djorgovski S. and Spinrad H. Searches for optical counterparts of the two millisecond pulsars, **306,** 569
Dobzhansky T. and Boesiger E. and Wallace B. *Editor,* Human Culture: A Moment in Evolution, *book review by* Attenborough R. **306,** 624
Donath C. *see under* Gallwitz D., **306,** 704
Downie A. Nitrogen fixation symposium: Molecular genetics: out of the laboratory into the field, *news and views,* **306,** 639
Draznin M. B. *see under* Rapoport R. M., **306,** 174
Dubner R. *see under* Gracely R. H., **306,** 264
Dubocovich M. L. Melatonin is a potent modulator of dopamine release in the retina, **306,** 782
Duesberg P. H. *see under* Nunn M. F., **306,** 391
Duncan G. Ciba Foundation symposium: Human cataract formation, *news and views,* **306,** 640
Durham F. and Purrington R. O. Frame of the Universe: A History of Physical Cosmology, *book review by* Barrow J. D. **306,** 123
Durkin J. T. Imperialist threat? *correspondence,* **306,** 420
Duval C. T. *see under* Chapman R. G., **306,** 512
Duverger D. *see under* Edvinsson L., **306,** 55
Dyer M. G. In-Depth Understanding: A Computer Model of Integrated Processing for Narrative Comprehension, *book review by* Boden M. **306,** 130
Edelbluth C. *see under* Diringer H., **306,** 476
Edlund T. *see under* Walker M. D., **306,** 557
Edmunds M. G. Astronomy: The decline of a superstar? *news and views,* **306,** 740
Edvinsson L., Degueurce A., Duverger D., MacKenzie E. T. and Scatton B. Central serotonergic nerves project to the pial vessels of the brain, **306,** 55
Edwards J. H. *see under* Palsdottir A., **306,** 615
Eichelberger J. C. *see under* Taylor B. E., **306,** 541
Eisenberg L. *book review of* How Humans Adapt: A Biocultural Odyssey, (Ortner D. J. *editor*) **306,** 293
Ekstrom A., Fookes C. J. R., Hambley T., Loeh H. J., Miller S. A. and Taylor J. C. Determination of the crystal structure of a petroporphyrin isolated from oil shale, **306,** 173
Elinson R. P. *see under* del Pino E. M., **306,** 589
Elkins N. Weather and Bird Behaviour, *book review by* Andrews J. H. **306,** 514
Elliott P. J. *see under* Bannon M. J., **306,** 791
Emanuel B. S. *see under* Nowell P., **306,** 494
Emerson D. L. *see under* Petrini M., **306,** 73
Estivariz F. E. *see under* Lowry P. J., **306,** 70
Evans C. *Editor,* Landscapes of the Night: How and Why We Dream, *book review by* Cohen D. **306,** 511
Evans M. C. W. *book review of* Photosynthesis Vol. 1 Energy Conversion by Plants and Bacteria. Vol. 2 Development, Carbon Metabolism, and Plant Productivity, (Govindjee *editor*) **306,** 96
Evans R. *see under* Barinaga M., **306,** 84
Evans R. M. *see under* Mayo K. E., **306,** 86
Ezzell R. M., Brothers A. J. and Cande W. Z. Phosphorylation-dependent contraction of actomyosin gels from amphibian eggs, **306,** 620
Fairall A. P. and Gaskell M. Electron densities in active galaxies—Reply, *matters arising,* **306,** 503
Fairbanks R. G. *see under* Miller K. G., **306,** 250
Falk D. and Conroy G. C. The cranial venous sinus system in *Australopithecus afarensis,* **306,** 779
Feigenbaum E. A. and McCorduck P. The Fifth Generation: Artificial Intelligence and Japan's Computer Challenge to the World, *book review by* Lavington S. **306,** 129
Fensom A. *see under* Olsen I., **306,** 75
Festinger L. The Human Legacy, *book review by* Jahoda M. **306,** 96
Fibiger H. C. *see under* Vincent S. R., **306,** 688
Fields H. L., Vanegas H., Hentall I. D. and Zorman G. Evidence that disinhibition of brain stem neurones contributes to morphine analgesia, **306,** 684
Finan J. *see under* Nowell P., **306,** 494
Fincham J. R. S. Molecular genetics: Transposable elements and plant gene structure, *news and views,* **306,** 425
Finlayson-Pitts B. J. Reaction of NO_2 with NaCl and atmospheric implications of NOCl formation, **306,** 676
Fisher L. M. *see under* Mills F. C., **306,** 809
Fisher M. M. *see under* Baldo B. A., **306,** 262
Fitton J. G., Kilburn C. R. J., Thirlwall M. F. and Hughes D. J. 1982 eruption of Mount Cameroon, West Africa, **306,** 327
Flaherty L. *see under* Shin H.-S., **306,** 380
Flavell R. A. *see under* Kioussis D., **306,** 662
see under Mellor A. L., **306,** 792
Fodor J. A. The Modularity of Mind, *book review by* Marshall J. C. **306,** 137
Foege W. H. AIDS and confidentiality, *correspondence,* **306,** 10
Fookes C. J. R. *see under* Ekstrom A., **306,** 173
Ford A. M. *see under* Mills F. C., **306,** 809
Forni G., Bindoni M., Santoni A., Belluardo N., Marchese A. E. and Giovarelli M. Radiofrequency destruction of the tuberoinfundibular region of hypothalamus permanently abrogates NK cell activity in mice, **306,** 181
Forster A. *see under* Rabbitts T. H., **306,** 806
Fortey R. Fossils: the Key to the Past, *book review by* Benton M. J. **306,** 515
Foster D. R., King G. A., Glaser P. H. and Wright H. E. Jr Origin of string patterns in boreal peatlands, **306,** 256
Fowler P. J. The Farming of Prehistoric Britain, *book review by* Bradley R. **306,** 716
Fox R. *book review of* The Montgolfier Brothers and the Invention of Aviation 1783-1784, (Gillispie C. C.) **306,** 122
Fraser J. T. The Genesis and Evolution of Time: A Critique of Interpretation in Physics, *book review by* Clarke C. J. S. **306,** 131
Frazer D. Reptiles and Amphibians in Britain, *book review by* Groombridge B. **306,** 515
Freeman S. A Treasury for Word Lovers, *book review by* Gratzer W. **306,** 814
Freund B. *see under* Lazar R. M., **306,** 54
Fritzsch H. Quarks: The Stuff of Matter, *book review by* Calder N. **306,** 508
Fry B., Gest H. and Hayes J. M. Sulphur isotopic compositions of deep-sea hydrothermal vent animals, **306,** 51
Fullmer J. Z. *book review of* The Revolution in Science 1500-1750, (Hall A. R.) **306,** 121
Gabbay K. H. *see under* Jansen M., **306,** 609
Gadsby D. C. β-Adrenoceptor agonists increase membrane K^+ conductance in cardiac Purkinje fibres, **306,** 691
Gainer H. *see under* Salzberg B. M., **306,** 36
Galbraith R. M. *see under* Petrini M., **306,** 73
Gallo R. C. *see under* Nowell P., **306,** 494
Gallwitz D., Donath C. and Sander C. A yeast gene encoding a protein homologous to the human c-*has/bas* proto-oncogene product, **306,** 704
Gardner M. The Whys of a Philosophical Scrivener, *book review by* Kemp P. **306,** 132
Garon C. F. *see under* Rabson A. B., **306,** 604
Garrison D. L., Ackley S. F. and Buck K. R. A physical mechanism for establishing algal populations in frazil ice, **306,** 363
Gaskell M. *see under* Fairall A. P., **306,** 503
Gattoni-Celli S., Hsiao W.-L. W. and Weinstein I. B. Rearranged c-*mos* locus in a MOPC 21 murine myeloma cell line and its persistence in hybridomas, **306,** 795
Gazdar A. F. *see under* Little C. D., **306,** 194
Gebhart W. *see under* Schuller-Petrovic S., **306,** 179
Gee C. E., Chen C.-L. C., Roberts J. L., Thompson R. and Watson S. J. Identification of proopiomelanocortin neurones in rat hypothalamus by *in situ* cDNA-mRNA hybridization, **306,** 374
Gegonne A. *see under* Leprince D., **306,** 395
Gelderblom H. *see under* Diringer H., **306,** 476
Gelmann E. P., Psallidopoulos M. C., Papas T. S. and Dalla Favera R. Identification of reciprocal translocation sites within the c-*myc* oncogene and immunoglobulin μ locus in a Burkitt lymphoma, **306,** 799
Genova F. *see under* Zarka P., **306,** 767
Georgi H., Ginsparg P. and Glashow S. L. Photon oscillations and cosmic background radiation, **306,** 765
Germain R. N., Norcross M. A. and Margulies D. H. Functional expression of a transfected murine class II MHC gene, **306,** 190
Gershenson S. M. Principles of Modern Genetics, 2nd Edn., *book review by* Trifonov E. N. **306,** 815
Gest H. *see under* Fry B., **306,** 51
Getzoff E. D. *see under* Tainer J. A., **306,** 284
Getzoff E. D., Tainer J. A., Weiner P. K., Kollman P. A., Richardson J. S. and Richardson D. C. Electrostatic recognition between superoxide and copper, zinc superoxide dismutase, **306,** 287
Ghatei M. A. *see under* Adrian T. E., **306,** 584
Gierasch P. J. *see under* Conrath B. J., **306,** 571
Gilden D. H., Vafai A., Shtram Y., Becker Y., Devlin M. and Wellish M. Varicella zoster virus DNA in human sensory ganglia, **306,** 478
Gill A. E. and Rasmusson E. M. The 1982–83 climate anomaly

in the equatorial Pacific, **306,** 229
Gillespie R., Street-Perrott F. A. and Switsur R. Post-glacial arid episodes in Ethiopia have implications for climate prediction, **306,** 680
Gillette R. *see under* Green D. J., **306,** 784
Gilliam A. C. *see under* Richards J. E., **306,** 483
Gillispie C. C. The Montgolfier Brothers and the Invention of Aviation 1783-1784, *book review by* Fox R. **306,** 122
Gilmore G. and Hewett P. A new limit on the nature of the galactic missing mass, **306,** 669
Gilmore T. and Martin G. S. Phorbol ester and diacylglycerol induce protein phosphorylation at tyrosine, **306,** 487
Gimlich R. L. and Cooke J. Cell lineage and the induction of second nervous systems in amphibian development, **306,** 471
Ginsburg S. and Rahamimoff R. Is extracellular calcium buffering involved in regulation of transmitter release at the neuromuscular junction?, **306,** 62
Ginsparg P. *see under* Georgi H., **306,** 765
Giovarelli M. *see under* Forni G., **306,** 181
Givol D. *see under* Cohen J. B., **306,** 797
see under Zakut-Houri R., **306,** 594
Glaros D. *see under* Kress M., **306,** 602
Glaser P. H. *see under* Foster D. R., **306,** 256
Glashow S. L. *see under* Georgi H., **306,** 765
Goedert M. *see under* Bannon M. J., **306,** 791
Goldberg E. D. *Editor*, Atmospheric Chemistry, *book review by* Chameides W. L. **306,** 714
see under Wong C. S., **306,** 623
Golenetskii S. V., Mazets E. P., Aptekar R. L. and Ilyinskii V. N. Correlation between luminosity and temperature in γ-ray burst sources, **306,** 451
Gomperts B. D. Involvement of guanine nucleotide-binding protein in the gating of Ca^{2+} by receptors, **306,** 64
Goodbourn S. E. Y. *see under* Higgs D. R., **306,** 398
Goodfellow P. Immunology Mitochondria and the major histocompatibility complex, *news and views*, **306,** 539
see under Solomon E., **306,** 223
Goss R. J. Deer Antlers: Regeneration, Function and Evolution, *book review by* Maden M. **306,** 405
Gossler M. Numerology, *correspondence*, **306,** 530
Gould H. *see under* Kimura T., **306,** 709
Gould H. J. *see under* Mills F. C., **306,** 809
Govindjee *Editor*, Photosynthesis Vol. 1 Energy Conversion by Plants and Bacteria. Vol. 2 Development, Carbon Metabolism, and Plant Productivity, *book review by* Evans M. C. W. **306,** 96
Gow G. F. Snakes of Australia, 2nd Edn., *book review by* McCarthy C. **306,** 514
Gowen M., Wood D. D., Ihrie E. J., McGuire M. K. B. and Russell R. G. G. An interleukin 1 like factor stimulates bone resorption *in vitro*, **306,** 378
Gowers Sir E. Fowler's Modern English Usage, 2nd edn, *book review by* Gratzer W. **306,** 134
Gracely R. H., Dubner R., Wolskee P. J. and Deeter W. R. Placebo and naloxone can alter post-surgical pain by separate mechanisms, **306,** 264
Graf T. EMBLs programme, *correspondence*, **306,** 726
Grainger R. M., Hazard-Leonards R. M., Samaha F., Hougan L. M., Lesk M. R. and Thomsen G. H. Is hypomethylation linked to activation of δ-crystallin genes during lens development? **306,** 88
Gratzer W. *book review of* A Treasury for Word Lovers, (Freeman S.) **306,** 814
book review of Fowler's Modern English Usage, 2nd edn, (Gowers Sir E.) **306,** 134
book review of The Language of Medicine: Its Evolution, Structure, and Dynamics, 2nd Edn., (Dirckx J. H.) **306,** 814
Grayson D. K. The Establishment of Human Antiquity, *book review by* Piggott S. **306,** 814
Green D. J. and Gillette R. Patch- and voltage-clamp analysis of cyclic AMP-stimulated inward current underlying neurone bursting, **306,** 784
Green N. *see under* Alexander H., **306,** 697
Gregg M. C. *see under* Larson N. G., **306,** 26
Grisolia S. Sutton's role, *correspondence*, **306,** 110
Groffen J. *see under* Heisterkamp N., **306,** 239
Groombridge B. *book review of* Reptiles and Amphibians in Britain, (Frazer D.) **306,** 515
Grosveld F. G. *see under* Kioussis D., **306,** 662
Grosveld G. *see under* Heisterkamp N., **306,** 239
Groyne F. *see under* Mekalanos J. J., **306,** 551
Guild B. C. Yellow raine, *correspondence*, **306,** 420
Guroff G., *Editor*, Growth and Maturation Factors, Vol. 1, *book review by* Taylor-Papadimitriou J. **306,** 295
Gusella J. F., Wexler N. S., Conneally P. M., Naylor S. L., Anderson M. A., Tanzi R. E., Watkins P. C., Ottina K., Wallace M. R., Sakaguchi A. Y., Young A. B., Shoulson I., Bonilla E. and Martin J. B. A polymorphic DNA marker genetically linked to Huntington's disease, **306,** 234
Güttler F. *see under* Woo S. L. C., **306,** 151
Gwei-Djen Lu *see under* Needham J., **306,** 120
Haas I. G. and Wabl M. Immunoglobulin heavy chain binding protein, **306,** 387
Haines D. A. and Smith M. C. Wind tunnel generation of horizontal roll vortices over a differentially heated surface, **306,** 351
Hall A. *see under* Reitsma P. H., **306,** 492
Hall A. J. *see under* Thorpe S. A., **306,** 167
Hall A. R. *book review of* Telescopes, Tides, and Tactics: A Galilean Dialogue about the *Starry Messenger* and Systems of the World, (Stillman Drake) **306,** 124
The Revolution in Science 1500-1750, *book review by* Fullmer J. Z. **306,** 121
Hall M. A. *see under* Shackleton N. J., **306,** 319
Hallam A. Great Geological Controversies, *book review by* Ager D. **306,** 128
Halliday T. Behavioural ecology: Do frogs and toads choose their mates? *news and views*, **306,** 226
Halstead B. Science in Africa: Historical science in Sokoto, Nigeria, *news and views*, **306,** 316
Hambley T. *see under* Ekstrom A., **306,** 173
Hamlyn P. H. *see under* Rabbitts T. H., **306,** 760
see under Rabbitts T. H., **306,** 806
Hammer R. E. *see under* Brinster R. L., **306,** 332
Hansen P. F. *see under* Heisterkamp N., **306,** 239
Hapel A. J. *see under* Djeu J. Y., **306,** 788
Harding A. F. *book review of* The Earliest Wheeled Transport: From the Atlantic Coast to the Caspian Sea, (Piggott S.) **306,** 121
Hardy R. R., Hayakawa K., Parks D. R. and Herzenberg L. A. Demonstration of B-cell maturation in X-linked immunodeficient mice by simultaneous three-colour immunofluorescence, **306,** 270
Harford N. *see under* Mekalanos J. J., **306,** 551
Harrison P. Seabirds: An Identification Guide, *book review by* Andrews J. H. **306,** 513
Harvey P. H. and Bennett P. M. Evolutionary biology: Brain size, energetics, ecology and life history patterns, *news and views*, **306,** 314
Harvey S. C. BeliketheGerman, *correspondence*, **306,** 530
Haselkorn R. *see under* Tumer N. E., **306,** 337
Hausmann B. *see under* Lindahl K. F., **306,** 383
Hauswirth W. W. *see under* Olivo P. D., **306,** 400
Hayakawa K. *see under* Hardy R. R., **306,** 270
Hayashida H. *see under* Horikawa S., **306,** 611
Hayes J. M. *see under* Fry B., **306,** 51
Hazard-Leonards R. M. *see under* Grainger R. M., **306,** 88
Hazen W. E. Not fit, *correspondence*, **306,** 220
Hazum S. *see under* Zakut-Houri R., **306,** 594
Heimann R. B., Kleiman J. and Salansky N. M. A unified structural approach to linear carbon polytypes, **306,** 164
Heinegg P. (*Trans.*) *see under* Ditfurth H. v. **306,** 403
Heisterkamp N., Stephenson J. R., Groffen J., Hansen P. F., de Klein A., Bartram C. R. and Grosveld G. Localization of the c-*abl* oncogene adjacent to a translocation break point in chronic myelocytic leukaemia, **306,** 239
Heitmann H. and Arnold F. Composition measurements of tropospheric ions, **306,** 747
Helfand S. L. *see under* McGarry R. C., **306,** 376
Henderson P., Marlow C. A., Molleson T. I. and Williams C. T. Patterns of chemical change during bone fossilization, **306,** 358
Hentall I. D. *see under* Fields H. L., **306,** 684
Herzenberg L. A. *see under* Hardy R. R., **306,** 270
see under Kavathas P., **306,** 385
Hesketh R. Biochemistry: Inositol trisphosphate: link or liability?, *news and views*, **306,** 16
Hewett P. *see under* Gilmore G., **306,** 669
Hewish A. In-flight movies, *correspondence*, **306,** 118
Heymans H. S. A., Schutgens R. B. H., Tan R., van den Bosch H. and Borst P. Severe plasmalogen deficiency in tissues of infants without peroxisomes (Zellweger syndrome), **306,** 69
Hidaka H. *see under* Naka M., **306,** 490
Higgs D. R., Goodbourn S. E. Y., Lamb J., Clegg J. B., Weatherall D. J. and Proudfoot N. J. α-Thalassaemia caused by a polyadenylation signal mutation, **306,** 398
Hilmert H. *see under* Diringer H., **306,** 476
Hinton G. E. *see under* Ballard D. H., **306,** 21
Hirose T. *see under* Horikawa S., **306,** 611
see under Nawa H., **306,** 32
Hirsh R. F. Glimpsing an Invisible Universe: The Emergence of X-Ray Astronomy, *book review by* Hughes D. W. **306,** 508
Hobbs R. W. *see under* Dent W. A., **306,** 41
Hoffman-Liebermann B. *see under* Liebermann, D., **306,** 342
Hofmann A. W. *see under* Jochum K. P., **306,** 431
Hogan B. Molecular biology: Enhancers, chromosome position effects, and transgenic mice, *news and views*, **306,** 313
Högstedt G. *see under* Alerstam T., **306,** 463
Holets V. R. *see under* Monaghan D. T., **306,** 176
Holliday R. Cancer and cell senescence, *correspondence*, **306,** 742
Holligan P. M. *book review of* Oceanography: The Present and Future, (Brewer P. G. *editor*) **306,** 815
Horak Z. A better value for Earth's mass, *correspondence*, **306,** 428
Horikawa S., Takai T., Toyosato M., Takahashi H., Noda M., Kakidani H., Kubo T., Hirose T., Inayama S., Hayashida H., Miyata T. and Numa S. Isolation and structural organization of the human preproenkephalin B gene, **306,** 611
Hougan L. M. *see under* Grainger R. M., **306,** 88
Houghten R. A. *see under* Rowlands D. J., **306,** 694
Howard R. J. *see under* Dent W. A., **306,** 41
Howgate M. Palaeontology: *Archaeopteryx* — no new finds after all, *news and views*, **306,** 644
Hoyle F. and Wickramasinghe N. C. Bacterial life in space, *correspondence*, **306,** 420
Hsiao W.-L. W. *see under* Gattoni-Celli S., **306,** 795
Hsü, K. J. The Mediterranean was a Desert: A Voyage of the Glomar Challenger, *book review by* Smith P. J. **306,** 507
Huffman D. R. *see under* Bohren C. F., **306,** 625
Hughes D. J. *see under* Fitton J. G., **306,** 327
Hughes D. W. Astronomy: 1983 TB and the Geminids, *news and views*, **306,** 116
book review of Glimpsing an Invisible Universe: The Emergence of X-Ray Astronomy, (Hirsh R. F.) **306,** 508
History of science: Scientific instruments and history, *news and views*, **306,** 20
book review of The Moment of Creation: Big Bang Physics from before the First Millisecond to the Present Universe, (Trefil J. S.) **306,** 508
Hughes V. M. *see under* Datta N., **306,** 616
Hull D. L. *book review of* The Eclipse of Darwinism: Anti-Darwinian Evolution Theories in the Decades around 1900, (Bowler P. J.) **306,** 714
Hummel E. *see under* van der Hulst J. M., **306,** 566
Humphreys C. J. and Waddington W. G. Dating the Crucifixion, *review article*, **306,** 743
Hunt G. *see under* Moore P., **306,** 507
Hunt S. *see under* Thorpe S. A., **306,** 167
Huston D. P. *see under* Smith III R., **306,** 599
Huston M. M. *see under* Smith III R., **306,** 599
Hutter K. *see under* Mysak L. A., **306,** 46
Huxley A. *see under* Davies P. and J., **306,** 626
Ihrie E. J. *see under* Gowen M., **306,** 378
Ilyinskii V. N. *see under* Golenetskii S. V., **306,** 451
Inayama S. *see under* Horikawa S., **306,** 611
see under Nawa H., **306,** 32
Incoronato A., Addison F. T., Tarling D. H., Nardi G. and Pescatore T. Magnetic fabric investigations of pyroclastic deposits from Phlegrean Fields, southern Italy, **306,** 461
Ingham P. W. Differential expression of *bithorax complex* genes in the absence of the *extra sex combs* and *trithorax* genes, **306,** 591
Irvine R. F. *see under* Streb H., **306,** 67
Ise N. and Tabushi I., *Editors*, An Introduction to Speciality Polymers, *book review by* Calvert P. **306,** 295
Ish-Horowicz D. *see under* Sinclair J. H., **306,** 198
Israelachvili J. N. and Pashley R. M. Molecular layering of water at surfaces and origin of repulsive hydration forces, **306,** 249
Ito E. *see under* Jochum K. P., **306,** 431
Iversen L. L. *see under* Bannon M. J., **306,** 791
Iversen S. D. *see under* Bannon M. J., **306,** 791
Jahoda M. *book review of* The Human Legacy, (Festinger L.) **306,** 96
Janeway Jr, C. A., Conrad P. J., Tite J., Jones B and Murphy D. B. Efficiency of antigen presentation differs in mice differing at the *IMls* locus, **306,** 80
Jangoux M. and Lawrence J. M., *Editors*, Echinoderm Studies I, *book review by* Nichols D. **306,** 296
Jansen M., van Schaik F. M. A., Ricker A. T., Bullock B., Woods D. E., Gabbay K. H., Nussbaum A. L., Sussenbach J. S., and Van den Brande J. L. Sequence of cDNA encoding human insulin-like growth factor I precursor, **306,** 609
Jasin M., Regan L. and Schimmel P. Modular arrangement of functional domains along the sequence of an aminoacyl tRNA synthetase, **306,** 441
Jay G. *see under* Kress M., **306,** 602
Jaye M. *see under* Camerino G., **306,** 701
Jenkins R. N. *see under* Smith III R., **306,** 599
Jerabek L. *see under* Alexander H., **306,** 697
Jessel T. *see under* Barnstable C., **306,** 14
Jochum K. P., Hofmann A. W., Ito E., Seufert H. M. and White W. M. K, U and Th in mid-ocean ridge basalt glasses and heat production, K/U and K/Rb in the mantle, **306,** 431
Johnson D. A. *see under* Alexander H., **306,** 697
Jones B *see under* Janeway Jr, C. A., **306,** 80
Jones D. Source of saturnian myriametric radiation, **306,** 453
Jones J. S. *book review of* Charles Darwin: A Centennial Commemorative, (Chapman R. G. and Duval C. T. *editors*) **306,** 512
see under Barton N., **306,** 317
Jones P. D. *see under* Raper S. C. B., **306,** 458
Jones R. Franklin Valley sites, *correspondence*, **306,** 726
Jordan C. Electron densities in active galaxies, *matters arising*, **306,** 503
Joyce C. R. B. Gripes, *correspondence*, **306,** 726
Joyner A. L. *see under* Rubin J. S., **306,** 206
Kahn A. J. *see under* Reitsma P. H., **306,** 492
Kakidani H. *see under* Horikawa S., **306,** 611
Kam Z. *book review of* Absorption and Scattering of Light by Small Particles, (Bohren C. F. and Huffman D. R.) **306,** 625
Kapoor N. *see under* Lazar R. M., **306,** 54
Karle I. *see under* Camerman A., **306,** 447
Karle J. *see under* Camerman A., **306,** 447
Kasamatsu T. *see under* Kuppermann B. D., **306,** 465
Kavanagh M. A Complete Guide to Monkeys, Apes and Other Primates, *book review by* Martin R. D. **306,** 512
Kavathas P. and Herzenberg L. A. Amplification of a gene coding for human T-cell differentiation antigen **306,** 385
Kedes L. *see under* Liebermann, D., **306,** 342
Keir N. H. The Yogi in *Nature*, *correspondence*, **306,** 310
Kelekar A. India's science base, *correspondence*, **306,** 636
Kelly P. M. *see under* Raper S. C. B., **306,** 458
Kemp D. J. *see under* Coppel R. L., **306,** 751
Kemp P. *book review of* The Whys of a Philosophical Scrivener, (Gardner M.) **306,** 132
Keren D. F. *see under* Tagliabue A., **306,** 184
Kershaw P. J., Swift D. J., Pentreath R. J. and Lovett M. B. Plutonium redistribution by biological activity in Irish Sea sediments, **306,** 774
Khoury G. *see under* Kress M., **306,** 602
Kilburn C. R. J. *see under* Fitton J. G., **306,** 327
Kimberlin R. H. *see under* Diringer H., **306,** 476
Kimura H. *see under* Okada H., **306,** 203
Kimura M. The Neutral Theory of Molecular Evolution, *book review by* Smith J. M. **306,** 713
Kimura T., Mills F. C., Allan J. and Gould H. Selective unfolding of erythroid chromatin in the region of the active β-globin gene, **306,** 709
King G. A. *see under* Foster D. R., **306,** 256
King G. and Brewer J. Fault related folding near the Wind River thrust, Wyoming, USA, **306,** 147
King G. M. First mammal-like reptile from Australia, *matters arising*, **306,** 209
Kioussis D., Vanin E., deLange T., Flavell R. A. and Grosveld F. G. β-Globin gene inactivation by DNA translocation in γβ-thalassaemia, **306,** 662
Kirkpatrick D. *see under* Lazar R. M., **306,** 54
Kleiman J. *see under* Heimann R. B., **306,** 164
Klein G. *see under* Rosén A., **306,** 189
see under Sümegi J., **306,** 497
Klein S. A. *see under* Levi D. M., **306,** 468
Kletz T. A. *book review of* How to Write and Publish a Scientific Paper, 2nd edn, (Day R. A.) **306,** 133
Knight P. *book review of* The Quantum Theory of Light, 2nd Edn, (Loudon R.) **306,** 404
Knowles B. B. *see under* Carlin C. R., **306,** 617
Koenig W. D. *see under* Mumme R. L., **306,** 583
Koller R. *see under* DeFeo-Jones D., **306,** 707
Kollman P. A. *see under* Getzoff E. D., **306,** 287
Kominz M. A. *see under* Bond G. C., **306,** 775
Kraft D. *see under* Schuller-Petrovic S., **306,** 179
Krebs J. R. and May R. M. International ethological conference: The evolutionary basis of behaviour, *news and views*, **306,** 533
Kress M., Glaros D., Khoury G. and Jay G. Alternative RNA splicing in expression of the *H-2K* gene, **306,** 602
Krupp E. C. Echoes of the Ancient Skies: The Astronomy of Lost Civilizations, *book review by* Ruggles C. **306,** 123
Kubo T. *see under* Horikawa S., **306,** 611
Kuhar M. J. *see under* Lo M. M. S., **306,** 57
Kuijpers A. *see under* Weaver P. P. E., **306,** 360
Kuppermann B. D. and Kasamatsu T. Changes in geniculate cell size following brief monocular blockade of retinal activity in kittens, **306,** 465
Kuroda P. K. The Origin of the Chemical Elements and the Oklo Phenomenon, *book review by* Viola V. E. **306,** 626
Kuroda R. *see under* Mills F. C., **306,** 809
Lagrou C. *see under* Leprince D., **306,** 395
Laipis P. J. *see under* Olivo P. D., **306,** 400
Lamb J. *see under* Higgs D. R., **306,** 398
Lambert D. Collins Guide to Dinosaurs, *book review by* Benton

M. J. **306,** 515
Landsberg P. T. The Enigma of Time, *book review by* Clarke C. J. S. **306,** 131
Lanza E. *see under* Djeu J. Y., **306,** 788
Larson N. G. and Gregg M. C. Turbulent dissipation and shear in thermohaline intrusions, **306,** 26
Lassmann H. *see under* Schuller-Petrovic S., **306,** 179
Latchman D. S. *see under* Brickell P. M., **306,** 756
Lavie V. *see under* Zakut-Houri R., **306,** 594
Lavington S. *book review of* The Fifth Generation: Artificial Intelligence and Japan's Computer Challenge to the World, (Feigenbaum E. A. and McCorduck P.) **306,** 129
book review of Towards Fifth-Generation Computers, (Simons G. L.) **306,** 129
Lawrence J. M. *see under* Jangoux M., **306,** 296
Lawrence P. Developmental biology: A new homoeotic gene, *news and views,* **306,** 643
Lawton J. H. and May R. M. Ecology: The birds of Selborne, *news and views,* **306,** 732
Lazar R. M., Tamaroff M., Nir Y., Freund B., O'Reilly R., Kirkpatrick D. and Kapoor N. Language recovery following isolation for severe combined immunodeficiency disease, **306,** 54
Leahy C. Birdwatcher's Companion, *book review by* Andrews J. H. **306,** 514
Lebofsky M. J. *see under* Middleditch J., **306,** 163
Leff S. E. and Cresse I. Dopaminergic D-3 binding sites are not presynaptic autoreceptors, **306,** 586
Lefkowitz R. J. *see under* Cerione R. A., **306,** 562
Leg 94 staff Deep Sea Drilling Project: Sediment drifts and intraplate tectonics in the North Atlantic, *news and views,* **306,** 532
Deep Sea Drilling Project: Testing the Vail depositional model, *news and views,* **306,** 641
Deep Sea Drilling Project: On the Mississippi Fan, **306,** 736
Leigh E. G., Rand A. S. and Windsor D. M. *Editors,* The Ecology of a Tropical Forest: Seasonal Rhythms and Long Term Changes, *book review by* Coe M. **306,** 625
Leprince D., Gegonne A., Coll J., de Taisne C., Schneeberger A., Lagrou C. and Stehelin D. A putative second cell-derived oncogene of the avian leukaemia retrovirus E26, **306,** 395
Lerner R. A. *see under* Alexander H., **306,** 697
see under Rowlands D. J., **306,** 694
Lesk M. R. *see under* Grainger R. M., **306,** 88
Levan G. *see under* Sümegi J., **306,** 497
Levi D. M., Manny R. E., Klein S. A. and Steinman S. B. Electrophysiological correlates of hyperacuity in the human visual cortex, **306,** 468
Levinson A. D. *see under* Alitalo K., **306,** 274
Lewis F. A., Magennis J. P., McKee S. G. and Ssebuwufu P. J. M. Hydrogen chemical potentials and diffusion coefficients in hydrogen diffusion membranes, **306,** 673
Lewis J. *book review of* From Egg to Embryo: Determinative Events in Early Development, (Slack J. M. W.) **306,** 294
Lewis N. F. *see under* Rahn K. A., **306,** 459
Lidsky A. S. *see under* Woo S. L. C., **306,** 151
Liebermann, D., Hoffman-Liebermann B., Weinthal J., Childs G., Maxson R., Mauron A., Cohen S. N. and Kedes L. An unusual transposon with long terminal inverted repeats in the sea urchin *Strongylocentrotus purpuratus,* , **306,** 342
Liesi P., Rechardt L. and Wartiovaara J. Nerve growth factor induces adrenergic neuronal differentiation in F9 teratocarcinoma cells, **306,** 265
Limbert D. W. S. *see under* Raper S. C. B., **306,** 458
Lindahl K. F., Hausmann B. and Chapman V. M. A new *H-2*-linked class I gene whose expression depends on a maternally inherited factor, **306,** 383
Lindsten J. Nobel Assembly, *correspondence,* **306,** 420
Line J. *see under* Shackleton N. J., **306,** 319
Lingelbach K. R. *see under* Coppel R. L., **306,** 751
Linton E. A. *see under* Lowry P. J., **306,** 70
Lipson H. *book review of* Fourier Optics: An Introduction, (Steward E. G.) **306,** 715
Liss P. *book review of* Trace Metals in Sea Water, (Wong C. S., Boyle E., Bruland K. W., Burton J. D., Goldberg E. D. *editors*) **306,** 623
Little C. D., Nau M. M., Carney D. N., Gazdar A. F. and Minna J. D. Amplification and expression of the *c-myc* oncogene in human lung cancer cell lines, **306,** 194
Litvak S. Survival and revival: Chilean universities under Pinochet, **306,** 11
Lo K. Y. and Claussen M. J. High-resolution observations of ionized gas in central 3 parsecs of the Galaxy: possible evidence for infall, **306,** 647
Lo M. M. S., Niehoff D. L., Kuhar M. J. and Snyder S. H. Differential localization of type I and type II benzodiazepine binding sites in substantia nigra, **306,** 57
Loeh H. J. *see under* Ekstrom A., **306,** 173
Logan J. *see under* Cairns J., **306,** 742
Lohmann G. P. *see under* Curry W. B., **306,** 577
Long J. V. P. *see under* Reed S. J. B., **306,** 172
Loudon R. The Quantum Theory of Light, 2nd Edn, *book review by* Knight P. **306,** 404
Lovett M. B. *see under* Kershaw P. J., **306,** 774
Low G. and Mansell W. North American Marsh Birds, *book review by* Andrews J. H. **306,** 513
Lowell G. H. *see under* Tagliabue A., **306,** 184
Lowenthal D. H. *see under* Rahn K. A., **306,** 459
Lowry P. J., Silas L., McLean C., Linton E. A. and Estivariz F. E. Pro-γ Melanocyte-stimulating hormone cleavage in adrenal gland undergoing compensatory growth, F. E. **306,** 70
Luckins A. G. *see under* Crowe J. S., **306,** 389
Lumsden A. G. S. and Davies A. M. Earliest sensory nerve fibres are guided to peripheral targets by attractants other than nerve growth factor, **306,** 786
Lynn R. IQ in Japan and the United States—Reply, *matters arising,* **306,** 291
McCarthy C. *book review of* Snakes of Australia, 2nd Edn., (Gow G. F.) **306,** 514
McClintock P. Knudsen effects in liquid helium, *news and views,* **306,** 422
McCorduck P. *see under* Feigenbaum E. A., **306,** 129
McDonald M. J. *see under* Bunn H. F., **306,** 498
McDowell J. *see under* Carr B. J., **306,** 666
McGarry R. C., Helfand S. L., Quarles R. H. and Roder J. C. Recognition of myelin-associated glycoprotein by the monoclonal antibody HNK-1, **306,** 376
McGraw J. T. *see under* Middleditch J., **306,** 163
McGuire M. K. B. *see under* Gowen M., **306,** 378
MacKay D. *book review of* Are Computers Alive? Evolution and New Life Forms, (Simons G.) **306,** 510
McKee S. G. *see under* Lewis F. A., **306,** 673
McKenna M. C. *see under* Novacek M. J., **306,** 683
MacKenzie E. T. *see under* Edvinsson L., **306,** 55
McLean C. *see under* Lowry P. J., **306,** 70
McLennan S. M. and Taylor S. R. Continental freeboard, sedimentation rates and growth of continental crust, **306,** 169
Macleod D. and Bird A. Transcription in oocytes of highly methylated rDNA from *Xenopus laevis* sperm, **306,** 200
McMichael A. *see under* Shaw S., **306,** 538
Maddox J. Can observation prevent decay? *leading article, news and views,* **306,** 111
Fuzzy sets make fuzzy logic, *news and views, leading article,* **306,** 637
Great greenhouse in the sky? *news and views, leading article,* **306,** 221
Is biology now part of physics? *leading article, news and views,* **306,** 311
Snowflakes are far from simple, *leading article, news and views,* **306,** 13
Yrast phenomena in fashion, *leading article, news and views,* **306,** 531
Maden M. *book review of* Deer Antlers: Regeneration, Function and Evolution, (Goss R. J.) **306,** 405
Magennis J. P. *see under* Lewis F. A., **306,** 673
Malviya A. N. Indian expatriates, *correspondence,* **306,** 10
Mandel J. L. *see under* Camerino G., **306,** 701
Manny R. E. *see under* Levi D. M., **306,** 468
Mansell W. *see under* Low G., **306,** 513
Manuelidis E. E. *see under* Merz P. A., **306,** 474
Manuelidis L. *see under* Merz P. A., **306,** 474
Mappin G. *book review of* DNA for Beginners, (Rosenfield I., Ziff E., Van Loon B.) **306,** 505
Marchese A. E. *see under* Forni G., **306,** 181
Margulies D. H. *see under* Germain R. N., **306,** 190
Marlow C. A. *see under* Henderson P., **306,** 358
Marsden G. M. *book review of* The Origins of Life: Evolution as Creation, (Ditfurth H. v. Heinegg P. (*Trans.*)) **306,** 403
Marshall J. C. *book review of* The Modularity of Mind, (Fodor J. A.) **306,** 137
Martin G. S. *see under* Gilmore T., **306,** 487
Martin J. B. *see under* Gusella J. F., **306,** 234
Martin M. A. *see under* Rabson A. B., **306,** 604
Martin R. D. *book review of* A Complete Guide to Monkeys, Apes and Other Primates, (Kavanagh M.) **306,** 512
Martin-Zanca D. *see under* Sukumar S., **306,** 658
Marzec C. J. and Spiegel E. A. The 1/*f* distribution for tuneable oscillators, **306,** 248
Mastropaolo D. *see under* Camerman A., **306,** 447
Masuda A. *see under* Tanaka T., **306,** 503
Mattei J. F. *see under* Camerino G., **306,** 701
Mattei M. G. *see under* Camerino G., **306,** 701
Matthews P. T. *book review of* Selected Papers 1945–1980 With Commentary, (Yang Chen Ning) **306,** 623
Mauro A. *see under* Young, J. D.-E., **306,** 186
Mauron A. *see under* Liebermann, D., **306,** 342
Maxson R. *see under* Liebermann, D., **306,** 342
May R. M. *see under* Krebs J. R., **306,** 533
see under Lawton J. H., **306,** 732
Mayes P. R. *see under* Raper S. C. B., **306,** 458
Mayo K. E., Vale W., Rivier J., Rosenfeld M. G. and Evans R. M. Expression-cloning and sequence of a cDNA encoding human growth-hormone-releasing factor, **306,** 86
Mazets E. P. *see under* Golenetskii S. V., **306,** 451
Medawar P. B. *book review of* The Return to Cosmology: Postmodern Science and the Theology of Nature, (Toulmin S.) **306,** 126
Mekalanos J. J., Swartz D. J., Pearson G. D. N., Harford N., Groyne F. and de Wilde M. Cholera toxin genes: nucleotide sequence, deletion analysis and vaccine development, **306,** 551
Mellor A. L., Weiss E. H., Ramachandran K. and Flavell R. A. A potential donor gene for the *bm1* gene conversion event in the C57BL mouse, **306,** 792
Merz P. A., Somerville R. A., Wisniewski H. M., Manuelidis L. and Manuelidis E. E. Scrapie-associated fibrils in Creutzfeldt-Jakob disease, **306,** 474
Meves H. A new view of the sodium channel, *news and views,* **306,** 424
Middleditch J., Cudaback D., Oliver B., Pennypacker C., Lebofsky M. J., Rieke G. H., McGraw J. T., Dearborn D., Wisniewski W. and Chini R. A search for pulsed IR and near-IR emission from PSR1937+214, **306,** 163
Mikkola H. Owls of Europe, *book review by* Andrews J. H. **306,** 513
Miles R. and Wong R. K. S. Single neurones can initiate synchronized population discharge in the hippocampus, **306,** 371
Miller E. N. *see under* Young J. R., **306,** 196
Miller K. G. and Fairbanks R. G. Evidence for Oligocene-Middle Miocene abyssal circulation changes in the western North Atlantic, **306,** 250
Miller S. A. *see under* Ekstrom A., **306,** 173
Mills D. L. *see under* Agranovich V. M., **306,** 95
Mills F. C. *see under* Kimura T., **306,** 709
Mills F. C., Fisher L. M., Kuroda R., Ford A. M. and Gould H. J. DNase I hypersensitive sites in the chromatin of human μ immunoglobulin heavy-chain genes, **306,** 809
Minna J. D. *see under* Little C. D., **306,** 194
Miyata T. *see under* Horikawa S., **306,** 611
Molleson T. I. *see under* Henderson P., **306,** 358
Monaghan D. T., Holets V. R., Toy D. W. and Cotman C. W. Anatomical distributions of four pharmacologically distinct ^{3}H-L-glutamate binding sites, **306,** 176
Moore P. B. and Shen J. An X-ray structural study of cacoxenite, a mineral phosphate, **306,** 356
Moore P. D. Ecology: Ecological diversity and stress, *news and views,* **306,** 17
Ecology: The decline of the chough, *news and views,* **306,** 644
Moore P., Hunt G., Nicolson I. and Cattermole P. The Atlas of the Solar System, *book review by* Darius J. **306,** 507
Moore R. L., Readhead A. C. S. and Baath L. Superluminal acceleration in 3C345, **306,** 44
Morrison P. *see under* Morrison P., **306,** 125
Morrison P. and Morrison P. Powers of Ten: About the Relative Size of Things in the Universe, *book review by* Winfree A. T. **306,** 125
Moscovici C. *see under* Nunn M. F., **306,** 391
Muir H. *see under* Olsen I., **306,** 75
Mulvaney D. J. Franklin Valley defended, *correspondence,* **306,** 636
Mumme R. L., Koenig W. D. and Pitelka F. A. Reproductive competition in the communal acorn woodpecker: sisters destroy each other's eggs, **306,** 583
Munro A. Immunology The I–J paradox remains unsolved, *news and views,* **306,** 537
Murad F. *see under* Rapoport R. M., **306,** 174
Murphy D. *see under* Brickell P. M., **306,** 756
Murphy D. B. *see under* Janeway Jr, C. A., **306,** 80
Mysak L. A., Salvadè G., Hutter K. and Scheiwiller T. Lake of Lugano and topographic waves, **306,** 46
Nagata Y., Diamond B. and Bloom B. R. The generation of human monocyte/macrophage cell lines, **306,** 597
Naka M., Nishikawa M., Adelstein R. S. and Hidaka H. Phorbol-ester induced activation of human platelets is associated with protein kinase C phosphorylation of myosin light chains, **306,** 490
Nakamura S. *see under* Okada H., **306,** 203
Nakanishi S. *see under* Nawa H., **306,** 32
Nardi G. *see under* Incoronato A., **306,** 461
Nash C. and Sen S. Topology and Geometry for Physicists, *book review by* Atiyah M. **306,** 296
Nau M. M. *see under* Little C. D., **306,** 194
Nawa H., Hirose T., Takashima H., Inayama S. and Nakanishi S. Nucleotide sequences of cloned cDNAs for two types of bovine brain substance P precursor, **306,** 32
Naylor S. L. *see under* Gusella J. F., **306,** 234
Nedrud J. G. *see under* Sixbey J. W., **306,** 480
Needham J. and Gwei-Djen Lu Science and Civilisation in China: Vol. 5, Part 5. Spagyrical Discovery and Invention: Physiological Alchemy, *book review by* Spence J. **306,** 120
Neff N. A. *see under* Novacek M. J., **306,** 683
Negoro S. *see under* Okada H., **306,** 203
Nelson M. A. *see under* Norman E. B., **306,** 503
Nencioni L. *see under* Tagliabue A., **306,** 184
Newmark P. Oncogenic intelligence: Viral oncogene permutations, *news and views,* **306,** 426
Oncogenic intelligence: More *ras*matazz, *news and views,* **306,** 642
Nicholls N. Predicting Indian monsoon rainfall from sea-surface temperature in the Indonesia–north Australia area, **306,** 576
Nichols D. *book review of* Echinoderm Studies I, (Jangoux M. and Lawrence J. M., *editors*) **306,** 296
Nicholson B. H. *see under* Rowlands D. J., **306,** 694
Nicolson I. *see under* Moore P., **306,** 507
Niehoff D. L. *see under* Lo M. M. S., **306,** 57
Nir Y. *see under* Lazar R. M., **306,** 54
Nishikawa M. *see under* Naka M., **306,** 490
Noda M. *see under* Horikawa S., **306,** 611
Norcross M. A. *see under* Germain R. N., **306,** 190
Norell M. A. Homology defined, *correspondence,* **306,** 530
Norman E. B. and Nelson M. A. On the half life of ^{138}La, *matters arising,* **306,** 503
Normanton J. R. *see under* Parish D. C., **306,** 267
Notario V. *see under* Sukumar S., **306,** 658
Novacek M. J., McKenna M. C., Neff N. A. and Cifelli R. L. Evidence from earliest known erinaceomorph basicranium that insectivorans and primates are not closely related, **306,** 683
Novakov T. *see under* Rosen H., **306,** 768
Nowell P., Finan J., Dalla Favera R., Gallo R. C., ar-Rushdi A., Romanczuk H., Selden J. R., Emanuel B. S., Rovera G. and Croce C. M. Association of amplified oncogene *c-myc* with an abnormally banded chromosome 8 in a human leukaemia cell line, **306,** 494
Noyes H. P. *see under* Rubenstein E., **306,** 118
Numa S. *see under* Horikawa S., **306,** 611
Nunn M. F., Seeburg P. H., Moscovici C. and Duesberg P. H. Tripartite structure of the avian erythroblastosis virus E26 transforming gene, **306,** 391
Nussbaum A. L. *see under* Jansen M., **306,** 609
Obaid A. L. *see under* Salzberg B. M., **306,** 36
O'Brien R. L. Arp B. *see under* Brinster R. L., **306,** 332
O'Dea C. P. *see under* Dent W. A., **306,** 41
Okada H., Negoro S., Kimura H. and Nakamura S. Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers, **306,** 203
Olashaw N. E. and Pledger W. J. Association of platelet-derived growth factor-induced protein with nuclear material, **306,** 272
Oliver B. *see under* Middleditch J., **306,** 163
Olivo P. D., Van de Walle M. J., Laipis P. J. and Hauswirth W. W. Nucleotide sequence evidence for rapid genotypic shifts in the bovine mitochondrial DNA D-loop, **306,** 400
Olsen I., Muir H., Smith R., Fensom A. and Watt D. J. Direct enzyme transfer from lymphocytes is specific, **306,** 75
O'Malley B. W. *see under* Ciejek E. M., **306,** 607
O'Nions R. K. and Oxburgh E. R. Heat and helium in the Earth, **306,** 429
O'Reilly R. *see under* Lazar R. M., **306,** 54
Oren M. *see under* Zakut-Houri R., **306,** 594
O'Riordan M. C. Radiation protection: Natural radiation risks, *news and views,* **306,** 225
Ortner D. J. *Editor,* How Humans Adapt: A Biocultural Odyssey, *book review by* Eisenberg L. **306,** 293
Ottina K. *see under* Gusella J. F., **306,** 234
Oxburgh E. R. *see under* O'Nions R. K., **306,** 429
Oxnard C. E. *see under* Rukang W., **306,** 258
Ozel M. *see under* Diringer H., **306,** 476
Padayatty J. D. *see under* Thomas G., **306,** 82
Pagano J. S. *see under* Sixbey J. W., **306,** 480
Pain R. H. Biochemistry: Dressing the SOD, *news and views,* **306,** 228
Palsdottir A., Cross S. J., Edwards J. H. and Carroll M. C. Correlation between a DNA restriction fragment length polymorphism and C4A6 protein, **306,** 615
Panganiban A. T. and Temin H. M. The terminal nucleotides of retrovirus DNA are required for integration but not virus production, **306,** 155
Papas T. S. *see under* Gelmann E. P., **306,** 799
Paquin C. E. and Adams J. Relative fitness can decrease in evolving asexual populations of *S. cerevisiae,* **306,** 368
Parish D. C., Smyth D. G., Normanton J. R. and Wolstencroft J. H. Glycyl glutamine, an inhibitory neuropeptide derived from β-endorphin, **306,** 267
Parks D. R. *see under* Hardy R. R., **306,** 270

Pashley R. M. *see under* Israelachvili J. N., **306,** 249
Pastore S. *see under* Djeu J. Y., **306,** 788
Paterson A. *see under* Broda P., **306,** 737
Pauliny-Toth I. I. K. *see under* Biretta J. A., **306,** 42
Pearson G. D. N. *see under* Mekalanos J. J., **306,** 551
Pedersen H., Tarenghi M., Cristiani S. and Salvati M. CCD images of the 6.1 ms pulsar field, **306,** 568
Pedlar A. *see under* van der Hulst J. M., **306,** 566
Pennypacker C. *see under* Middleditch J., **306,** 163
Penrose R. *book review of* The Mathematical Theory of Black Holes, (Chandrasekhar S.) **306,** 404
Pentreath R. J. *see under* Kershaw P. J., **306,** 774
Pescatore T. *see under* Incoronato A., **306,** 461
Petersen N. *see under* Bleil U., **306,** 92
Peterson R. T. *Editor,* A Field Guide to the Birds of Britain and Europe, *book review by* Andrews J. H. **306,** 513
Petrini M., Emerson D. L. and Galbraith R. M. Linkage between surface immunoglobulin and cytoskeleton of B lymphocytes may involve Gc protein, **306,** 73
Pfeifer S. O. *see under* Alitalo K., **306,** 274
Phadnis S. H. *see under* Seshi B., **306,** 310
Phillips P. D. *see under* Carlin C. R., **306,** 617
Pickford M. *see under* Shipman P., **306,** 365
Piggott S. The Earliest Wheeled Transport: From the Atlantic Coast to the Caspian Sea, *book review by* Harding A. F. **306,** 121
book review of The Establishment of Human Antiquity, (Grayson D. K.) **306,** 814
Pilbeam D. *see under* Raza S. M., **306,** 52
Pitelka F. A. *see under* Mumme R. L., **306,** 583
Pledger W. J. *see under* Olashaw N. E., **306,** 272
Polak J. M. *see under* Adrian T. E., **306,** 584
Porter R. *book review of* The Great Chain of History: William Buckland and the English School of Geology (1814–1849), (Rupke N. A.) **306,** 813
Postgate J. Nitrogen fixation: A zoocoenotic symbiosis? *news and views,* **306,** 19
see under Cannon F., **306,** 290
Potts R. *see under* Shipman P., **306,** 365
Press W. H. *book review of* The Roving Mind, (Asimov I.) **306,** 510
Price N. J. and Audley-Charles M. G. Plate rupture by hydraulic fracture resulting in overthrusting, **306,** 572
Proudfoot N. J. *see under* Higgs D. R., **306,** 398
Psallidopoulos M. C. *see under* Gelmann E. P., **306,** 799
Purrington R. O. *see under* Durham F., **306,** 123
Quarles R. H. *see under* McGarry R. C., **306,** 376
Queen C. *see under* Stafford J., **306,** 77
Raab-Traub N. *see under* Sixbey J. W., **306,** 480
Rabbitts T. H., Forster A., Baer R. and Hamlyn P. H. Transcription enhancer identified near the human C_μ immunoglobulin heavy chain gene is unavailable to the translocated *c-myc* gene in a Burkitt lymphoma, **306,** 806
Rabbitts T. H., Hamlyn P. H. and Baer R. Altered nucleotide sequences of a translocated c-*myc* gene in Burkitt lymphoma, **306,** 760
Rabson A. B., Steele P. E., Garon C. F. and Martin M. A. mRNA transcripts related to full-length endogenous retroviral DNA in human cells, **306,** 604
Radhakrishnamurty C. Variations in magnetization intensity of ocean floor basalts, *matters arising,* **306,** 92
Rahamimoff R. *see under* Ginsburg S., **306,** 62
Rahn K. A., Lewis N. F., Lowenthal D. H. and Smith D. L. Noril'sk only a minor contributor to Arctic haze, **306,** 459
Ralston R. and Bishop J. M. The protein products of the *myc* and *myb* oncogenes and adenovirus Ela are structurally related, **306,** 803
Ramachandran K. *see under* Mellor A. L., **306,** 792
Ramdahl T. Retene — a molecular marker of wood combustion in ambient air, **306,** 580
Ramsay G. *see under* Alitalo K., **306,** 274
Rand A. S. *see under* Leigh E. G., **306,** 625
Raper S. C. B., Wigley T. M. L., Jones P. D., Kelly P. M., Mayes P. R. and Limbert D. W. S. Recent temperature changes in the Arctic and Antarctic, **306,** 458
Rapoport R. M., Draznin M. B. and Murad F. Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation, **306,** 174
Rasmusson E. M. *see under* Gill A. E., **306,** 229
Ravetch J. *see under* Shin H.-S., **306,** 380
Raza S. M., Barry J. C., Pilbeam D., Rose M. D., Shah S. M. I. and Ward S. New hominoid primates from the middle Miocene Chinji Formation, Potwar Plateau, Pakistan, **306,** 52
Readhead A. C. S. *see under* Moore R. L., **306,** 44
Rechardt L. *see under* Liesi P., **306,** 265
Rechavi G. *see under* Cohen J. B., **306,** 797
Reed S. J. B., Smith D. G. W. and Long J. V. P. Rare earth elements in chondritic phosphates — implications for ^{244}Pu chronology, **306,** 172
Regan L. *see under* Jasin M., **306,** 441
Reitsma P. H., Rothberg P. G., Astrin S. M., Trial J., Bar-Shavit Z., Hall A., Teitelbaum S. L. and Kahn A. J. Regulation of *myc* gene expression in HL-60 leukaemia cells by a vitamin D metabolite, **306,** 492
Rich R. R. *see under* Smith III R., **306,** 599
Richards J. E., Gilliam A. C., Shen A., Tucker P. W. and Blattner F. R. Unusual sequences in the murine immunoglobulin μ-δ heavy-chain region, **306,** 483
Richards W. G. *book review of* Computer Images: State of the Art, (Deken J.) **306,** 509
Richardson D. C. *see under* Getzoff E. D., **306,** 287
see under Tainer J. A., **306,** 284
Richardson J. S. *see under* Getzoff E. D., **306,** 287
see under Tainer J. A., **306,** 284
Ricker A. T. *see under* Jansen M., **306,** 609
Rieke G. H. *see under* Middleditch J., **306,** 163
Rigby P. W. J. *see under* Brickell P. M., **306,** 756
Ritchie K. A. *see under* Brinster R. L., **306,** 332
Rivier C. *see under* Barinaga M., **306,** 84
Rivier J. *see under* Mayo K. E., **306,** 86
Roberts G. W. *see under* Adrian T. E., **306,** 584
Roberts J. L. *see under* Gee C. E., **306,** 374
Roberts T. D. M. "Microgravity", *correspondence,* **306,** 742
Robertson M. Huntington's disease: The beginning of the end of a dilemma? *news and views,* **306,** 222
Oncogenesis: Paradox and paradigm: the message and meaning of *myc*, *news and views,* **306,** 733
see under Barnstable C., **306,** 14
Robinson A. B. Pauling institute, *correspondence,* **306,** 10
Robinson S. J. *see under* Tumer N. E., **306,** 337
Robson K. J. H. *see under* Woo S. L. C., **306,** 151
Roche J. J. *book review of* Ludwig Boltzmann. Man. Physicist. Philosopher, (Broda E.) **306,** 128
Roder J. C. *see under* McGarry R. C., **306,** 376
Rogers J. Molecular biology: A straight LINE story, *news and views,* **306,** 113
Romanczuk H. *see under* Nowell P., **306,** 494
Römer H. Tonotopic organization of the auditory neuropile in the bushcricket ettigonia viridissima, **306,** 60
Rose M. D. *see under* Raza S. M., **306,** 52
Rose S. Schizophrenic terms, *correspondence,* **306,** 110
Rosén A. and Klein G. UV light-induced immunoglobulin heavy-chain class switch in a human lymphoblastoid cell line, **306,** 189
Rosen H. and Novakov T. Combustion-generated carbon particles in the Arctic atmosphere, **306,** 768
Rosen J. *see under* Alexander H., **306,** 697
Rosenfeld M. G. *see under* Barinaga M., **306,** 84
see under Mayo K. E., **306,** 86
Rosenfield I., Ziff E. and Van Loon B. DNA for Beginners, *book review by* Mappin G. **306,** 505
Ross C. A. *see under* Crowe J. S., **306,** 389
Rossor M. N. *see under* Adrian T. E., **306,** 584
Rothberg P. G. *see under* Reitsma P. H., **306,** 492
Rothschild M. Dear Lord Rothschild: Birds, Butterflies & History, *book review by* Cain A. J. **306,** 506
Rovera G. *see under* Nowell P., **306,** 494
Rowlands D. J., Clarke B. E., Carroll A. R., Brown F., Nicholson B. H., Bittle J. L., Houghten R. A. and Lerner R. A. Chemical basis of antigenic variation in foot-and-mouth disease virus, **306,** 694
Rubenstein E., Bonner W. A., Noyes H. P. and Brown G. S. Supernovae and life, *correspondence,* **306,** 118
Rubin J. S., Joyner A. L., Bernstein A. and Whitmore G. F. Molecular identification of a human DNA repair gene following DNA-mediated gene transfer, **306,** 206
Ruggles C. *book review of* Echoes of the Ancient Skies: The Astronomy of Lost Civilizations, (Krupp E. C.) **306,** 123
Rukang W. and Oxnard C. E. Ramapithecines from China: evidence from tooth dimensions, **306,** 258
Rumpold H. *see under* Schuller-Petrovic S., **306,** 179
Rupke N. A. The Great Chain of History: William Buckland and the English School of Geology (1814–1849), *book review by* Porter R. **306,** 813
Russell M. J. *see under* Boyce A. J., **306,** 545
Russell R. G. G. *see under* Gowen M., **306,** 378
Rutherfurd-Dyer R. The "wine-dark" sea, *correspondence,* **306,** 110
Rutter W. J. *see under* Walker M. D., **306,** 557
Saint R. B. *see under* Coppel R. L., **306,** 751
Sakaguchi A. Y. *see under* Gusella J. F., **306,** 234
Salamsky N. M. *see under* Heimann R. B., **306,** 164
Saliwanchik R. Legal Protection for Microbiological and Genetic Engineering Inventions, *book review by* Cole M., Smither R. **306,** 94
Salvadè G. *see under* Mysak L. A., **306,** 46
Salvati M. *see under* Pedersen H., **306,** 568
Salzberg B. M., Obaid A. L., Senseman D. M. and Gainer H. Optical recording of action potentials from vertebrate nerve terminals using potentiometric probes provides evidence for sodium and calcium components, **306,** 36
Samaha F. *see under* Grainger R. M., **306,** 88
Sander C. *see under* Gallwitz D., **306,** 704
Sanes J. *see under* Barnstable C., **306,** 14
Sang J. H. *see under* Sinclair J. H., **306,** 198
Santoni A. *see under* Forni G., **306,** 181
Sato H. *see under* Carr B. J., **306,** 666
Satoh K. *see under* Vincent S. R., **306,** 688
Scatton B. *see under* Edvinsson L., **306,** 55
Scheiwiller T. *see under* Mysak L. A., **306,** 46
Scherrer P. H. *see under* Delache P., **306,** 651
Schilling J.-G. *see under* Verma S. P., **306,** 654
Schimmel P. *see under* Jasin M., **306,** 441
Schneeberger A. *see under* Leprince D., **306,** 395
Schuller-Petrovic S., Gebhart W., Lassmann H., Rumpold H. and Kraft D. A shared antigenic determinant between natural killer cells and nervous tissue, **306,** 179
Schulz I. *see under* Streb H., **306,** 67
Schutgens R. B. H. *see under* Heymans H. S. A., **306,** 69
Scolnick E. M. *see under* DeFeo-Jones D., **306,** 707
Seeburg P. H. *see under* Nunn M. F., **306,** 391
Sejnowski T. J. *see under* Ballard D. H., **306,** 21
Selden J. R. *see under* Nowell P., **306,** 494
Sen S. *see under* Nash C., **306,** 296
Senseman D. M. *see under* Salzberg B. M., **306,** 36
Seshi B., Phadnis S. H. and Shamala N. Indian institute idea, *correspondence,* **306,** 310
Seufert H. M. *see under* Jochum K. P., **306,** 431
Shackleton N. J., Hall M. A., Line J. and Shuxi C. Carbon isotope data in core V19-30 confirm reduced carbon dioxide concentration in the ice age atmosphere, **306,** 319
Shah S. M. I. *see under* Raza S. M., **306,** 52
Shamala N. *see under* Seshi B., **306,** 310
Shaw S. and McMichael A. Immunology HLA class II antigens and monoclonal antibodies, **306,** 538
Shen A. *see under* Richards J. E., **306,** 483
Shen J. *see under* Moore P. B., **306,** 356
Shin H.-S., Flaherty L., Artzt K., Bennett D. and Ravetch J. Inversion in the *H–2* complex of *t*-haplotypes in mice, **306,** 380
Shipman P., Potts R. and Pickford M. Lainyamok, a new middle Pleistocene hominid site, **306,** 365
Shoulson I. *see under* Gusella J. F., **306,** 234
Shtram Y. *see under* Gilden D. H., **306,** 478
Shuxi C. *see under* Shackleton N. J., **306,** 319
Sibatani A. Scientists quiet in Japan, *correspondence,* **306,** 220
Silas L. *see under* Lowry P. J., **306,** 70
Simons G. Are Computers Alive? Evolution and New Life Forms, *book review by* MacKay D. **306,** 510
Simons G. L. Towards Fifth-Generation Computers, *book review by* Lavington S. **306,** 129
Simpson E. Immunology: Antigens associated with H–2, embryos and tumours, *news and views,* **306,** 738
SimpsonR. How the PhD came to Britain: A Century of Struggle for Postgraduate Education, *book review by* Dainton F. **306,** 93
Sinclair J. H., Sang J. H., Burke J. F. and Ish-Horowicz D. Extrachromosomal replication of *copia*-based vectors in cultured *Drosophila* cells, **306,** 198
Sixbey J. W., Vesterinen E. H., Nedrud J. G., Raab-Traub N., Walton L. A. and Pagano J. S. Replication of Epstein–Barr virus in human epithelial cells infected *in vitro*, **306,** 480
Slack J. M. W. From Egg to Embryo: Determinative Events in Early Development, *book review by* Lewis J. **306,** 294
Smith D. G. W. *see under* Reed S. J. B., **306,** 172
Smith D. L. *see under* Rahn K. A., **306,** 459
Smith III R., Huston M. M., Jenkins R. N., Huston D. P. and Rich R. R. Mitochondria control expression of a murine cell-surface antigen, **306,** 599
Smith J. M. *book review of* The Neutral Theory of Molecular Evolution, (Kimura M.) **306,** 713
Smith K. A. *Editor,* Soil Analysis: Instrumental Techniques and Related Procedures, *book review by* West T. S. **306,** 94
Smith M. C. *see under* Haines D. A., **306,** 351
Smith P. J. *book review of* The Mediterranean was a Desert: A Voyage of the Glomar Challenger, (Hsü, K. J.) **306,** 507
Smith R. *see under* Olsen I., **306,** 75
Smither R. *see under* Cole M., **306,** 94
see under Cole M., **306,** 94
Smyth D. G. *see under* Parish D. C., **306,** 267
Snyder S. H. *see under* Lo M. M. S., **306,** 57
Solomon E. and Goodfellow P. Human genetics: Human gene mapping rolls along, *news and views,* **306,** 223
Somerville R. A. *see under* Merz P. A., **306,** 474
Sonett C. P. Sunspot index infers a small relict magnetic field in the Sun's core, **306,** 670
Sparks R. S. J. Earth sciences: Mixed-up magmas, *news and views,* **306,** 315
Spence J. *book review of* Science and Civilisation in China: Vol. 5, Part 5. Spagyrical Discovery and Invention: Physiological Alchemy, (Needham J. and Gwei-Djen Lu) **306,** 120
Spiegel E. A. *see under* Marzec C. J., **306,** 248
Spinrad H. *see under* Djorgovski S., **306,** 569
Spira J. *see under* Sümegi J., **306,** 497
Ssebuwufu P. J. M. *see under* Lewis F. A., **306,** 673
Stafford J. and Queen C. Cell-type specific expression of a transfected immunoglobulin gene, **306,** 77
Steele P. E. *see under* Rabson A. B., **306,** 604
Stebelin D. *see under* Leprince D., **306,** 395
Steinman S. B. *see under* Levi D. M., **306,** 468
Stephenson J. R. *see under* Heisterkamp N., **306,** 239
Stevens C. *see under* Barnstable C., **306,** 14
Stevens C. F. *see under* Aldrich R. W., **306,** 436
Stevenson H. W. and Azuma H. IQ in Japan and the United States, *matters arising,* **306,** 291
Steward E. G. Fourier Optics: An Introduction, *book review by* Lipson H. **306,** 715
Stewart I. *book review of* The Visual Display of Quantitative Information, (Tufte E. R.) **306,** 135
Stillman Drake Telescopes, Tides, and Tactics: A Galilean Dialogue about the *Starry Messenger* and Systems of the World, *book review by* Hall A. R. **306,** 124
Stinebring D. R. and Cordes J. M. Low-frequency polarimetry of the millisecond pulsar PSR1937+214, **306,** 349
Storb U. *see under* Brinster R. L., **306,** 332
Streb H., Irvine R. F., Berridge M. J. and Schulz I. Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate, **306,** 67
Street-Perrott F. A. *see under* Gillespie R., **306,** 680
Strulovici B. Benovic J. L. *see under* Cerione R. A., **306,** 562
Sukumar S., Notario V., Martin-Zanca D. and Barbacid M. Induction of mammary carcinomas in rats by nitrosomethylurea involves malignant activation of H-*ras*-1 by single point mutations, **306,** 658
Sümegi J., Spira J., Bazin H., Szpirer J., Levan G. and Klein G. Rat c-*myc* oncogene is located on chromosome 7 and rearranges in immunocytomas with t(6:7) chromosomal translocation, **306,** 497
Sussenbach J. S. *see under* Jansen M., **306,** 609
Swanson C. P. Ever-Expanding Horizons: The Dual Informational Sources of Human Evolution, *book review by* Attenborough R. **306,** 624
Swartz D. J. *see under* Mekalanos J. J., **306,** 551
Swift D. J. *see under* Kershaw P. J., **306,** 774
Switsur R. *see under* Gillespie R., **306,** 680
Szpirer J. *see under* Sümegi J., **306,** 497
Tabushi I. *see under* Ise N., **306,** 295
Tagliabue A., Nencioni L., Villa L., Keren D. F., Lowell G. H. and Boraschi D. Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA, **306,** 184
Taieb M. *see under* Aronson J. L., **306,** 210
Tainer J. A. *see under* Getzoff E. D., **306,** 287
Tainer J. A., Getzoff E. D., Richardson J. S. and Richardson D. C. Structure and mechanism of copper, zinc-superoxide dismutase, **306,** 284
Takahashi H. *see under* Horikawa S., **306,** 611
Takai T. *see under* Horikawa S., **306,** 611
Takashima H. *see under* Nawa H., **306,** 32
Tamaroff M. *see under* Lazar R. M., **306,** 54
Tan R. *see under* Heymans H. S. A., **306,** 69
Tanaka T. and Masuda A. On the half life of ^{138}La—Reply, *matters arising,* **306,** 503
Tanzi R. E. *see under* Gusella J. F., **306,** 234
Tarenghi M. *see under* Pedersen H., **306,** 568
Tarling D. H. *see under* Incoronato A., **306,** 461
Tatemoto K. *see under* Adrian T. E., **306,** 584
Taylor B. E., Eichelberger J. C. and Westrich H. R. Hydrogen isotopic evidence of rhyolitic magma degassing during shallow intrusion and eruption, **306,** 541
Taylor F. W. Atmospheric physics: Natural lasers on Venus and Mars, *news and views,* **306,** 640
Taylor J. C. *see under* Ekstrom A., **306,** 173
Taylor S. R. *see under* McLennan S. M., **306,** 169
Taylor-Papadimitriou J. *book review of* Growth and Maturation Factors, Vol. 1, (Guroff G., *editor*) **306,** 295
Tease C. *book review of* Aneuploidy, (Bond D. J. and Chandley A. C.) **306,** 816
Teitelbaum S. L. *see under* Reitsma P. H., **306,** 492
Temin H. M. *see under* Panganiban A. T., **306,** 155
Terzian Y. and Bilson E. M. *Editors,* Cosmology and Astrophysics: Essays in Honor of Thomas Gold, *book review by* Bath G. T. **306,** 127
Tetens V. *see under* Wells R. M. G., **306,** 500

Thiemens M. H. Meteoritics: New isotopic evidence of early Solar System processes, *news and views*, **306**, 18
Thirlwall M. F. *see under* Fitton J. G., **306**, 327
Thomas G. and Padayatty J. D. Organization and bidirectional transcription of H2A, H2B and H4 histone genes in rice embryos, **306**, 82
Thompson R. *see under* Gee C. E., **306**, 374
Thomsen G. H. *see under* Grainger R. M., **306**, 88
Thornton J. *see under* blundell T., **306**, 281
Thorpe S. A., Hall A. J. and Hunt S. Bouncing internal bores of Ardmucknish Bay, Scotland, **306**, 167
Thulborn R. A. First mammal-like reptile from Australia—Reply, *matters arising*, **306**, 209
Tite J. *see under* Janeway Jr, C. A., **306**, 80
Tomeoka K. and Buseck P. R. A new layered mineral from the Mighei carbonaceous chondrite, **306**, 354
Toulmin S. The Return to Cosmology: Postmodern Science and the Theology of Nature, *book review by* Medawar P. B. **306**, 126
Toy D. W. *see under* Monaghan D. T., **306**, 176
Toyosato M. *see under* Horikawa S., **306**, 611
Trefil J. S. The Moment of Creation: Big Bang Physics from before the First Millisecond to the Present Universe, *book review by* Hughes D. W. **306**, 508
Trial J. *see under* Reitsma P. H., **306**, 492
Trifonov E. N. *book review of* Principles of Modern Genetics, 2nd Edn., (Gershenson S. M.) **306**, 815
Trimble V. Astronomy: Formation and fate of interacting binaries, *news and views*, **306**, 115
Tsai M.-J. *see under* Ciejek E. M., **306**, 607
Tucker P. W. *see under* Richards J. E., **306**, 483
Tufte E. R. The Visual Display of Quantitative Information, *book review by* Stewart I. **306**, 135
Turner N. E., Robinson S. J. and Haselkorn R. Different promoters for the *Anabaena* glutamine synthetase gene during growth using molecular or fixed nitrogen, **306**, 337
Turner M. J. *see under* Young J. R., **306**, 196
Turner M. S. The end may be hastened by magnetic monopoles, **306**, 161
Unger T. *see under* Cohen J. B., **306**, 797
Unkeless J. C. *see under* Young, J. D.-E., **306**, 186
Unwin S. C. *see under* Biretta J. A., **306**, 42
Vafai A. *see under* Gilden D. H., **306**, 478
Vale W. *see under* Barinaga M., **306**, 84
see under Mayo K. E., **306**, 86
van Albada G. D. *see under* van der Hulst J. M., **306**, 566
Van de Walle M. J. *see under* Olivo P. D., **306**, 400
van den Bosch H. *see under* Heymans H. S. A., **306**, 69
Van den Brande J. L. *see under* Jansen M., **306**, 609
van der Hulst J. M., Hummel E., Davies R. D., Pedlar A. and van Albada G. D. Radio discovery of a young supernova, **306**, 566
Van Loon B. *see under* Rosenfield I., **306**, 505
van Schaik F. M. A. *see under* Jansen M., **306**, 609
Vanegas H. *see under* Fields H. L., **306**, 684
Vanin E. *see under* Kioussis D., **306**, 662
Verma S. P., Schilling J.-G. and Waggoner D. G. Neodymium isotopic evidence for Galapagos hotspot-spreading centre system evolution, **306**, 654
Vértes A. *see under* Burger K., **306**, 353
Vesterinen E. H. *see under* Sixbey J. W., **306**, 480
Vickerman K. *see under* Barry J. D., **306**, 699
see under Crowe J. S., **306**, 389
Villa L. *see under* Tagliabue A., **306**, 184
Vincent S. R., Satoh K., Armstrong D. M. and Fibiger H. C. Substance P in the ascending cholinergic reticular system, **306**, 688
Vining L. ***Editor***, Biochemistry and Genetic Regulation of Commercially Important Antibiotics, *book review by* Cole M., Smither R. **306**, 94
Viola V. E. *book review of* The Origin of the Chemical Elements and the Oklo Phenomenon, (Kuroda P. K.) **306**, 626
Wabl M. *see under* Haas I. G., **306**, 387
Wabl M. R. *see under* Burrows P. D., **306**, 243
Waddington W. G. *see under* Humphreys C. J., **306**, 743
Wade N. *book review of* Exploring the Earth and the Cosmos, (Asimov I.) **306**, 133
Waggoner D. G. *see under* Verma S. P., **306**, 654
Walker M. D., Edlund T., Boulet A. M. and Rutter W. J. Cell-specific expression controlled by the 5'-flanking region of insulin and chymotrypsin genes, **306**, 557
Wall P. D. *see under* Woolf C. J., **306**, 739
Wall R. Terminal guidance, *correspondence*, **306**, 220
Wallace B. *see under* Dobzhansky T., **306**, 624
Wallace M. R. *see under* Gusella J. F., **306**, 234
Walls D. F. Squeezed states of light, *review article*, **306**, 141
Walter R. C. *see under* Aronson J. L., **306**, 210
Walton L. A. *see under* Sixbey J. W., **306**, 480
Ward S. *see under* Raza S. M., **306**, 52
Wardhaugh T. Owls of Britain and Europe, *book review by* Andrews J. H. **306**, 513
Wartiovaara J. *see under* Liesi P., **306**, 265
Wasser S. ***Editor***, Social Behavior of Female Vertebrates, *book review by* Clutton-Brock T. **306**, 716
Watkins P. C. *see under* Gusella J. F., **306**, 234
Watson S. J. *see under* Gee C. E., **306**, 374
Watt D. J. *see under* Olsen I., **306**, 75
Wdowczyk J. and Wolfendale A. W. Mass composition of high-energy cosmic rays and scaling violation in their interactions, **306**, 347
Weatherall D. J. *see under* Higgs D. R., **306**, 398
Weaver P. P. E. and Kuijpers A. Climatic control of turbidite deposition on the Madeira Abyssal Plain, **306**, 360
Webber J. A. *see under* Cooke J., **306**, 423
Weiner P. K. *see under* Getzoff E. D., **306**, 287
Weinstein I. B. *see under* Gattoni-Celli S., **306**, 795
Weinthal J. *see under* Liebermann, D., **306**, 342
Weiss E. H. *see under* Mellor A. L., **306**, 792
Weissman I. L. *see under* Alexander H., **306**, 697
Wellish M. *see under* Gilden D. H., **306**, 478
Wells R. M. G., Tetens V. and Brittain T. Absence of cooperative haemoglobin–oxygen binding in *Sphenodon*, a reptilian relict from the Triassic, **306**, 500
West A. R. *see under* Almond D. P., **306**, 456
West T. S. *book review of* Soil Analysis: Instrumental Techniques and Related Procedures, (Smith K. A. *editor*) **306**, 94
Westrich H. R. *see under* Taylor B. E., **306**, 541
Wexler N. S. *see under* Gusella J. F., **306**, 234
Whaler K. A. Earth sciences: Geomagnetic impulses and deep mantle conductivity, *news and views*, **306**, 117
White W. M. *see under* Jochum K. P., **306**, 431
Whitehead D. L. Invertebrate endocrinology Steroids ... the earliest hormones, *news and views*, **306**, 540
Whitehouse H. L. K. Genetics: Duplex breaks in DNA as recombination initiators, *news and views*, **306**, 645
Whitmore G. F. *see under* Rubin J. S., **306**, 206
Whittaker J. B. *book review of* Herbivory: The Dynamics of Animal–Plant Interactions, (Crawley M. J.) **306**, 816
Wickramasinghe N. C. *see under* Hoyle F., **306**, 420
Wigley T. M. L. *see under* Raper S. C. B., **306**, 458
Williams C. T. *see under* Henderson P., **306**, 358
Williams Cold Spring Harbor meeting: Building new vaccines, *news and views*, **306**, 427
Williams D. A. Bacterial life in space, *correspondence*, **306**, 420
Williams R. O. *see under* Young J. R., **306**, 196
Willison K. *see under* Brickell P. M., **306**, 756
Willson M. F. and Burley N. Mate Choice in Plants: Tactics, Mechanisms and Consequences, *book review by* Charlesworth D. **306**, 405
Windsor D. M. *see under* Leigh E. G., **306**, 625
Winfree A. T. *book review of* Powers of Ten: About the Relative Size of Things in the Universe, (Morrison P. and Morrison P.) **306**, 125
Wisniewski H. M. *see under* Merz P. A., **306**, 474
Wisniewski W. *see under* Middleditch J., **306**, 163
Wolfendale A. W. *see under* Wdowczyk J., **306**, 347
Wolskee P. J. *see under* Gracely R. H., **306**, 264
Wolstencroft J. H. *see under* Parish D. C., **306**, 267
Wong C. S., Boyle E., Bruland K. W., Burton J. D. and Goldberg E. D. ***Editors***, Trace Metals in Sea Water, *book review by* Liss P. **306**, 623
Wong R. K. S. *see under* Miles R., **306**, 371
Woo S. L. C., Lidsky A. S., Güttler F., Chandra T. and Robson K. J. H. Cloned human phenylalanine hydroxylase gene allows prenatal diagnosis and carrier detection of classical phenylketonuria, **306**, 151
Wood B. *book review of* New Interpretations of Ape and Human Ancestry, (Ciochon R. L. and Corruccini R. S. *editors*) **306**, 140
Wood D. D. *see under* Gowen M., **306**, 378
Wooden J. L. *see under* Ashwal L. D., **306**, 679
Woods D. E. *see under* Jansen M., **306**, 609
Woolf C. J. Evidence for a central component of post-injury pain hypersensitivity, **306**, 686
Woolf C. J. and Wall P. D. Neuropharmacology: Endogenous opioid peptides and pain mechanisms: a complex relationship, *news and views*, **306**, 739
Wright H. E. Jr *see under* Foster D. R., **306**, 256
Wullstein L. H. Garret Hardin, *correspondence*, **306**, 530
Yamonoto G. *see under* Barinaga M., **306**, 84
Yang Chen Ning Selected Papers 1945–1980 With Commentary, *book review by* Matthews P. T. **306**, 623
Yelton J. M. Particle physics: The F meson found at Cornell, *news and views*, **306**, 223
Young, J. D.-E., Unkeless J. C., Young T. M., Mauro A. and Cohn Z. A. Role for mouse macrophage IgG Fc receptor as ligand-dependent ion channel, **306**, 186
Young A. B. *see under* Gusella J. F., **306**, 234
Young J. R., Miller E. N., Williams R. O. and Turner M. J. Are there two classes of VSG gene in *Trypanosoma brucei*?, **306**, 196
Young T. M. *see under* Young, J. D.-E., **306**, 186
Zakut-Houri R., Oren M., Bienz B., Lavie V., Hazum S. and Givol D. A single gene and a pseudogene for the cellular tumour antigen p53, **306**, 594
Zarka P. and Genova F. Low-frequency jovian emission and solar wind magnetic sector structure, **306**, 767
Ziff E. *see under* Rosenfield I., **306**, 505
Zoback M. D. Geology: A new data source for *in situ* stress field orientations, *news and views*, **306**, 18
Zorman G. *see under* Fields H. L., **306**, 684
Zuckerkandl E. Homologyana, *correspondence*, **306**, 220

Nature Author Indexes

Nature Author Indexes are published bimonthly. Indexes for the previous 1983 volumes were published in the issues of 17 March (Vol.301), 10 May (Vol.302), 21 July (Vol.303), 15 September (Vol.304) and 24 November (Vol.305).

AN 'RT' THAT WORKS NEEDN'T BE AN 'ET' ONE

Introduction of an above 95% pure
AMV REVERSE TRANSCRIPTASE
which is totally RNase free

Door to door delivery
within 48 hours anywhere in Europe

Telephone (England) (0206) 44469
Telex 987181 BIOTEC G

Circle No.19 on Reader Service Card.



nature is keeping ahead. Are you being left behind?

New research results, the very latest international science news, key papers by the world's leading scientists and speed of publication ensure *Nature* remains one of the foremost weekly scientific journals in the world.

But if you're tied in to a circulation list at work, all this vital weekly information will undoubtedly reach you late, often incomplete and sometimes not at all.

With your own personal copy, you solve the problem. Every week you'll have all the latest research results and science news in your hands within days of publication:

News reports from throughout the world of science and technology.

A **News and Views** section, where new ideas are analysed and discussed by leading researchers.

Articles and Letters in which key investigators disclose their work for the first time.

Book Reviews – Eminent figures offer expert judgments on a wide variety of recent publications.

Special Issues providing an in-depth treatment of topics of importance.

Product Reviews – Detailed descriptions of laboratory equipment newly available.

Classified Advertisements for a wide variety of positions all over the world.

You will no longer be dependent upon other people passing down the 'list' copy, and because you will automatically receive every issue, you won't risk missing a single copy through absence from work. Moreover, you keep all this key information for permanent reference.

Through your door every week



Subscribe now – at Half Price

Become a personal subscriber now and you will receive a discount of 50% off the full rate.

All 51 weekly issues will be delivered to the address of your choice. You can start your annual subscription with any issue you choose. And you will receive six bi-monthly author indexes, together with the annual index of subject and author.

All this for just 94p a week.

Place your order now and make certain that you receive the scientific news and research results crucial to your work when you want them.

☐ I accept: Please send me a personal subscription to *Nature* for one year (51 issues) at the special half-price rate of £49.00.

☐ I enclose my personal cheque for £49.00 made payable to *Nature*. (Note: Institutional cheques cannot be accepted at this special rate).

☐ Please charge to my credit card account.
Access/American Express/Visa/Diners Club.

Account No._____

Card Expiry Date_____

Signature_____

Name_____

Address_____

_____

_____

_____

Date_____ 7B18

Please return this form to:
Circulation Manager
Nature
Brunel Road, Houndmills
Basingstoke
Hants RG21 2XS

This offer applies to UK and North America only. Please allow approximately 4 weeks for delivery of the first issue in your subscription year.

nature

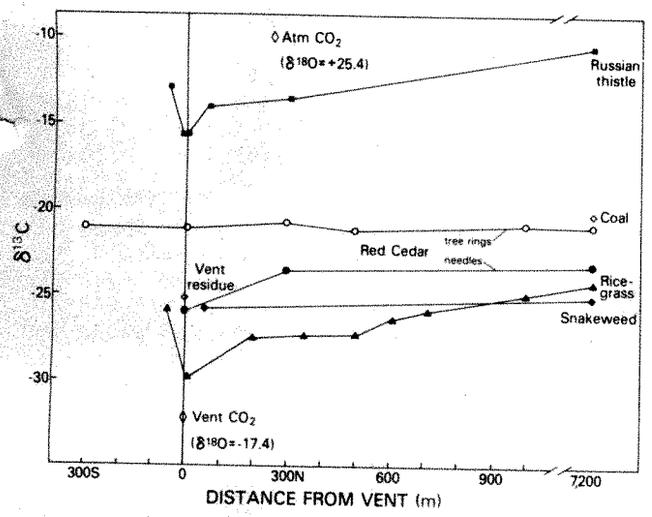


Fig. 1 Variations in $\delta^{13}C$ values of various materials as a function of their proximity to the vent. CO_2 issuing from the vent has a $\delta^{13}C$ value of −32.0 and a $\delta^{18}O$ value of −17.4, whereas atmospheric CO_2 300 m north of the vent has a $\delta^{13}C$ value of −10.3 and a $\delta^{18}O$ value of +25.4. Residue collected inside the vent has a $\delta^{13}C$ value of −25.0, and coal samples collected away from the vent area at two separate sites have identical $\delta^{13}C$ values of −21.0. Wood from tree rings of red cedar exhibits no isotopic effects from burning coal contamination, whereas needles of a red cedar on the vent trend do show some contamination. Roots from Russian thistle (a C_4 plant), stems from ricegrass, and roots from snakeweed (both C_3 plants) exhibit decreasing contamination in their $\delta^{13}C$ values as a function of their distance north of vents (prevailing wind is from the south). The plants collected south of the vent exhibit very little contamination from the vent gas in their $\delta^{13}C$ values.

Table 1 Stable isotope compositions of materials and gases associated with naturally burning coal

Sample	Location	$\delta^{13}C$	$\delta^{18}O$
Coal	Bed 1*	−21.0	
Coal	Bed 2*	−21.0	
CO_2	Large vent	−32.5	−17.4
CO_2	Atmosphere 300 m north of vents	−10.3	+25.4
Carbonaceous material	Wall of large vent	−25.1	

* Coal beds 1 and 2 are two separate veins collected south of the vent trend from an area where there was no evidence of burning.

the response of different species to local effects of fossil fuel burning because the CO_2 produced from the combustion of coal should be extremely depleted in ^{13}C relative to normal atmospheric CO_2. In addition, stable isotope ratios of the gases emanating from the vents may provide information on the mechanisms by which coal burns naturally.

Carbon isotope ratios of organic material were determined using carbon dioxide gas that was produced by the complete combustion of the organic material at 800–1,000 °C in purified oxygen at 1 atmosphere. The carbon dioxide was purified and then analysed in a 6″–60° sector isotope ratio mass spectrometer. Before combustion, the wood samples were chopped in a Wiley mill, then extracted with benzene and ethanol in a Soxhlet extractor to remove soluble components. The resulting sample was composed primarily of cellulose and lignin. Differences between the $^{13}C/^{12}C$ ratios of wood treated in this manner and untreated wood were small (<0.2‰). The wood samples were collected from tree rings representing the past 20 yr of growth. The height of the trees was 1–2 m. Plant samples were dried and combusted directly, without any chemical treatment. The herbaceous plants were all ~0.5–1 m in height. Atmospheric and vent gases were collected in evacuated stainless steel cylinders and CO_2 was separated and purified by differential freezing

Table 2 $\delta^{13}C$ values of trees and herbaceous plants

Tree	Distance and direction from main vent	$\delta^{13}C$	
Red cedar (*Juniperus scopulorum*)	300 m S	−21.0(TR)	
	10 m N (on east part of vent trend)	−21.3(TR)	−25.9(N)
	300 m N	−21.3(TR)	−23.6(N)
	500 m N	−21.4(TR)	
	1,000 m N	−20.8(TR)	
	7,200 m NW (control)	−21.0(TR)	−23.2(N)
Herb			
Russian thistle (*Salsola kali* L.)	1 m S	−15.5(R)	−16.8(L)
	50 m SW	−12.4(R)	
	2 m N	−15.6(R)	−17.4(L)
	50 m N	−13.5(R)	−14.1(L)
	300 m N	−13.1(R)	
	7,200 m NW (control)	−11.0(R)	−12.4(L)
Ricegrass (*Oryzopsis webberi*)	2 m S	−25.8(ST)	−24.9(L)
			−29.6(R)
	2 m W	−29.7(ST)	
	100 m W	−27.6(ST)	
	2 m E	−30.4(ST)	
	100 m NE	−26.1(ST)	
	1 m N	−29.9(ST)	−30.5(R)
	200 m N	−27.5(ST)	
	325 m N	−27.5(ST)	−25.8(L)
			−28.4(R)
	500 m N	−27.3(ST)	
	600 m N	−26.4(ST)	
	700 m N	−26.1(ST)	
	1,000 m N	−25.2(ST)	
	7,200 m NW (control)	−24.8(ST)	−24.7(L)
			−24.7(R)
Snakeweed (*Gutierrezia sarothrae*)	50 m N	−25.7(R)	−24.7(L)
	50 m NW (control)	−25.2(R)	−24.2(L)

TR, Tree rings; N, needles; R, roots; L, leaf; ST, stem.

techniques. The results are reported in units of ‰ relative to SMOW standard for oxygen or relative to PDB standard for carbon and are accurate to ±0.2‰ based on replicate analysis. The $\delta^{13}C$ value of NBS-21 in this laboratory is −28.1 relative to PDB.

The burning coal beds are part of the Straight Cliffs Formation located at 37°17′30′ N, 111°22′ W in the Escalante Quadrangle near the Utah–Arizona border. This formation consists of interbedded, Upper Cretaceous marine and nonmarine sandstones, mudstones, shales and coal[5]. Areas where coal is burning or has burned are easily recognized because the sandstones, which are usually yellow, are turned red by the heat and gases from the burning coal. The collection site is located on the southern edge of the arid Kaiparowits Plateau in the Smoky Mountains. A 200-m long, east–west-trending series of small vents each ~4 cm in diameter, breach the red sandstone surface of this area. Two large vents (1–2 m in diameter) on the western edge of this trend are the most active and, therefore, served as the focus of the study. The local topography was such than the vents were ~2 m higher in elevation than the surrounding area. Approximately 10 l min^{-1} of steam and carbonaceous gases issue from each of the large vents. The gas collected from the vents consists of ~70% air, 7% steam, 20% CO_2, 2% CH_4 and 1% saturated hydrocarbons. These gases are blown northwards by prevailing winds.

Coal from different beds in this area have $\delta^{13}C$ values near −21.0 (Table 1, Fig. 1). Carbonaceous material which precipitated on the walls of the vents has a lighter value of −25.1 and CO_2 from the vent is even lighter at −32.5 (Table 1, Fig. 1). Modern atmospheric CO_2 normally has $\delta^{13}C$ values between −7.5 and 8.0 (ref. 6 and I. Friedman and J.D.G., in preparation), so that the CO_2 issuing from the vent is not atmospheric but is derived from the oxidation of the coal. Furthermore, equilibrium fractionation factors indicate that ^{13}C is always concentrated in

Table 3 Calculated carbon isotope fractionations between certain plant species and atmospheric CO_2

Common name	Species	Organ	$\Delta^{13}C$ plant–CO_2
Ricegrass	*Oryzopsis webberi*	Stem	−17
Russian thistle	*Salsola kali*	Root	−3

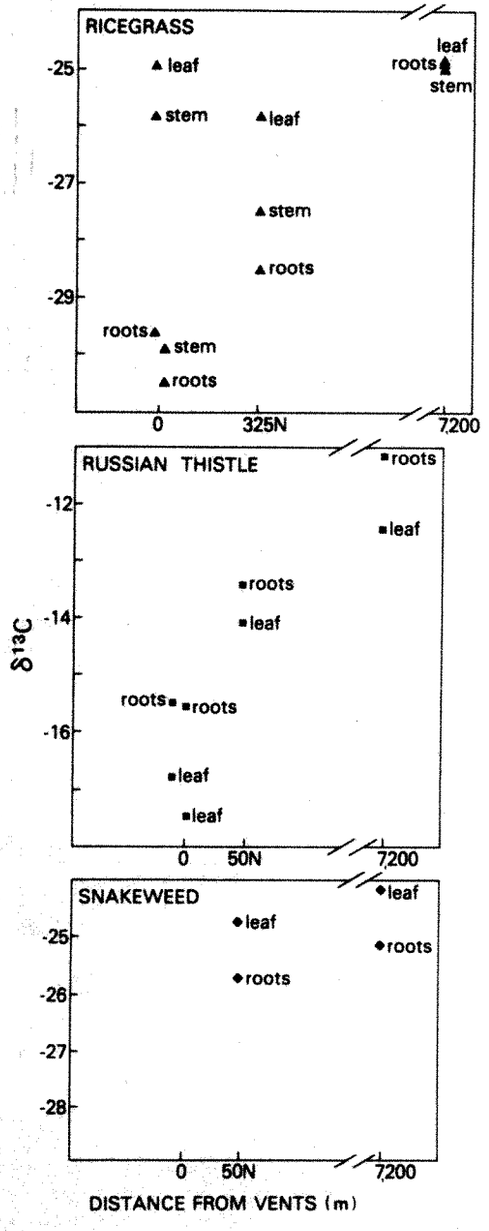


Fig. 2 $\delta^{13}C$ values of various parts of plants as a function of their proximity to the vents. Snakeweed and ricegrass, both C_3 plants, have more negative $\delta^{13}C$ values in their roots than in their leaves or stems, whereas Russian thistle, a C_4 plant, shows the inverse trend. Although the difference between the $\delta^{13}C$ values of roots and leaves from Russian thistle is constant, those plants growing near the vents have $\delta^{13}C$ values which indicate that they have incorporated vent CO_2 during photosynthesis. Ricegrass exhibits large variations among the $\delta^{13}C$ values of various organs of plants collected in areas where there is contamination from vent CO_2, and very little variation in organs from plants collected from the control area. The roots of ricegrass collected south of the vent (prevailing wind is from the south) exhibit contamination by vent CO_2, whereas the leaf and stem do not.

CO_2 relative to a reduced-carbon phase even at high temperatures[7] so that the CO_2 from the vent is not in isotopic equilibrium with either the carbon in the coal or the carbonaceous material from the walls of the vent. Instead, the isotope data indicate that the coal burns by a kinetic process wherein ^{12}C in the coal is preferentially oxidized relative to ^{13}C.

The oxygen isotopic composition of the vent CO_2 is −17.4 (Table 1, Fig. 1), a value only moderately more ^{18}O-depleted than the isotopic composition of −15 for groundwater that would be expected from this area[8]. Atmospheric O_2 has a $\delta^{18}O$ value of +23.5 (ref. 9). The extreme ^{18}O depletion of the vent CO_2 relative to that expected from the kinetic oxidation of the coal by atmospheric O_2 leads us to conclude that the source of most of the oxygen in the naturally burning coal must come from a reservoir having a low $\delta^{18}O$ value such as that of the groundwater.

Red cedars (*Juniperus scopulorum* Sarg.) were collected with the anticipation that the ^{13}C-depleted CO_2 issuing from vents would produce wood having low $\sigma^{13}C$ values. The $\delta^{13}C$ values of wood from trees 5 and 10 m north of the vents were identical to the values of control trees >7.2 km away (Table 2, Fig. 1). Evidence that the red cedars were exposed to the vent CO_2 is observed in the $\delta^{13}C$ values of some of the needles of a cedar located 10 m north of the main vent (Fig. 1). These were depleted in $\delta^{13}C$ by 2‰ relative to needles from trees further from the vents, suggesting that they may be more sensitive indicators of local isotopic anomalies than are the tree rings. The absence of low $\delta^{13}C$ values of the tree rings from samples collected near the vents suggests that the fractionation of carbon isotopes between red cedars and atmospheric CO_2 is not always constant.

Unlike the red cedars, the isotopic compositions of certain herbaceous plants were affected by the CO_2 issuing from the burning coal vents (Table 2, Fig. 1). The effect was observed in the carbon isotopic compositions of plants using each of the two common pathways of photosynthesis and in plants with different physiognomies. For example, the $\delta^{13}C$ values of samples of Russian thistle, a herbaceous annual C_3 plant, and ricegrass, a C_4 plant, are both strongly dependent on their position relative to the vents. Samples of snakeweed, a woody perennial C_3 plant, have $\delta^{13}C$ values which are less dependent on their position relative to the vents.

Although different organs of each plant have different carbon isotopic compositions because of variations in their chemical components[10], the $^{13}C/^{12}C$ ratio of the vent CO_2 is reflected in the $\delta^{13}C$ values of all the organs in plants collected near the vents (Fig. 2). Differences among the $\delta^{13}C$ values of the organs of snakeweed and Russian thistle are constant although organs of these plants closest to the vents are depleted in ^{13}C relative to corresponding organs of the control plant. However, the difference between the roots of snakewood collected 50 m north of the vents is depleted in ^{13}C by only 0.5‰ relative to the roots of the control plant whereas the roots of Russian thistle 50 m north of the vents is depleted by 2.5‰ relative to the control plant. Differences among the $\delta^{13}C$ values of various organs of ricegrass are not constant: the roots and stems have more variable $\delta^{13}C$ values than do the leaves.

Russian thistle and ricegrass appear to be the species most sensitive to changes in the isotopic composition of local atmospheric CO_2. With a $\delta^{13}C$ value of atmospheric CO_2 of −8.0 surrounding the control plants, the fractionation factor between the roots of Russian thistle (C_3 plant) and CO_2 is −3.0 whereas the fractionation factor between the stems of ricegrass (C_4 plant) and CO_2 is −16.8. Atmospheric CO_2 collected 300 m north of the vents above the zone that may have been affected by CO_2 from the local flora and fauna has a $\delta^{13}C$ value of −10.3 (Table 1, Fig. 1). The fractionation factor between roots of Russian thistle collected 300 m north of the vents and the local atmospheric CO_2 having a $\delta^{13}C$ of −10.3 is −2.8 whereas the corresponding factor between the stems of the ricegrass 325 m north of the vent and this CO_2 is −17.2. Although the fractionation factors for these two species differ greatly, the fractionation

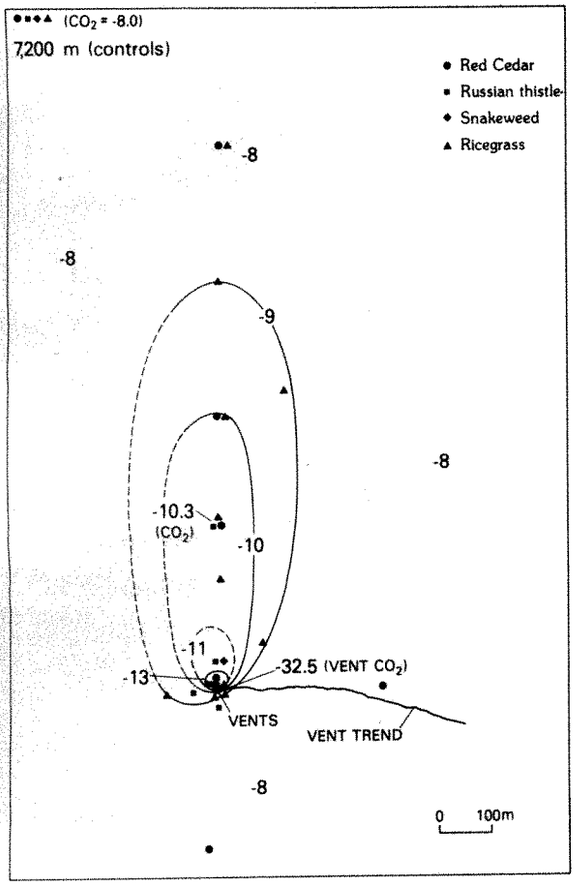


Fig. 3 Estimated $\delta^{13}C$ contours of local atmospheric CO_2 in the area surrounding the vents. From a $\delta^{13}C$ value of −8.0 for CO_2 in equilibrium with the roots of Russian thistle and stems of ricegrass from the control area and a value of −10.3 for atmospheric CO_2 in equilibrium with these plants collected 300 m north of the vents, the fractionation of $\delta^{13}C$ between the organs of these plants and atmospheric CO_2 was calculated (Table 3). By applying these calculated isotope fractionations to the $\delta^{13}C$ values measured in plants collected from the area surrounding the vents, contours can be drawn showing average $\delta^{13}C$ values of atmospheric CO_2. The $\delta^{13}C$ values of −32.5 for vent CO_2 and −10.3 for atmospheric CO_2 collected 300 m north of the vents are also shown.

factors between the control plants and local CO_2 are similar to those of plants of the same species under the influence of ^{13}C-depleted CO_2 from the vents. This suggests that the $\delta^{13}C$ values of Russian thistle and ricegrass reflect the isotopic composition of the local atmospheric CO_2.

Because the $\delta^{13}C$ values of tissues of Russian thistle and ricegrass depend on the $\delta^{13}C$ value of atmospheric CO_2 surrounding the plants during photosynthesis, it should conversely be true that the $\delta^{13}C$ value of atmospheric CO_2 can be determined from the $\delta^{13}C$ value of the plant. By using the fractionation factors listed in Table 3 and the $\delta^{13}C$ values of the plants from the area around the vents, $\delta^{13}C$ contours of atmospheric CO_2 surrounding the vents have been estimated (Fig. 3). Contamination effects of the vent CO_2 on the isotopic composition of the plants are detected up to a distance of 1 km to the north.

The burning coal area located in the Escalante Quadrangle near the Utah–Arizona border is a natural laboratory for studying the effects of the burning of fossil fuels on the atmosphere and on the biosphere. Our results suggest that: (1) the source of most of the oxygen in gases from naturally burning coal may be from a low $\delta^{18}O$ reservoir such as groundwater; (2) wood from tree rings does not necessarily record small variations in the $\delta^{13}C$ values of atmospheric CO_2; and (3) some C_4 and C_3 plants do record small variations in the value of atmospheric CO_2 so that by analysing the $\delta^{13}C$ value of selected organs of some carefully chosen plants, the extent of any contamination to normal atmospheric CO_2 can be estimated.

Received 7 June; accepted 20 October 1983.

1. Park, R. & Epstein, S., *Geochim. cosmochim. Acta* **27,** 110–126 (1960).
2. Bender, M. M. *Phytochemistry* **10,** 1239–1244 (1971).
3. Mazany, T., Lerman, J. C. & Long, A. *Nature* **187,** 432–435 (1980).
4. Sternberg, L. & DeNiro, M. J. *Science* **220,** 947–949 (1983).
5. Hackman, R. J. & Wyant, D. G. *U.S. geol. Surv. Misc. Geol. Inv. Map.* I-744 (1973).
6. Keeling, C. D., Mook, W. G. & Tans, P. P. *Nature* **277,** 121–123 (1971).
7. Bottinga, Y. *Geochim. cosmochim. Acta* **33,** 49–64 (1969).
8. *IAEA Tech. Rep. Ser.* **192** (IAEA, Vienna, 1978).
9. Kroopnick, P. M. & Craig, H. *Science* **175,** 54–55 (1972).
10. Galimov, E. M. in *Advances in Organic Geochemistry,* (eds Tissot, B. & Brenner, R.) 439–452 (Technip, Paris, 1973).

Persistence of organic phosphates in buried soils

J. H. Ottaway

Biochemistry Department, Edinburgh University Medical School, Edinburgh EH8 9XD, UK

Studies of the occurrence of phosphate in soil on archaeological sites have sometimes measured total phosphate[1], but more frequently the inorganic or 'available' phosphate[2,3]—that extractable by brief treatment with acid or by inorganic solutions in the cold. The nature of the organic phosphate component does not seem to have been examined. Here we present an analysis of total phosphate and inorganic phosphate in 50 samples of soil taken from various levels of a site with a continuous settlement record from 1,600 to 6,800 yr BP. We could detect no mineralization of organic phosphate, at least over the period 1,600–5,200 yr BP. But in the upper soils, corresponding to the interval 1600–2800 BP, there was much more organic phosphate than in the lower samples, the transition being quite sharp. We tentatively conclude that the lower soils belonged to an island site relatively sparsely inhabited and often covered with natural vegetation. The upper soils corresponded to a more dense settlement in which organic phosphate, possibly phytic acid, was deposited on the transient surface and was not incorporated into the soil phosphate cycle.

Three groups of workers have compared the proportions of organic and inorganic phosphate in soil buried beneath an ancient structure: a Roman villa in England[4] (~1,800 yr BP), a megalithic tomb in Ireland[5], (~4,000 yr BP), and a Kurgan burial mound in Moldavia[6] (~4,500 yr BP). In each instance the findings were essentially similar. When the phosphate content of soil layers just below the present-day soil surface were compared with equivalent layers below the buried structure (a layer 25–50 cm below the surface was used for comparison in order to eliminate any phosphate that might have been added by human activity such as a burial), it was found that the total phosphate content of the two soil layers was not markedly different, but the organic phosphate content had markedly decreased. In the present-day soil samples the organic phosphate was about 30–40% of the total, a value which is characteristic of many soils[7], while in the buried soil the organic phosphate fraction was only 10–15%. As far as can be determined from these three observations, the extent to which organic phosphate had been lost does not seem to depend on the length of time that the soil had been buried. Possibly partial hydrolysis took place quite quickly[5], but the occlusion was in each case a single event, and it is not possible to say anything about the kinetics.

The collection of a large number of soil samples from a 2 × 2 m sondage excavated in a Yugoslav tell site has enabled phosphate analyses to be made on soils which have been buried for various times, thus making it possible in principle to plot the progress of mineralization against time. The site, tell Gomolava on the Sava River about 80 km west of Belgrade, lies in a district of

© 1984 Macmillan Journals Ltd

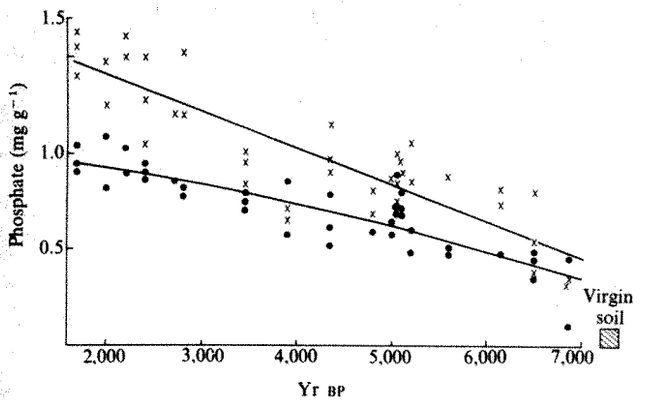


Fig. 1 Total phosphate (× and upper line) and inorganic phosphate (● and lower line) in soil samples from tell Gomolava, Yugoslavia. The lines show the best fit to the points predicted by an optimization routine. The basis of the model used for fitting is described in the text.

calcareous loess (*p*H 8–8.5), so that it is possible completely to exclude leaching. Unlike the three sites mentioned above, the total phosphate content of the buried virgin soil is very low, about 0.15 mg g^{-1}, so that almost all the phosphate in the archaeological samples must have come directly or indirectly from human activity. This has introduced complications in the interpretation of the results, but there are several points of interest that do not appear in a straight comparison of natural soils. The sondage was taken down about 6 m to virgin soil; the layer immediately above this is estimated to have been deposited in the early Vinča–Pločnik period (about 6,850 yr BP). The tell was occupied more or less continuously until sub-Roman times, so that the samples span a range of rather more than 5,000 calendar years (1,600–6,850 yr BP). The dating of the layers, by radiocarbon dating and by traditional archaeological methods (for example, by ceramic typology and by coin dating in the La Tène and Roman eras) is discussed in detail elsewhere[8]. It can be regarded as fairly reliable.

The analytical methods have been described earlier[1]. Total phosphate was measured by a nitrate incineration procedure, and inorganic phosphate by a 15-min extraction with 2 M HCl at 100 °C. About 130 soil samples were assayed for total phosphate. Approximately 50, selected to cover all the 19 archaeological strata, each about 30 cm deep, were chosen at random within the replicate samples available for each layer and assayed for inorganic phosphate. Organic phosphate was taken as the difference between the two measurements on a single soil sample.

The overall results for total and inorganic phosphate, for the soil samples that were subjected to both analyses, are shown in Fig. 1. Because the occupation density was markedly greater in Iron Age and Roman times than in the Neolithic, the total phosphate content of the excavated soil decreases with age, although even in the earliest Vinča–Pločnik layers it is clearly greater than that of the buried virgin soil, as indicated by the block in the lower right-hand corner of Fig. 1. (No measurements for present-day 'virgin' soil are available because the entire surrounding district is now heavily cultivated.) This variation makes it difficult to interpret changes in the relative inorganic phosphate content of soil samples of different ages. A simple mathematical model, with a straight line fitted to the total phosphate points, and assuming that the fraction of organic phosphate would be the same no matter when the soil was deposited, but that the organic fraction would be mineralized at a rate proportional to its concentration in the soil, produced the lower curve in Fig. 1. This fits the data reasonably well, but it gives a half-life for the organic phosphate of ~7,000 yr, with a decay constant that cannot be statistically distinguished from zero. This implies an infinite life for the organic phosphate fraction. Sections of the complete data were therefore examined in more detail (Figs 2, 3).

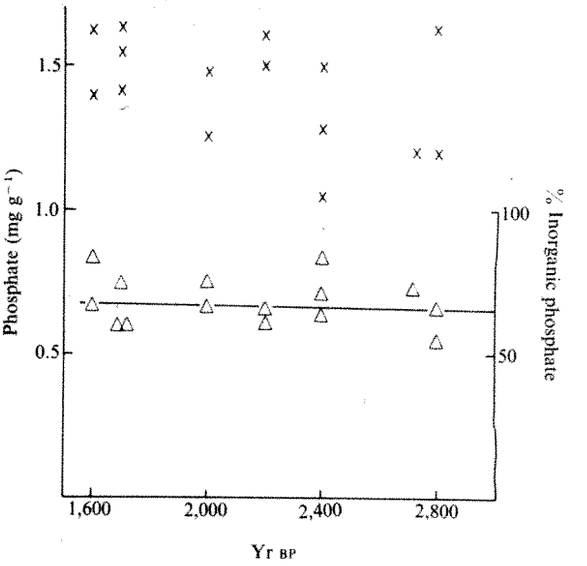


Fig. 2 Total phosphate (×) and inorganic phosphate as per cent of the total (△) over the period 1,600–2,800 yr BP at tell Gomolava. The line is the regresssion of the percentage points with time. It is not significantly different from zero, and the intercept lies at 66% (=34% organic phosphate).

Figure 2 shows the results for the period 1,600–2,800 yr BP, during which time the total phosphate content of the samples was reasonably constant, averaging 1.3 mg P per g dry soil. It can be seen that over this 1,200-yr period there is no evidence for mineralization. The inorganic phosphate content if anything decreased slightly with increasing age of sample. The same picture emerges from Fig. 3, which covers the period from 3,450 to 5,200 yr BP. (Allocation of the data to Figs 2 and 3 was dictated primarily by the lower total phosphate content of the samples shown in Fig. 3, averaging 0.9 mg P per g, but it corresponds also to a change in the nature of the settlements on the site, and of the palaeobotanical evidence, which is discussed in detail below.)

Consideration of Figs 2 and 3 suggests that over a total period of almost 4,000 yr, there is no evidence for conversion of the remanent organic phosphate into inorganic phosphate. It is however remarkable that the average organic phosphate content of the soils dated between 1,600 and 2,800 yr (Fig. 2) is about 34%, while that of the older samples (Fig. 3) is only about 12%. The difference is very consistent, and suggests that the two sets of soil samples had somewhat different histories, almost certainly before they were buried. Possible reasons for the difference are now discussed.

The transition point corresponds to the archaeological transition between Eneolithic and Copper Age cultures on the site (for example Baden and Kostalač) to full Bronze Age cultures (Vučedol, followed later by the Iron Age, Hallstatt and later cultures). It also corresponds almost exactly with a massive increase in the number of cereal seeds (mostly millet, einkorn and barley[9]) obtained from the sondage layers by flotation. It seems likely from the two pieces of evidence that there was a change from a subsistence still partly supplemented by hunting and fishing to one overwhelmingly based on cereal agriculture. A high proportion of the seeds found in the upper layers were charred, and thus brittle, so that one may realistically assume that the deposited soil will, from 3,000 yr BP onwards, contain a greater proportion of abraded grain than in earlier levels, whether the abrasion was caused by treading underfoot or by grinding into flour. The major organic phosphate compounds present in seeds, especially cereal seeds, are *myo*-inositol polyphosphates, principally phytic acid[7]. It is generally agreed that this class of compound is very stable in natural soil; it is thus reasonable to suppose that an important component of the organic phosphate fraction of the soil samples from the period

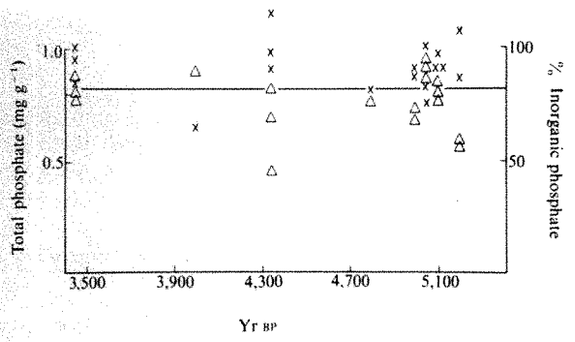


Fig. 3 Total phosphate (×) and inorganic phosphate as per cent of the total (△) over the period 3,400–5,600 yr BP at tell Gomolava. The line has the same significance as in Fig. 2, and the intercept lies at 88% (equivalent to 12% organic phosphate).

1,600–2,800 BP at tell Gomolava is comprised of inositol polyphosphates. As Fig. 2 provides no evidence of mineralization, it may be presumed that inositol polyphosphates are indeed completely resistant to biological attack in the conditions in which they were preserved. They would have been buried rather quickly, by collapse of mudbrick walls and the like[11], under as much as 30 cm of soil, on which a new phase of the settlement was then established. If confirmation can be obtained by direct analysis phytate would become an important addition to the list of resistant organic compounds whose identification can be of assistance to archaeologists and palaeoecologists.

In discussing the possible reasons for the lower proportion of organic phosphate found in the earlier buried soils, the results shown in Fig. 3, conclusions must be more tentative, because the argument has to be by analogy. There is no way of establishing what was the organic phosphate content of those soils when they were buried. One can only say that it must have been much higher in absolute amount than that of the buried virgin soil, because the organic phosphate content of the former is even now as great as the total phosphate of the latter. If the original organic phosphate of the soils buried earlier than ~3,000 yr BP did not come predominantly from cereal grains, it must nevertheless in some way have been synthesized. Almost certainly much of it was synthesized from the phosphate, largely inorganic, added to the soil by human and animal excretion. Except in the very earliest habitation levels on the site, the total phosphate of the soil was sixfold greater than that of the virgin soil. The soil phosphate cycle is sufficiently well understood[10] for it to be extremely probable that the organic components were synthesized as the result of a symbiosis between plant growth and soil microbial activity. If this reasoning is correct, the original organic phosphate may well have been considerably higher than 12% of the total, with fairly rapid mineralization of much of it. Gardiner and Walsh[5] concluded that this had been the case, both in the soil that they studied, and in the English buried soil[4].

This interpretation of events is of interest because it implies that until the middle of the fourth millennium the site was covered, at least in part, by vegetation. This idea is attractive for the following reasons. The density of settlement was much less in Neolithic times than later, and it seems unlikely that the whole tell surface, originally some 2–3 hectares in extent, was continuously occupied. It has been suggested that parts of the tell were in Neolithic times covered by local vegetation for quite long periods[9]. It is even possible that some cereal culture was carried out on the tell itself, as it was originally surrounded by extensive marshes bordering the Sava. The composition of the buried virgin soil, and of the clay exposed on the river bank, suggests that phosphate may have been rate-limiting for growth, so that vegetation would have grown rapidly when the tell surface received deposits of phosphate and also of nitrogen, from the settlers and their domestic animals.

This analysis of the results therefore suggests two phases in the development of the tell. In an earlier phase the tell resembled an island slightly raised above the surrounding marsh, and patchily settled by people who raised the fertility of the soil but did not prevent it from metabolizing phosphates in a way characteristic of normal soils. This was followed by a later phase in which settlement was much more intense, so that vegetation was necessarily much sparser. In addition, it seems possible that in the later phase, pre-formed organic phosphates (probably phytate) may have been deposited on the transient surface by changes in dietary and agricultural habits, but were not incorporated into the soil structure. This analysis agrees closely with what has already been deduced about the history of the tell from geological and palaeoecological observations[9,12]. The transition between the occurrence of shade-loving and open-country snails at the end of the Neolithic[9] is particularly significant in this regard.

It may be felt that this interpretation of the analytical results depends very heavily on inference and analogy. It is, however, susceptible to test in the following way. Although the reasons are not understood, it is known that the inositol in phytic acid is entirely the *myo*-isomer[7], while that in the inositol polyphosphates extracted from a variety of natural soils contains a mixture of the *myo*-, *chiro*-, *scyllo*- and *neo*-isomers of inositol[10,13]. Thus extraction of the inositol polyphosphates from earlier and later soil samples from tell Gomolava, followed by identification of the isomers, should make it possible to demonstrate whether the inositol in the later soils came predominantly from cereal grains, while that in the earlier soils has the isomeric composition characteristic of a stable natural soil, even though it is now part of an artificial mound. A programme of research to this end has been initiated in this laboratory.

Received 11 July; accepted 21 October 1983.

1. Ottaway, B. S. & Ottaway, J. H. *Proc. 22nd Symp. Archaeometry*, 28–37 (Bradford University Press, 1983).
2. Cook, S. F. & Heizer, R. F. *Univ. Calif. Publ. Anthrop.* **2** (1965).
3. Schwarz, G. T. *Archaeometry* **10**, 57–63 (1967).
4. Mattingley, G. E. G. & Williams, R. J. B. *J. Soil Sci.* **13**, 254–258 (1962).
5. Gardiner, M. J. & Walsh, T. *Proc. R. Irish Acad.* **65**(C), 29–35 (1966).
6. Koltun, V. D. & Bessonova, A. S. *Plodorodie i Obrab. Pochvy v Sevoobrorotakh, Kishinev*, 99–103 (1980).
7. Cosgrove, D. J. *Rev. pure appl. Chem.* **16**, 209–224 (1966).
8. Brukner, B. *Actes du VIIe Congrès Int. des Sci. Préhist. et Protohist.* **2**, 317–324 (1973).
9. Bottema, S. & Ottaway, B. S. *J. archaeol. Sci.* **9**, 221–246 (1982).
10. Cosgrove, D. J. *Adv. microbial Ecol.* **1**, 95–134 (1977).
11. Davidson, D. A. in *Geoarchaeology* (eds Davidson, D. A. & Strackley, M. L.) 255–266 (Duckworth, London, 1976).
12. Van Zeist, W. *J. archaeol. Sci.* **2**, 315–325 (1975).
13. McKercher, R. B. & Anderson, G. *J. Soil Sci.* **19**, 302–310 (1968).

Presence of NADPH-cytochrome P450 reductase in central catecholaminergic neurones

Lena Haglund*, Christer Köhler†, Tapio Haaparanta*, Menek Goldstein‡ & Jan-Åke Gustafsson*

* Department of Medical Nutrition, Karolinska Institutet, Huddinge University Hospital F69, S-141 86 Huddinge, Sweden
† Department of Pharmacology, Astra Research Laboratories, Astra, S-15185 Södertälje, Sweden
‡ Department of Neurochemistry, New York University Medical Center, New York, New York 10003, USA

The cytochrome P450-containing mixed function oxidases metabolize a variety of endogenous and exogenous compounds including drugs, carcinogens, fatty acids and steroids[1]. Mixed function oxidases have been detected in several tissues[2], including brain[3]. The enzyme system consists of a lipid fraction (phosphatidylcholine), cytochrome P450 and NADPH-cytochrome P450 reductase[4]. NADPH-cytochrome P450 reductase has been purified to apparent homogeneity and demonstrated to supply reducing equivalents from NADPH to cytochrome P450 (refs 5–7). Detection of NADPH-cytochrome P450 reductase thus represents an indirect means of demonstrating the presence

© 1984 Macmillan Journals Ltd

of cytochrome P450. Although the role of cytochrome P450 in the central nervous system (CNS) is not known, it may include such different functions as metabolism of xenobiotics[8], aromatization of androgens to oestrogens[9] and the formation of catecholoestrogens[10]. Despite the potentially very important role(s) of cytochrome P450 in brain function, its exact regional distribution remains essentially unknown. Using a specific antibody against rat liver NADPH-cytochrome P450 reductase in combination with immunohistochemical techniques, we have now localized this enzyme to defined catecholamine (CA)-containing structures of the rat and monkey brain.

NADPH-cytochrome P450 reductase purified from rat liver microsomes according to the method of Guengerich and Martin[11] with minor modifications[12], was >95% pure as judged by SDS-polyacrylamide gel electrophoresis[13]. Protein concentrations were determined by the method of Lowry *et al.*[14]. Antibodies to the enzyme were raised in rabbits and IgG fractions were isolated from the serum by affinity chromatography using a protein A–Sepharose CL-4B column[15], then concentrated to contain 25 mg protein ml^{-1}. IgG was isolated in a similar way from preimmune serum. The IgG fractions were dialysed against phosphate-buffered saline (PBS; 10 mM potassium phosphate buffer *p*H 7.4, containing 0.15 M NaCl). The antibodies were specific for the antigen as judged by the following criteria[12]: inhibition of NADPH-cytochrome *c* reductase activity of the purified antigen and in rat liver microsomes; Ouchterlony immunodiffusion; and immunoblotting[16] (described in Fig. 3 legend).

For the immunohistochemical studies, 15 rats (Sprague–Dawley, 180 g; Anticimex, Stockholm) of both sexes and two female monkeys (Talapoin, 1 kg) were perfused with 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (*p*H 7.4), their brains removed and cut in a cryostat (at 10 μm thickness) or on a Vibratome (at 70 μm). The sections were incubated with anti-NADPH-cytochrome P450 reductase antibody (diluted 1:300 to 1:1,000 in PBS containing 0.3% Triton X-100) for 1–6 days and the antibody–antigen complex visualized using either the immunofluorescence method of Coons[17] or the avidin–biotin complex method of Hsu *et al.*[18]. In a separate study of the possible coexistence of NADPH-cytochrome P450 reductase and tyrosine hydroxylase (TH) within the same neurone, sections were incubated with either antibody and reacted with 4-chloronaphthol according to the methods of Nakane[19], Gu *et al.*[20] and Tramu *et al.*[21] (Fig. 2).

Neurones containing a strong NADPH-cytochrome P450 reductase-like immunoreactivity are present within neuronal cell bodies of the following brain areas: the substantia nigra (SN), the ventral tegmental area of Tsai, the rostral part of dorsal raphe, the nucleus locus coeruleus and the ventrolateral medulla region of both rats and monkeys. NADPH-cytochrome P450 reductase-positive neurones are present also in the periventricular nucleus of the hypothalamus, the nucleus arcuatus (including the tanycytes of this nucleus) and the medial aspect of the zona incerta (Fig. 1).

All the brain areas listed above are known to harbour neurones containing the catecholamines dopamine (DA), noradrenaline (NA) or adrenaline (A)[22].

As the catecholamines are dependent on TH for their synthesis[23], alternating sections were incubated with a well characterized anti-TH antibody[24,25]. Analysis of serial sections revealed an overlapping distribution of cell bodies containing TH- and NADPH-cytochrome P450 reductase-like immunoreactivity in the mes- and diencephalon and nerve terminals in the following forebrain regions: the striatum, nucleus accum-

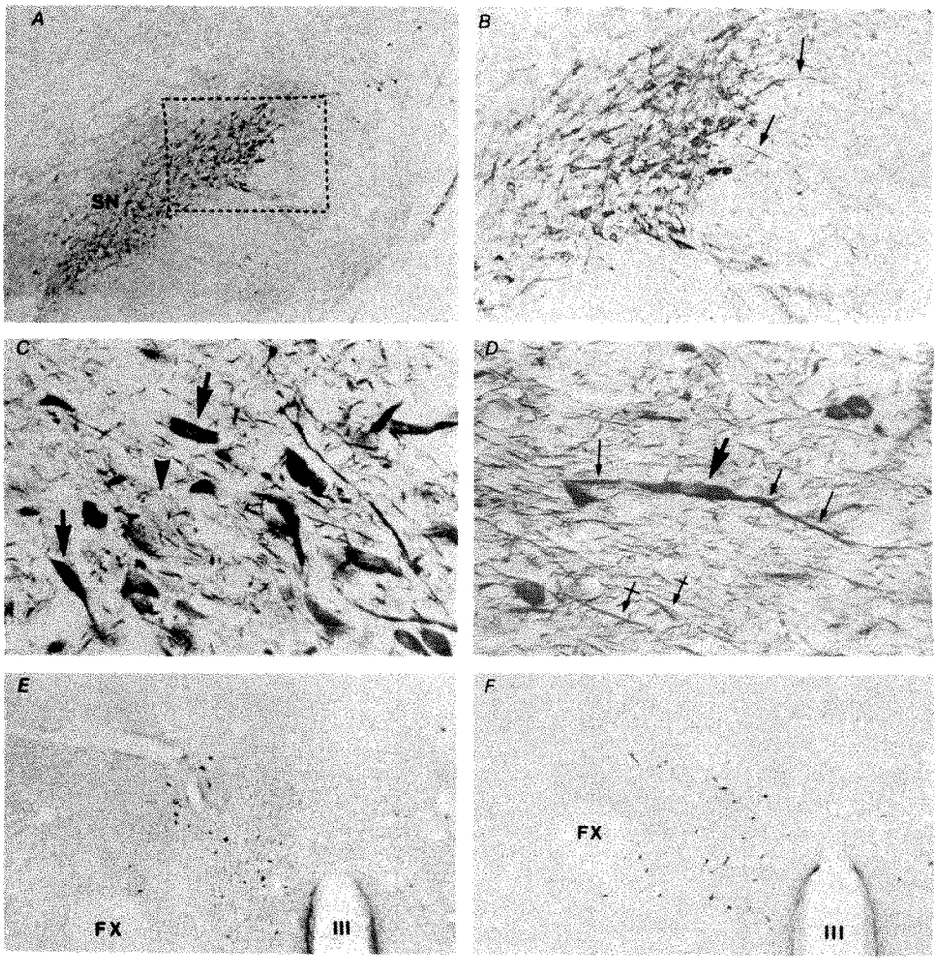


Fig. 1 *A–F*, Photomicrographs showing neurones in the substantia nigra (*A–D*) and hypothalamus (*E, F*) containing strong NADPH-cytochrome P450 reductase-like immunoreactivity. A large number of NADPH-cytochrome P450 reductase-positive cell bodies are present throughout the SN of the rat (*A*). The photomicrograph in *B* is an enlarged view of the enclosed area in *A*; arrows mark dendrites containing immunoreactivity. *C* And *D* show NADPH-cytochrome P450 reductase-positive neurones in pars compacta (arrows in *C*) and reticulata (large arrow in *D*) of the monkey SN. Small arrows in *D* indicate dendrites, and crossed arrows indicate dendrites of pars compacta cells that extend into reticulata. The substantia nigra of both rats and monkeys contains numerous cells lacking NADPH-cytochrome P450 reductase-like immunoreactivity (arrowhead in *C*). Incubations of sections in preimmune IgG or anti-NADPH-cytochrome P450 reductase IgG preabsorbed with the pure antigen (100 μg per 1,000 μl diluted antibody at 4 °C for 24 h) resulted in no staining in any of the brain regions examined. *E* And *F* show NADPH-cytochrome P450 reductase-positive neurones in the hypothalamus after incubation with the antibody alone (*E*) or antibody preabsorbed with pure TH (150 μg per 1,000 μl diluted antibody at 4 °C for 24 h (*F*). Note that preabsorption with TH did not block the staining of neurones with NADPH-cytochrome P450 reductase antibody. The peroxidase–anti-peroxidase method was used. Nomarski phase interference contrast for *A–D*. *A*, *E*, *F*, ×56; *B*, ×230; *C*, *D*, ×360. FX, fornix; III, third ventricle.

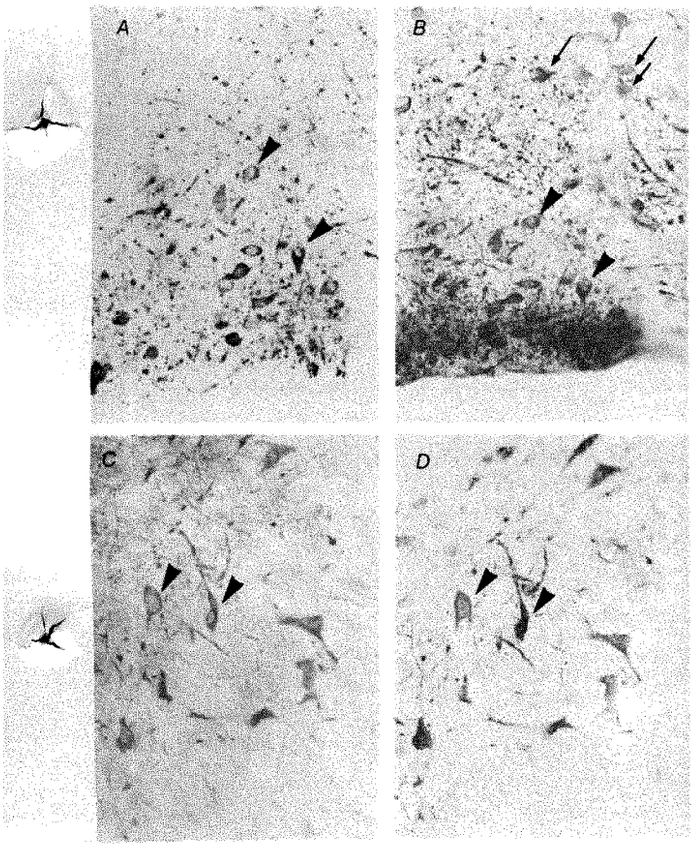


Fig. 2 (*A–D*), Photomicrographs of neurones in the ventral tegmental area of Tsai (*A*, *B*) and the pars reticulata of the substantia nigra (*C*, *D*) in rat, demonstrating the simultaneous localization of TH (*A*, *C*) and NADPH-cytochrome P450 reductase-like immunoreactivity (*B*, *D*) within the same neurones.

Methods: Vibratome sections (70 μm) were incubated for 7 days in anti-TH antibody (diluted 1:2,000) and the antibody–antigen complex visualized using the avidin-biotin complex method with 4-chloronaphthol as a chromogen. The stained sections were coverslipped in PBS and glycerol (1:3) and the areas containing TH-like immunoreactivity photographed. The sections were subsequently decolourized in alcohol, eluted according to the method of Tramu *et al.*[21] and re-incubated in NADPH-cytochrome P450 reductase IgG (diluted 1:2000) for 5 days. The sections were reacted with 3′, 3′-diaminobenzidine and H_2O_2 to produce a brown reaction product. The stained sections were defatted, coverslipped in Pertex, re-photographed and analysed for the presence of double-labelled cells. Large arrowheads indicate cells containing both antigens. Small arrows in *B* mark cells containing NADPH-cytochrome P450 reductase but lacking TH-like immunoreactivity. Nomarski optics. ×500.

bens, olfactory tubercle and the septum. Lesions of the DA-containing axons ascending from the SN to the striatum in the lateral hypothalamus by infusions of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA, 8 μg per 4 μl; survival time of 10 days) resulted in a loss of NADPH-cytochrome P450 reductase and TH-like immunoreactivity from the striatum, accompanied by an increased intensity of immunoreactivity in the cell bodies of the SN ipsilateral to the lesion. These experiments suggest a coexistence of NADPH-cytochrome P450 reductase and TH in dopaminergic neurones of the SN. In separate experiments where the anti-NADPH-cytochrome P450 reductase antibody was preabsorbed with pure TH before incubation of the sections, staining of neurones in the SN and hypothalamus occurred to the same extent as after incubations with non-absorbed antiserum. Taken together, these findings show that the anti-NADPH-cytochrome P450 reductase antibody does not cross-react with TH and suggest that NADPH-cytochrome P450 reductase and TH occur in the same population of neurones. This hypothesis was confirmed by direct double

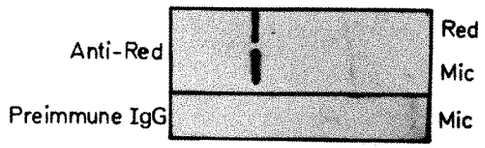


Fig. 3 Autoradiogram of an immunoblot showing the presence of NADPH-cytochrome P450 reductase (Red) in a microsomal fraction isolated from rat brain. Microsomes were isolated from the substantia nigra, ventral tegmental area of Tsai, striatum, nucleus accumbens and olfactory tubercle. Microsomes were prepared according to the procedure described by Holtzman and Desautel[31]. Immunoblotting was performed by the method of Towbin *et al.*[16] with minor modifications[12]. SDS-polyacrylamide gel electrophoresis[13] was performed using 8% acrylamide and 2.7 mm-thick slab gels. Proteins were transferred to nitrocellulose sheets which were then incubated for 2 h with antibody (or preimmune IgG) against rat liver NADPH-cytochrome P450 reductase (IgG fraction). The immunoreactive bands were visualized on the nitrocellulose filters using ^{125}I-labelled protein A followed by autoradiography. In the above experiment 0.1 μg of purified NADPH-cytochrome P450 reductase and 42 μg of brain microsomal protein (Mic) were run on the gel. The top part of the nitrocellulose filter was incubated with anti-NADPH-cytochrome P450 reductase IgG whereas the lower part was incubated with preimmune IgG. NADPH-cytochrome P450 reductase having a molecular weight similar to purified rat liver NADPH-cytochrome P450 reductase was detected in brain microsomes.

labelling experiments in which the same neurone in the SN (and hypothalamus) was found to contain both NADPH-cytochrome P450 reductase and TH-like immunoreactivity. A closer inspection revealed that while most, if not all, TH-positive cells contained NADPH-cytochrome P450 reductase, many reductase-positive neurones contained no TH immunoreactivity (Fig. 2).

The present study represents the first histochemical demonstration of NADPH-cytochrome P450 reductase in brain. The presence of NADPH-cytochrome P450 reductase-like immunoreactivity in central neurones supports earlier biochemical findings of this enzyme in brain[26]. Our study further extends the biochemical findings by identifying those neurones in the rat and monkey brain that contain the NADPH-cytochrome P450 reductase and thus possibly also cytochrome P450. It seems unlikely that the presence of NADPH-cytochrome P450 reductase and TH-like immunoreactivity in the same cell is due to cross-reactivity of the antibodies as preabsorption of anti-NADPH-cytochrome P450 reductase IgG with excess pure TH (and, conversely, anti-TH antiserum with excess pure NADPH-cytochrome P450 reductase) failed to block the subsequent immunostaining of central neurones.

Figure 3 shows that a protein having the same apparent molecular weight as the purified rat liver NADPH-cytochrome P450 reductase was found in brain microsomes. The part of the immunoblot which was incubated with preimmune IgG showed no reaction. This experiment supports the immunohistochemical data indicating the presence of NADPH-cytochrome P450 in the above-mentioned areas of the brain.

The presence of NADPH-cytochrome P450 reductase immunoreactivity in TH-positive cells probably indicates the existence of a cytochrome P450 system within these neurones. This is of particular interest as cytochrome P450 has been implicated in the formation of catecholoestrogens. The conversion of oestradiol and oestrone to catecholoestrogens has been demonstrated in the CNS of rat[27] and it is probable that the enzyme responsible for this conversion in the brain is similar to the cytochrome P450 found in peripheral tissue[10]. The action of the catecholoestrogens in the CNS has been linked to their interaction with biogenic amines as competitive inhibitors of catechol-*O*-methyltransferase (COMT)[28] and as possible inhibitors of TH[29] although it could also imply that catecholamines are being synthesized through an alternative pathway involving cytochrome P450.

Although the presence of NADPH-cytochrome P450 reductase in central catecholaminergic neurones strongly indicates

the existence of cytochrome P450 within these neurones, it cannot be excluded that this enzyme may have functions not directly linked to cytochrome P450. This could involve the donation of electrons from NADPH to cytochrome b_5—another electron transfer component identified in brain microsomes[30]. It has also been proposed[26] that NADPH-cytochrome P450 reductase could be involved in the formation of the catecholamine neurotoxin 6-OHDA. It may be speculated that the presence of large amounts of NADPH-cytochrome P450 reductase in central dopaminergic neurones may be of relevance for the formation of endogenous 6-OHDA in the development of neurodegenerative disorders such as Parkinson's disease.

We thank Dr James Halpert for providing the purified NADPH-cytochrome P450 reductase. This study was supported by grants from the Swedish MRC (13X-2819).

Received 27 July; accepted 24 October 1983.

1. Conney, A. H. *Cancer Res.* **42,** 4875–4917 (1982).
2. Burke, M. D. & Orrenius, S. in *Characteristics of the Mammalian Hepatic Mixed Function Oxidase. International Encyclopedia of Pharmacology and Therapeutics* (eds Schenkman, J. B. & Kupfer, D.) 549–599 (Pergamon, Oxford, 1979).
3. Cohn, J. A., Alvares, A. P. & Kappas, A. *J. exp. Med.* **145,** 1607–1611 (1977).
4. Lu, A. Y. H., Junk, K. W. & Coon, M. J. *J. biol. Chem.* **244,** 3714–3721 (1969).
5. Dignam, J. D. & Strobel, H. W. *Biochemistry* **16,** 1116–1123 (1977).
6. Yasukochi, Y. & Masters, B. S. S. *J. biol. Chem.* **251,** 5337–5344 (1976).
7. Vermilion, J. L. & Coon, M. J. *J. biol. Chem.* **253,** 2694–2704 (1978).
8. Das, M., Seth, P. K. & Mukhtar, H. *J. Pharmac. exp. Ther.* **216,** 156–161 (1981).
9. Reddy, V. V., Naftolin, F. & Ryan, K. J. *Endocrinology* **91,** 117–121 (1974).
10. Paul, S. M., Axelrod, J. & Diliberto, E. J. Jr. *Endocrinology* **101,** 1604–1610 (1977).
11. Guengerich, F. P. & Martin, M. V. *Arch. Biochem. Biophys.* **205,** 365–379 (1980).
12. Haaparanta, T., Halpert, J., Glaumann, H. & Gustafsson, J.-Å. *Cancer Res.* **43,** 5131–5137 (1983).
13. Laemmli, U. K. *Nature* **227,** 680–685 (1970).
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193,** 265–275 (1951).
15. Goding, J. W. *J. immun. Meth.* **13,** 215–226 (1976).
16. Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76,** 4350–4354 (1979).
17. Coons, A. H. in *General Cytochemical Methods* (ed. Danielli, J. F.) 399–422 (Academic, New York, 1958).
18. Hsu, S.-M., Raine, L. & Fanger, H. *J. Histochem. Cytochem.* **29,** 577–580 (1981).
19. Nakane, P. K. *J. Histochem. Cytochem.* **16,** 557–560 (1968).
20. Gu, J., de Mey, J., Moeremans, M. & Polak, J. M. *Regul. Peptides* **1,** 365–374 (1981).
21. Tramu, G., Pillex, A. & Leonardelli, J. *J. Histochem. Cytochem.* **26,** 322–324 (1978).
22. Dahlström, A. & Fuxe, K. *Acta physiol. scand.* **62,** Suppl. 232, 1–55 (1964).
23. Patrick, R. L. in *Neuroregulators and Psychiatric Disorders* (eds Usdin, E. *et al.*) 88–94 (Oxford University Press, New York, 1976).
24. Markey, K. A., Kondo, H., Shenkman, L. & Goldstein, M. *Molec. Pharmac.* **17,** 79–85 (1980).
25. Hökfelt, T. *et al. Med. Biol.* **54,** 427–453 (1976).
26. Sasame, H. A., Ames, M. M. & Nelson, S. D. *Biochem. biophys. Res. Commun.* **78,** 919–926 (1977).
27. Fishman, J. & Norton, B. *Endocrinology* **96,** 1054–1058 (1975).
28. Ball, P. *et al. J. clin. Endocr. Metab.* **34,** 736–746 (1972).
29. Lloyd, T. & Weisz, J. *J. biol. Chem.* **253,** 4841–4843 (1978).
30. Inouye, A. & Shinagawa, Y. *J. Neurochem.* **12,** 803–813 (1965).
31. Holtzman, D. & Desautel, M. *J. Neurochem.* **14,** 1535–1537 (1980).

Effect of impulse blockage on cytochrome oxidase activity in monkey visual system

M. Wong-Riley & E. W. Carroll

Department of Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, USA

Cytochrome oxidase (cytochrome *c* oxidase; ferrocytochrome *c*: oxygen oxidoreductase, EC 1.9.2.1) has been introduced as an oxidative metabolic marker for neurones in the central nervous system[1]. Previous studies have shown that mature neurones remained sensitive to altered functional demands[2–9], and that both developing and adult neurones responded to sensory deprivation or deafferentation by reducing their cytochrome oxidase (Cyt.Ox.) activity[1,3,4,9–11]. More recently, we showed that the blockage of retinal impulse transmission with tetrodotoxin led to a reversible reduction in Cyt.Ox. staining of affected lateral geniculate (LGN) and striate neurones in adult cats[9]. The present study sought to extend these findings to adult monkeys, where Cyt.Ox. 'puffs' or 'blobs' are uniquely present in the visual cortex[10,12–14]. We found that, while the retina remained histologically intact, with only moderate decreases in Cyt.Ox. staining of large ganglion cells and the two plexiform layers, subtle changes occurred in the LGN as early as 1 day post-tetrodotoxin injection, and clear reduction in enzyme levels was evident in both the LGN and the visual cortex by 3 days. Changes became progressively more severe up to 4 weeks post-injection. Within area 17, alternating bands of high and low Cyt.Ox. staining occurred in lamina IV, with alternating rows of dark and lightly reactive puffs superimposed in exact register. Thus, the mature visual neurones in the primate remain extremely sensitive to the cessation of retinal impulse transmission, and plastic metabolic changes occur through several synapses along the sensory pathway.

Seven adult monkeys (*Macaca mulatta*) were used; six were experimental and one, plus a normal *Macaca fascicularis*, served as controls. The experimental monkeys were all anaesthetized with ketamine-HCl (15–30 mg per kg intramuscularly), and each received a unilateral intravitreal injection of tetrodotoxin (TTX, 19 μg in 10 μl of sterile water, a dosage found not to block axoplasmic transport)[9]. The pupillary light reflex was again used as a convenient indicator of the impulse blockage effect of TTX which, in the macaque, lasted for at least 4 days per injection. Injections were repeated twice weekly where applicable, and survivals were 1, 3, 6, 14 and 28 days. The animals were then perfused and all eyes and brains were frozen sectioned and processed for Cyt.Ox. histochemistry[1].

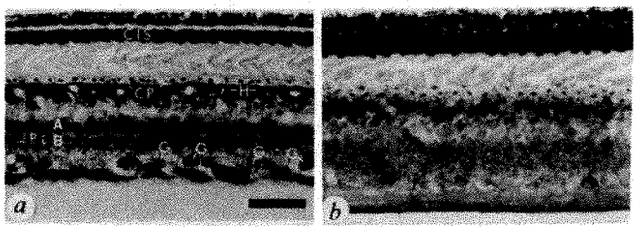


Fig. 1 Transverse sections through the normal (*a*) and TTX-injected (for 2 weeks) (*b*) retinae of a macaque. Sections have been reacted for cytochrome oxidase without counterstaining. In the normal retina, note the dense staining of cone inner segments (CIS), cone pedicles (CP), horizontal cells (H) and scattered large ganglion cells (G, arrows). The moderately stained inner plexiform layer (IPL) is subdivided into A and B, with B being slightly more reactive than A and exhibiting two thin bands of reactivity. The TTX-treated retina shows slightly decreased staining of the outer and inner plexiform layers and of the large ganglion cells. Scale bar, 50 μm.

The normal retinae of monkeys (as well as those of cats, ferrets and other mammals[15,16]) exhibited a distinct pattern of Cyt.Ox staining (Fig. 1*a*). From the photoreceptor layer inward, we found a highly reactive band of cone inner segments, a row of reactive cone pedicles and a row of reactive horizontal cell bodies with some moderately reactive bipolar cells. The inner plexiform layer contained a moderately reactive sublamina A and a slightly more reactive sublamina B, which included two thin reactive bands separated by a relatively clear zone. Finally, the large ganglion cells tended to be the most reactive, and they were scattered amongst slightly less reactive medium and small ganglion cells. The ganglion cell axons were reactive until they formed the optic nerve bundle, at which point they acquired myelin and lost their intense Cyt.Ox. activity.

In the normal macaque LGN, the two magnocellular layers and lamina 6 showed the highest level of Cyt.Ox. activity for both neurones and neuropil, with lamina 5 being moderately reactive and laminae 3 and 4 the least reactive (Fig. 2*a*) (see also ref. 15). Due to the greater cellular density in lamina 1, it appeared slightly more reactive than lamina 2. The S layer and the interlaminar zones contained relatively non-reactive neuropil and small neurones with occasional moderately reactive medium-sized neurones. In addition, all of the major cellular layers along the medial and lateral boundaries of the LGN, representing more peripheral portions of the visual field[17],

0028-0836/84/030262—03$01.00

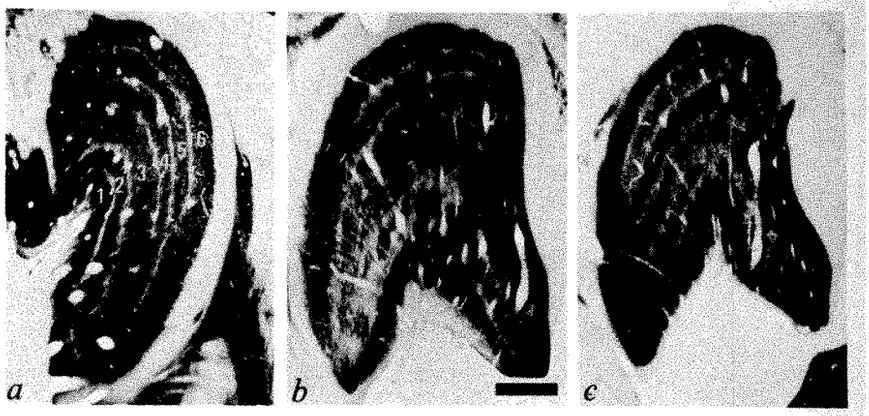


Fig. 2 Transverse sections through the macaque LGN reacted for Cyt.Ox. In the normal LGN (*a*), magnocellular laminae 1 and 2 and parvocellular lamina 6 are the most reactive. Lamina 5 is moderately reactive, while laminae 3 and 4 are the least reactive. After TTX injections, laminae 1, 4 and 6 that are contralateral, and 2, 3 and 5 that are ipsilateral to the injected eye exhibit decreased Cyt.Ox. activity. This pattern, shown here as ipsilateral to the injected eyes, can be seen by 3 days post-injection (*b*), but becomes more severe by week 4 (*c*). Scale bar, 1 mm.

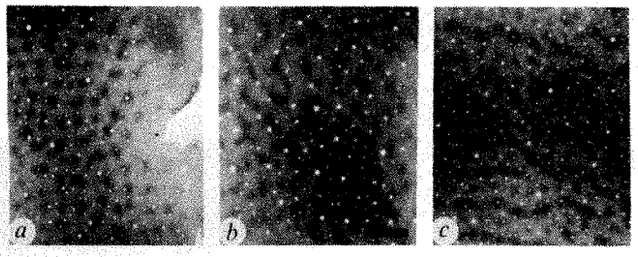


Fig. 3 Tangential sections through Cyt.Ox.-rich puffs in laminae II–III of macaque striate cortex reacted for Cyt.Ox. *a* Shows the normal distribution (see text). After 3 days of TTX treatment (*b*), a slight decrease in enzyme staining can be seen in some of the puffs which have been traced to be in register with lightly reactive bands in lamina IVC. After 4 weeks of TTX treatment (C), distinct rows of lightly reactive and shrunken puffs can be seen alternating with rows of darkly reactive puffs. These rows have been traced to line up in exact register with lightly reactive and darkly reactive bands respectively in lamina IVC (see Fig. 5). Scale bar, 1 mm.

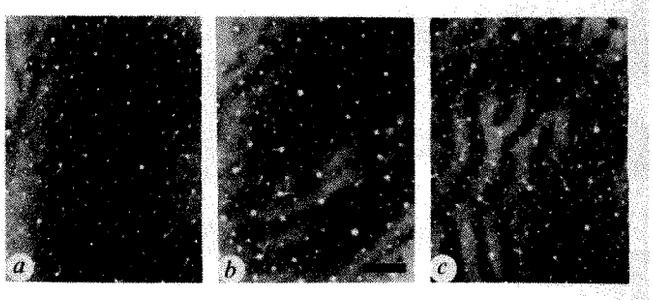


Fig. 4 Tangential sections through lamina IVC of macaque striate cortex reacted for Cyt.Ox. *a* Shows a homogeneous distribution of enzyme activity in the normal monkey. After 3 days of TTX injections (*b*), alternating bands of high and low Cyt.Ox. activity can be seen in IVC. This pattern becomes more distinct after 4 weeks of TTX injections (*c*). Scale bar, 1 mm.

exhibited a higher level of Cyt.Ox. activity than the middle portion of the nucleus.

The pattern of Cyt.Ox. staining in the normal striate cortex of the macaque was similar to that reported previously[10,18–20]. In the supragranular layers, the Cyt.Ox.-rich zones or puffs[13,14] were globular in shape, measuring ~262 μm × 377 μm (Fig. 3*a*). The puffs lined up in rows roughly orthogonal to the 17/18 border. The centre-to-centre spacing of puffs within the rows was 450 μm, while those between rows was about 353 μm. Lamina IV was also reactive (Fig. 4*a*), with lamina IVA having an intermittent punctate appearance, lamina IVCα being slightly more reactive than lamina IVCβ, and the lower edge of lamina IVCβ forming a thin band of darker staining. Laminae IVB and V were relatively non-reactive, while lamina VI was again moderately reactive. Reactive neurones, mainly of the stellate type, were seen in all of the cellular laminae. Reactive pyramidal cells were found mainly in laminae II–III, V and upper VI. These findings were comparable with our results in squirrel monkeys[13,14].

The effect of TTX on the retina was relatively mild. Even after 4 weeks of injections, the retina appeared remarkably intact histologically, and the pattern of staining remained essentially the same as normal. The major change was a decrease in the level of reactivity of large ganglion cells as well as those of the outer and inner plexiform layers (Fig. 1*b*).

As early as 1 day after TTX treatment, a mild decrease in the level of Cyt.Ox. staining was seen in laminae 2, 3 and 5 of the ipsilateral LGN, while laminae 1, 4 and 6 of the contralateral LGN appeared relatively normal. By 3 days changes were seen in all of the laminae innervated by the TTX-treated eye (Fig. 2*b*) and the severity increased with longer survivals. Because laminae 1 and 6 were normally quite reactive, they were the last ones to exhibit a level of Cyt.Ox. staining that was lower than that of non-TTX-treated laminae. After 4 weeks of TTX injections, distinct differences were seen between reactive and TTX-affected laminae (Fig. 2*c*). At all survival periods, the ipsilateral laminae suffered a greater reduction of Cyt.Ox. than the contralateral ones.

TTX treatment for 1 day did not reveal significant alterations in enzyme staining or overall dimensions of laminae II–III puffs in the striate cortex. Changes in lamina IV were subtle, with minor fluctuations in staining within lamina IVC. By 3 days, however, alternating bands of high and low Cyt.Ox activity were clearly visible within lamina IVC (Fig. 4*b*). The puffs exhibited some disarray, with slightly shrunken, paler puffs scattered amidst darkly reactive ones (Fig. 3*b*). Serial tracings of tangential sections (see Fig. 5 legend) confirmed that rows of reactive puffs stayed within the confines of reactive bands in lamina IVC, while rows of light puffs fell within those of light bands in lamina IVC. This pattern of alternating dark and light puffs/bands became more prominent with longer survivals up to 4 weeks (Figs 3*c*, 4*c*, 5). Moreover, light puffs appeared progressively smaller. In register with the pattern in the upper layers, laminae V and especially lamina VI showed alternating bands of dark and light Cyt.Ox reactivity. At each survival time, the overall dimensions of reactive puffs were comparable with those in control monkeys. However, even at 3 day survival, there was a significant difference ($P \ll 0.001$) in cross-sectional areas between reactive and non-reactive puffs (56,372.211 μm^2 compared with 39,870.51 μm^2). By 4 weeks, this difference was even more pronounced (55,504.347 μm^2 and 17,859.168 μm^2, $P \ll 0.001$). On the other hand, the centre-to-centre spacing between reactive puffs of the same row, between non-reactive puffs of the same row, and between puffs of adjacent rows remained essentially the same as the control.

The possibility that these transneuronal and trans-trans-neuronal enzymatic alterations might be due to retinal damage was deemed unlikely because: (1) All TTX-injected eyes

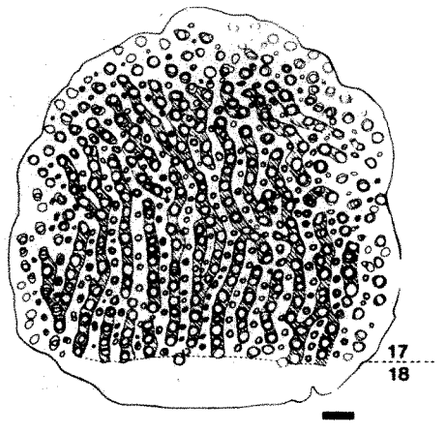


Fig. 5 Composite camera lucida tracings through nine serial tangential sections (spanning a depth of 550 μm) from area 17 of a macaque whose contralateral eye has been injected with TTX for 4 weeks. Note that the large, darkly reactive puffs lie in exact register with darkly reactive bands (hatched) in lamina IVC, while the shrunken, lightly reactive puffs are superimposed over lightly reactive bands in lamina IVC. Scale bar, 1 mm. Tracings such as this were done in the control as well as 11 of the experimental hemispheres. For each hemisphere, the areas of at least 100 puffs overlying the reactive bands, and 100 overlying the non-reactive bands in lamina IVC of each composite tracing were digitized with the aid of a computer. Statistical analyses were done for the mean areas of reactive and non-reactive puffs, and comparisons made between these two groups at different survival periods.

remained histologically intact, similar to the control; (2) the ganglion cells of the injected eyes retained their ability readily to transport tritium-labelled tracers to the LGN (our unpublished observations); (3) the observed response in the striate cortex was rapid (within 3 days and after only a single injection) and widespread rather than localized; and (4) our findings in the cat[9] and our preliminary results in the monkey indicated that these enzymatic adjustments were reversible. Moreover, histochemical changes appeared to be more dramatic in the brain itself than in the retina (see note added in proof).

The present study thus indicates that the mature primate visual system remains extremely sensitive to the cessation of retinal impulse transmission. Tetrodotoxin is known to cause altered functional properties in the developing kitten visual system[21,22], but the adult sensory system was thought to be refractory to the detrimental effect of sensory deprivation. Our present results demonstrate that, not only are changes pronounced at both the geniculate and cortical levels, but the swift onset of transneuronal and trans-transneuronal responses is much more dramatic than our earlier findings in the cat (1 week)[9], and of reported geniculate cell shrinkage and cortical enzyme changes after a more drastic procedure of enucleation (10 days)[11,23,24]. The primate visual neurones, then, respond briskly to the blockage of retinal input, even while the system remains structurally intact, and axoplasmic transport is retained. The metabolic changes do not occur uniformly throughout the LGN. Neurones which receive ipsilateral input appear to suffer the consequences slightly earlier and to a greater extent than those receiving contralateral input, as we have observed in the cat[9]. Likewise, neurones representing the central portion of the visual field seem to be more affected than the peripheral ones. Changes in cortical lamina IVC and the puffs indicate that their energy-coupled activities are very much controlled by retinal input. The dark and light bandings in lamina IVC correspond in spacing and dimensions to ocular dominance columns described by Hubel's group[25], and are the known sites for geniculocortical terminations[26–28]. The sources of input to the Cyt.Ox. puffs have been postulated to arise from the interlaminar and S layers of the LGN[29,30], although other inputs have not yet been ruled out. The histochemical 'shrinkage' of puffs suggests that, although the entire puff may suffer an overall lowered level of activity, there may be an additional tendency for the response to progress in a peripheral-to-central direction. In conclusion, we have demonstrated the dramatic and early response of mature primate neurones to the blockage of primary afferent impulses, indicating that the normal functional and enzymatic integrity of cortical neurones are very much governed by activity originating one to several synapses away.

Note added in proof: Our recent findings indicate that 2 weeks of unilateral intravitreal saline injections did not result in any histochemical changes in the visual system of the macaque.

This work was supported by NIH grant NS 18121. We thank J. Laursen and D. Sullivan for technical assistance.

Received 9 August; accepted 2 November 1983.

1. Wong-Riley, M. T. T. *Brain Res.* **171,** 11–28 (1979).
2. Merrill, E. G. & Wall, P. D. in *Neuronal Plasticity* (ed. Cotman, C. W.) 97–111 (Raven, New York, 1978).
3. Wong-Riley, M. T. T., Merzenich, M. M. & Leake, P. A. *Brain Res.* **141,** 185–192 (1978).
4. Wong-Riley, M. T. T. & Welt, C. *Proc. natn. Acad. Sci. U.S.A.* **77,** 2333–2337 (1980).
5. Frank, J. I. *Brain Res.* **186,** 458–462 (1980).
6. Merzenich, M. M. *et al. Soc. Neurosci. Abstr.* **6,** 651 (1980).
7. Robbins, N. *Trends Neurosci.* **3,** 120–122 (1980).
8. Dietrich, W. D., Durham, D., Lowry, O. H. & Woolsey, T. A. *J. Neurosci.* **1,** 929–935 (1981).
9. Wong-Riley, M. & Riley, D. A. *Brain Res.* **261,** 185–193 (1983).
10. Horton, J. C. & Hubel, D. H. *Nature* **292,** 762–764 (1981).
11. Graybiel, A. M. & Ragsdale, C. W. Jr *Nature* **299,** 439–442 (1982).
12. Humphrey, A. L. & Hendrickson, A. E. *Soc. Neurosci. Abstr.* **6,** 315 (1980).
13. Carroll, E. & Wong-Riley, M. *Soc. Neurosci. Abstr.* **8,** 706 (1982).
14. Carroll, E. W. & Wong-Riley, M. T. T. *J. comp. Neurol.* (in press).
15. Kageyama, G. H. & Wong-Riley, M. *Soc. Neurosci. Abstr.* **9,** 814 (1983).
16. Kageyama, G. H. & Wong-Riley, M. *J. Neurosci.* (submitted).
17. Polyak, S. in *The Vertebrate Visual System* (ed. Kluver, H.) 354–445 (University of Chicago Press, 1957).
18. Horton, J. C. & Hubel, D. H. *Soc. Neurosci. Abstr.* **6,** 315 (1980).
19. Tootell, R. B. H. & Silverman, M. S. *Soc. Neurosci. Abstr.* **7,** 356 (1981).
20. Hendrickson, A. E., Hunt, S. P. & Wu, J. Y. *Nature* **292,** 605–607 (1981).
21. Archer, S. M., Dubin, M. W. & Stark, L. A. *Science* **217,** 743–745 (1982).
22. Stryker, M. P. *Soc. Neurosci. Abstr.* **7,** 842 (1981).
23. Glees, P. & Le Gros Cbark, W. E. *J. Anat.* **75,** 295–308 (1941).
24. Matthews, M. R., Cowan, W. M. & Powell, T. P. S. *J. Anat.* **94,** 145–169 (1960).
25. Hubel, D. H., Wiesel, T. N. & LeVay, S. *Cold Spring Harb. Symp. quant. Biol.* **40,** 581–589 (1976).
26. Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **146,** 421–450 (1972).
27. Lund, J. S. *J. comp. Neurol.* **164,** 287–304 (1973).
28. Hendrickson, A. E., Wilson, J. R. & Ogren, M. P. *J. comp. Neurol.* **182,** 123–136 (1978).
29. Weber, J. T., Huerta, M. F., Kaas, J. H. & Harting, J. K. *J. comp. Neurol.* **213,** 135–145 (1983).
30. Fitzpatrick, D., Itoh, K. & Diamond, I. T. *J. Neurosci.* **3,** 673–702 (1983).

Community food webs have scale-invariant structure

Frédéric Briand* & Joel E. Cohen†

* University of Ottawa, Department of Biology, Ontario, Canada K1N 6N5
† The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA

We have analysed 62 community food webs drawn from published studies and have found a remarkable regularity in ecosystem structure: in biological communities, the proportions of top, intermediate and basal species are, on average, independent of the total number of species. Hence, there is a direct proportionality between the numbers of prey and predators.

The finding[1] that, in community food webs, the ratio of prey to predators is 3:4 may be challenged on two grounds: first, it is based on a relatively small set of 14 webs, and second it may indicate that taxonomists have exercised greater taxonomic refinement in classifying organisms at higher than at lower trophic levels[2].

A community food web involves the feeding, that is, trophic, relations among all organisms found in a well-defined habitat by the original investigator. Organisms are separated into 'trophic species', which may be a single biological species, or a size class or stage in the life cycle of a single biological species, or a collection of functionally or taxonomically related biological species, according to the original report. Throughout this paper a 'species' refers to a 'trophic species', not necessarily to a single

0028-0836/84/030264—04$01.00

biological species. A 'top' species is a predator that has no predator. An 'intermediate' species is a species that is both a predator and a prey. A 'basal' species is a prey that has no prey.

The community food webs analysed include 40 webs assembled and described by Briand[3]; of these, 13 are corrected and drawn from the 14 originally used by Cohen[1]. Details of the food webs not yet presented by Cohen[4] or Briand[3] will be published elsewhere (F.B., in preparation). We find that the number of prey is roughly proportional to the number of predators with a slope less than 1 (Fig. 1*a*). This is also true for webs from constant and fluctuating environments, although they are quite different in overall structure[3]. On the far right of Fig. 1*a*, four outliers emerge from the general relationship: a cluster of three constant food webs (C), all from Fryer's study[5] of littoral communities of Lake Nyasa, and one fluctuating food web (F) representing a salt meadow from New Zealand[6].

There can be no direct test of Pimm's conjecture[2] of why the slope is less than 1, without repeating the original field studies with a uniform attention to taxonomic detail. As an indirect test, we examined the ratio of prey to predators in the 62 food webs, after we had 'lumped' trophically identical species. That is, in each food web, whenever two or more species are preyed on by exactly the same set of predators, and prey upon exactly the same set of prey, we treated them as one. This procedure, which we call 'lumping', removes possible differences in the propensity to split, both among observers and among trophic levels.

Lumping moves the outliers into or much closer to the bulk of the remaining data points, for both fluctuating and constant webs (Fig. 1*b*). The correlation coefficient between numbers of predators and prey among the 43 fluctuating webs increases from 0.83 before lumping to 0.92 after, and from 0.58 to 0.64 among the 19 constant webs. In other words, eliminating predators or prey that are trophically identical tightens the relation between numbers of predators and prey.

Because individual observers tend to influence prey–predator ratios, we shall deal only with the lumped version of the webs. Because some observers contributed more than one food web to our collection, the assumption of independence that is required to justify attaching probability values to significance tests using the unlumped data is open to challenge. Though the assumption of independence is probably more acceptable with the lumped version of the webs, we shall base our statistical analysis primarily on descriptive statistics. Fortunately the patterns in the data are clear.

If a straight line, either with arbitrary intercept or through the origin, is fitted to a scatter plot of the 62 community food webs, the squared residuals increase with the number of predators. This reveals that the usual least-squares procedure, which assumes the variance of residuals constant regardless of the abscissa, is not appropriate to these data.

If a regression line is fitted through the origin on the assumption that the variance of the residuals is proportional to the number of predators, then the estimator of the slope is simply the ratio of the mean number of prey divided by the mean number of predators. Under this assumption, a straight line through the origin fitted to all 62 food webs has slope 0.8819 or approximately 0.9.

This slope is higher than the slope near 0.75 found by Cohen[1], so there appears to be some merit in Pimm's suggestion[2] that ecologists have exercised greater taxonomic refinement at high trophic levels than at low. This suggestion, however, is not quantitatively sufficient to account for the excess, that remains after lumping, in the number of predators over the number of prey.

Classical ecological theory views predators as generally limited by resources, and the diversity of predators in particular as being limited by the diversity of prey. From this perspective it would seem more natural to treat the number of prey as an independent variable and the number of predators as a dependent variable. However, when the number of predators is regressed against the number of prey, using a straight line through the origin with variance of residuals proportional to the abscissa, the standard error of estimate (with 61 degrees of freedom) increases from 3.1 to 3.5.

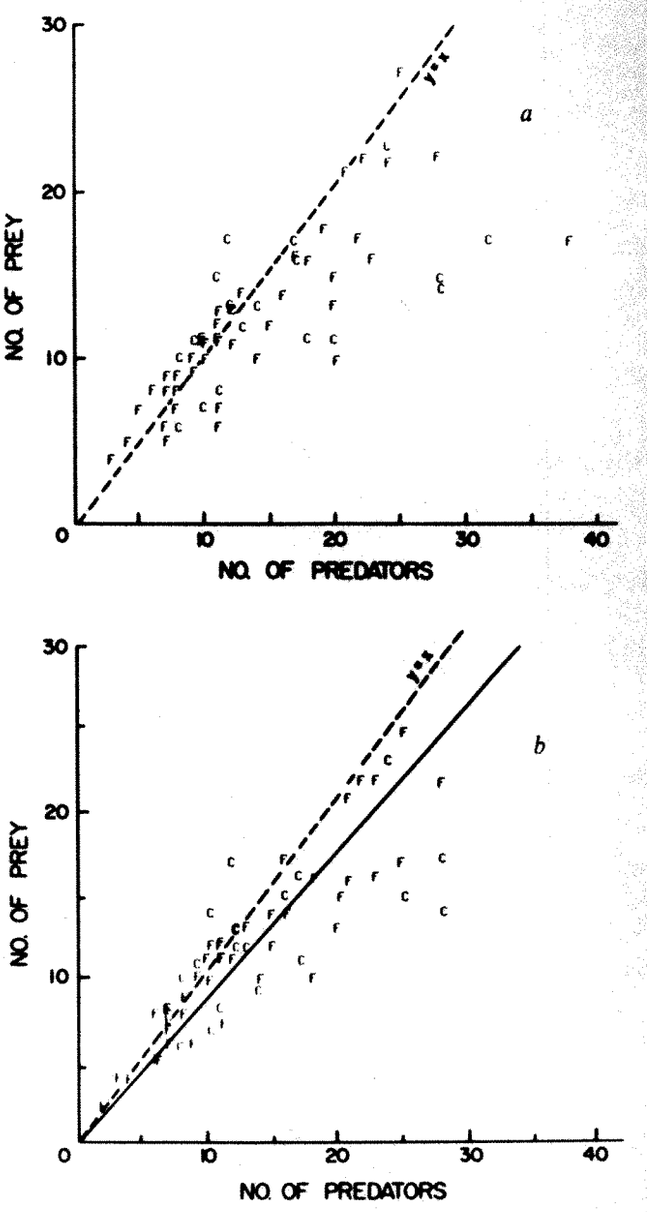


Fig. 1 Number of prey species as a function of number of predator species in 62 community food webs. F, fluctuating environment. C, constant environment. An environment is classified as 'fluctuating' if the original report indicates temporal variations of substantial magnitude in temperature, salinity, water availability, or any other major physical parameter. The magnitude, and not the predictability, of the fluctuations is the criterion of classification. The symbols F and C have been shifted from their exact locations by a small random amount to indicate when several food webs have exactly the same coordinates. *a*, Original data; *b*, after lumping. The solid line through the origin is fitted on the assumption that the variance of the residuals is proportional to the number of predators. The slope is 0.8819.

This observation means that the number of predators is a better predictor of the number of prey than the reverse. This raises the intriguing possibility that, in both constant and fluctuating environments, the number of predators is causally more important in controlling the number of prey than vice versa. Evidence and theory in favour of this suggestion have been independently reviewed by Jeffries and Lawton[7].

That we find a linear relationship between number of prey and number of predators is not too surprising, since the *x*- and

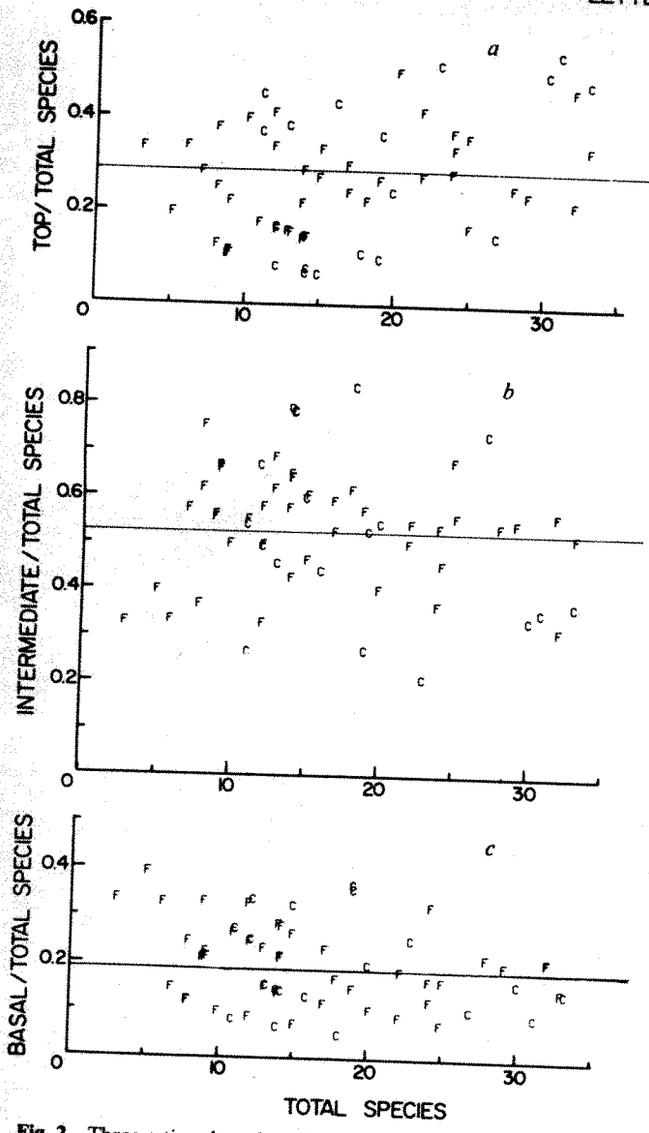


Fig. 2 Three ratios plotted as a function of the number of species. The fitted lines are constrained to be horizontal (slope = 0). *a*, Top species/total species. The height of the line is 0.2853. *b*, Intermediate species/total species. The height of the line is 0.5251. *c*, Basal species/total species. The height of the line is 0.1896.

y-axes share a similar quantity, namely the intermediate species, which are both prey and predators. What is surprising is the tightness of the fit, considering the size and heterogeneity of the sample examined. This suggests two possibilities: either the redundant variable, that is, the number of intermediate species, is very large compared with the number of basal and top species in most communities, or the proportions of all species in a food web that fall into each of these three categories are, overall, independent of the total number of species.

Figure 2 illustrates the reality of the second alternative: in the 62 webs examined, the fractions of top, intermediate and basal species are, on average, independent of the total number of species, although there is a slight tendency for the fraction of top species to increase and for the fraction of basal species to decrease as the total number of species increases. To obtain a global estimate of the proportions of species in each of the three categories (top, intermediate, basal), we summed over all food webs the observed numbers in each category, and divided by the sum total of species over all food webs. The global proportions of top, intermediate and basal species correspond to the heights of the horizontal lines in Fig. 2*a*–*c*.

The scatter of points about the horizontal lines in Fig. 2, when constant and fluctuating food webs are considered together, agrees with the hypothesis that in each food web the top, intermediate and basal species are multinomially sampled from the total species in proportions that are constant for all webs. If the species counts are arranged in a 3×62 contingency table with rows for top, intermediate and basal species and one column for each web, a homogeneity test yields a χ^2 of 138.9 with 122 d.f., which is not significant at the 0.1 level.

There is no evidence for a difference between constant and fluctuating food webs in the mean proportions of top, intermediate and basal species. A homogeneity test of a 3×2 contingency table with rows for top, intermediate and basal species and columns for constant and fluctuating food webs, and the summed species counts as cell entries, gives a χ^2 statistic of 1.4 with 2 d.f., which is not significant at the 0.1 level.

However, the proportions of top, intermediate and basal species in constant food webs, considered separately, are significantly more variable, and the proportions in fluctuating food webs, considered separately, are significantly less variable, than expected from multinomial sampling (using a 0.02 significance level). Separate homogeneity tests of the constant webs (in a 3×19 contingency table) and of the fluctuating webs (in a 3×43 contingency table) yield χ^2 of 82.8 with 36 d.f. and 56 with 84 d.f., respectively. The visual counterpart of this statistical result is the appearance in each panel of Fig. 2 of fluctuating food webs near the horizontal line and of constant food webs scattered above and below the band of fluctating webs. Food web structure appears more constrained in fluctuating than in constant environments, as previously noted[3,8].

We now show that the empirical regularities in Fig. 1*b* can be derived from the approximate scale-invariance shown in Fig. 2.

Let S be the total number of species in a single community food web, T the expected number of top species in that web, I the expected number of intermediate species, B the expected number of basal species, R the expected number of predators and Y the expected number of prey. By definition

$$S = T + I + B \tag{1}$$

$$R = T + I \tag{2}$$

$$Y = I + B \tag{3}$$

By observation

$$T/S = p \quad \text{or} \quad T = pS, \qquad \hat{p} = 0.2853 \text{ (Fig. 2}a\text{)} \tag{4}$$

$$I/S = q \quad \text{or} \quad I = qS, \qquad \hat{q} = 0.5251 \text{ (Fig. 2}b\text{)} \tag{5}$$

$$B/S = r \quad \text{or} \quad B = rS, \qquad \hat{r} = 0.1896 \text{ (Fig. 2}c\text{)} \tag{6}$$

Adding equations (5) and (6) and dividing by the sum of equations (4) and (5), we recover the observed regularity (Fig. 1*b*)

$$Y/R = a \quad \text{or} \quad Y = aR, \qquad a = (q+r)/(q+p) \tag{7}$$

The predicted value $(\hat{q}+\hat{r})/(\hat{q}+\hat{p}) = 0.8819$ is identical to the observed $\hat{a} = 0.8819$ because of the formulas we used to estimate the slope a and the proportions p, q and r. However, the observation in Fig. 1*b* that the average number of prey is a linear function of the number of predators is not a tautologous consequence of the estimation formulas. The proportionality of prey to predators follows from the scale-invariance we have discovered here. Were data available, it would be interesting to examine whether the distribution of biomass into top, intermediate and basal species is also scale-invariant.

We conclude that the values of any two of the three parameters p, q and r summarize succinctly a substantial amount of information about the empirical regularities found in community food webs and provide a factually grounded benchmark against which the deviations of particular food webs may be measured. Why these proportions take the values they do and why the proportions are scale-invariant remain open questions.

We thank Stuart Pimm, W. G. Sprules and P. Yodzis for their helpful comments. This work was supported by grants A9868 from the Natural Science and Engineering Research Council of Canada and DEB80-11026 from the USNSF.

Received 3 August; accepted 25 October 1983.

1. Cohen, J. E. *Nature* **270**, 165–167 (1977).
2. Pimm, S. L. *Food Webs* (Chapman & Hall, New York, 1982).
3. Briand, F. *Ecology* **64**, 253–263 (1983).
4. Cohen, J. E. *Food Webs and Niche Space* (Princeton University Press, 1978).
5. Fryer, G. *Proc. zool. Soc. Lond.* **132**, 153–281 (1959).
6. Paviour-Smith, K. *Trans. R. Soc. N.Z.* **83**, 525–554 (1956).
7. Jeffries, M. J. & Lawton, J. H. *J. Linn. Soc.* (in the press).
8. Yodzis, P. *J. theor. Biol.* **92**, 103–117 (1981).

Monoclonal antibody that identifies subsets of neurones in the central visual system of monkey and cat

S. H. C. Hendry*, S. Hockfield†, E. G. Jones* & R. McKay†

* James L. O'Leary Division of Experimental Neurology and Neurological Surgery and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St Louis, Missouri 63110, USA
† Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

Striking correlations between structure and function are found in the visual cortex of Old World primates[1–4]. These include the co-localization of glutamic acid decarboxylase (GAD, the biosynthetic enzyme of the inhibitory neurotransmitter, γ-aminobutyric acid) with the mitochondrial enzyme, cytochrome oxidase (CO)[5] in functionally distinct subcompartments[6,7] of ocular dominance columns[3,4,8]. We report here immunocytochemical studies with a monoclonal antibody (CAT 301)[9] showing that the antibody recognizes an uncharacterized antigen on surfaces of some neurones in certain layers of the monkey striate cortex (area 17), and in certain parts of the cat and monkey dorsal lateral geniculate nuclei (LGN). Patches of immunocytochemically stained neurones and neuropil, apparent in layers III, IVB and VI of the striate cortex of normal monkeys, become even more clearly delineated in animals from which one eye has been removed. The antibody-stained patches in the three layers line up radially with one another in lines passing through the centres of ocular dominance columns (demonstrable by CO staining in layers IVA and IVC). In layers III and VI the patches coexist with CO-positive patches[3,4] and, in the horizontal dimension, both antibody and CO-positive patches are aligned to form rows. Stained neurones in the monkey LGN are primarily in the magnocellular layers and in the cat LGN are confined to laminae A and A_1, the inter-laminar plexuses, the perigeniculate nucleus and the medial inter-laminar nucleus. The antigen we have localized is associated with particular cell populations, some of which may correspond to a specific, physiological class.

Three normal cynomolgus monkeys (*Macaca fascicularis*) and three cats were used. Three other monkeys were anaesthetized and one eye removed 8 or 10 days before they were re-anaesthetized and perfused with buffered 4% paraformaldehyde. Alternating frozen sections from both sides of the brains were processed immunocytochemically with CAT 301 (refs 9, 10) and histochemically for cytochrome oxidase[11].

CAT 301 stains pyramidal and nonpyramidal neurones particularly in layers IVB and VI of monkey striate cortex (Fig. 1); there is a diffuse staining of the neuropil around the stained cells (Fig. 1*a*). Some cells in layers II and III but very few in layers I, IVA, IVC or V are stained.

Within layers IVB and VI and deep in layer III, CAT 301-positive neurones form patches (Figs 2*a,c*, 3*a*). Patches in layers III and IVB are 200 μm × 150 μm; those in layer VI approach 300 μm. In tangential sections (Fig. 2) patches in layers IVB and VI form rows perpendicular to the border of areas 17 and 18. Gaps between patches vary in size from 300–500 μm along the rows to 200–400 μm between the rows (Fig. 2). Rows of patches are less distinct in layer III (Fig. 3).

In transverse sections most patches of CAT 301-positive cells in layer VI line up radially with patches in layers IVB and III (see Fig. 2). Less than 10% of those in layer VI are without overlying patches in layer IVB, but approximately 30% in layers VI and IVB have no corresponding patch in layer III.

Following the removal of one eye, the patchy distribution of CAT 301-positive cells in layer VI becomes much more distinct due to a sharp reduction in the neuropil staining at the edges of the patches.

Ocular dominance columns in area 17 are demonstrated by CO histochemistry after monocular enucleation (Fig. 2*b,d*). The columns are most obvious in layers IVA and IVC[2,12] but they can also be distinguished in layers III and VI.

By aligning radially oriented blood vessels, tangential sections in layer IVC reacted for CO can be superimposed on adjoining sections stained immunocytochemically with CAT 301. Patches of CAT 301-positive cells in layers III, IVB and VI are aligned with the centres of both CO-rich and CO-poor columns in layers IVA and IVC (Figs 2*a–c*), indicating that CAT 301-positive patches lie at the centres of both ipsilateral and contralateral ocular dominance columns. Where the stripes formed by ocular dominance columns branch, two side-by-side patches of CAT 301-positive cells appear in layers IVB and VI. Patches are also present where the monocular segment of the visual field is represented and ocular dominance columns are not demonstrable[2,12].

In layer III, 70% of the CO-positive periodicities of layer III of normal monkeys[3,4,7,8] (Fig. 3*a*) superimpose with CAT 301-positive patches (Fig. 3*b*). The remaining 30% are without corresponding CAT 301 patches. However, virtually all CO-positive periodicities in layer III line up radially with CAT 301 patches in layers IVB and VI.

In layer VI, with very few exceptions, patches of CAT 301-stained cells and patches of CO staining in layer VI are exactly superimposed (Fig. 2). Both lie at the centres of right- and left-eye dominance columns (Fig. 2*c,d*) and both are aligned in stripes.

In the LGN of enucleated and intact monkeys most cells (~95%) in the magnocellular layers and very few (<15%) in the parvocellular layers are CAT 301-positive (Fig. 4*a*). Stained cells vary from 18 to 24 μm in average diameter.

In the cat LGN, CAT 301-positive cells make up slightly more than 20% of the population of the A and A1 laminae and only 1 or 2% of the population of the C laminae. Stained cells average 20–25 μm in diameter. Most neurones in the inter-laminar zones, the perigeniculate nucleus and the ventral lateral geniculate nucleus, and approximately 80% of those in the medial interlaminar nucleus are labelled (Fig. 4*b*).

The striate cortex of Old World monkeys can be divided into a series of functional modules or 'hypercolumns'[1,2]. CO histochemistry has revealed a series of radially organized periodicities in layers II–III and V–VI[3,4] that occupy the centres of ocular dominance columns. Several reports, focused on the periodicities in layer III, document their structural[3,4,8] functional[6,7,10,13,14] and biochemical[5,15] properties and Livingstone and Hubel[4] have pointed out that they may be an indication that hypercolumns are structural entities with definite borders. We have demonstrated that neurones in monkey striate cortex stained with CAT 301, are also organized in radial arrays that align with the CO-rich periodicities. Like the CO-positive zones, CAT 301-positive patches occupy the centres of ocular dominance columns, further demonstrating that groups of neurones within the columns possess distinct properties.

CAT 301-positive patches in area 17 superficially resemble patches of cells described as giving rise to long intracortical axons[16] but, unlike CAT 301-positive patches, the long-axoned

© 1984 Macmillan Journals Ltd

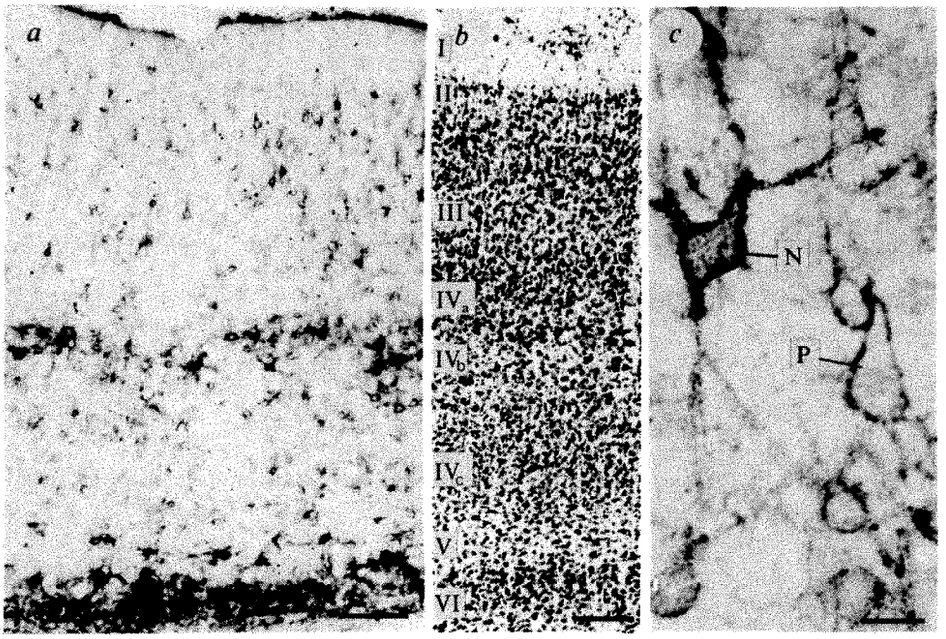


Fig. 1 *a*, Laminar distribution of CAT 301-positive neurones in a radial section through area 17 of a normal monkey. Dense bands of cells are stained in layers IVB and VI but very few cells are stained in other layers. *b*, Section adjacent to *a*, counterstained with thionin. Roman numerals indicate cortical laminae according to the scheme of Brodmann[26] as modified by Lund[27]. *c*, Higher magnification of CAT 301-positive cells in layer VI of same section as *a*. Both pyramidal (P) and non-pyramidal types are stained. Scale bars, 100 μm in *a* and *b*, 20 μm in *c*.

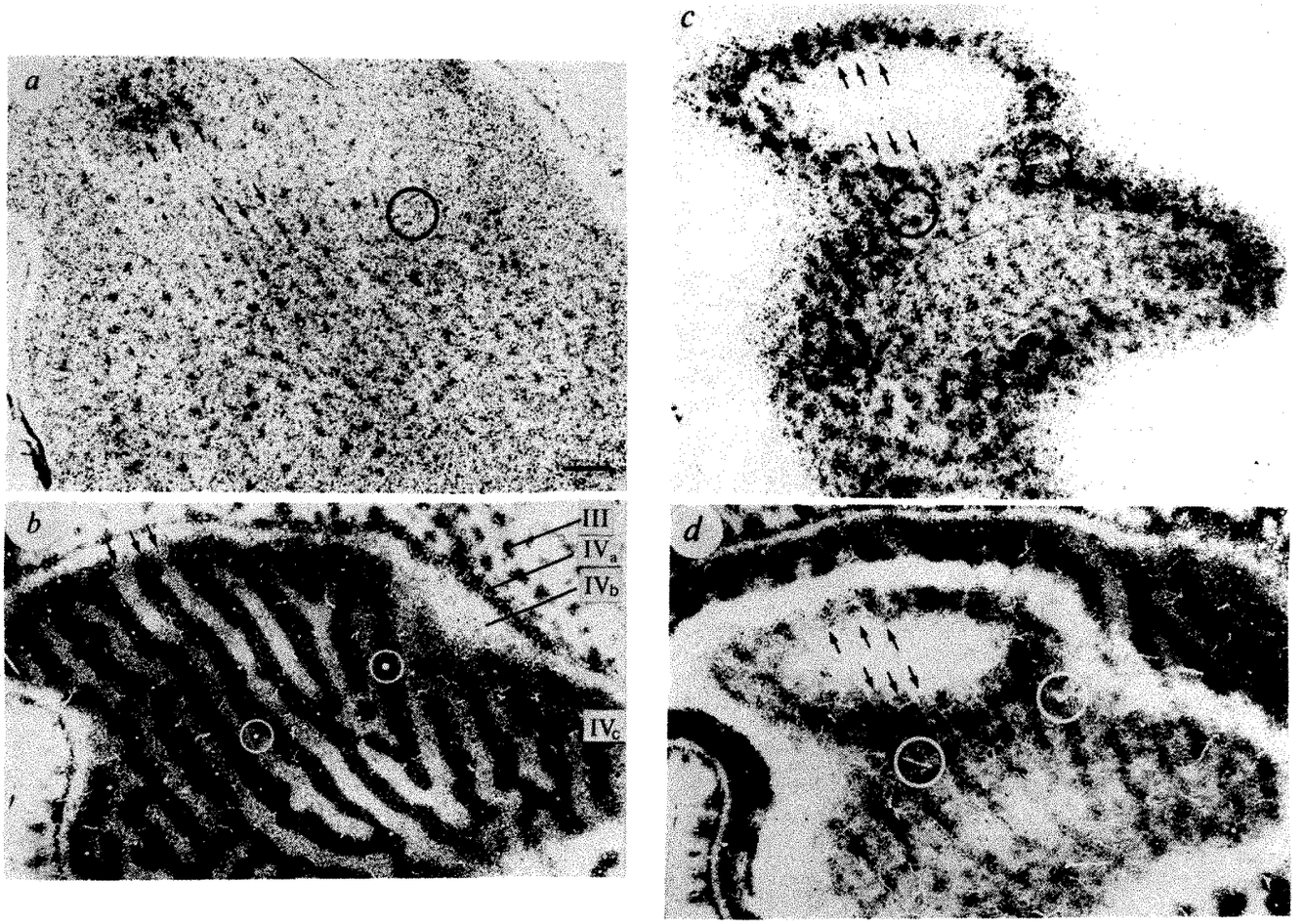


Fig. 2 Tangential sections (parallel to the brain surface) through area 17 of a monkey from which the contralateral eye was removed. *a*, Stained immunocytochemically by CAT 301, mainly through layer IVB; *b*, stained for CO, mainly through layer IVC. Alternating CO-rich and -poor bands represent ocular dominance stripes of intact and removed eyes, respectively. *c*, *d*, Stained by CAT 301 and for CO, respectively, mainly through layer VI. In *d* blanching of alternate rows of CO patches is an effect of the enucleation. Other layers indicated on *b* appear because plane of section is not perfectly parallel to the individual layers. The sections can be superimposed by aligning the penetrating blood vessels, two of which are circled. When this is done, the rows of CAT 301-stained periodicities can be seen to be in exact register with one another, with the ocular dominance stripes of layer IVC, and with the rows of CO-stained periodicities of layer VI. Three corresponding rows of patches and stripes are indicated by arrows. Note that the CO staining declines in ocular dominance stripes and patches related to the removed eye so that staining with CAT 301 is associated with both strongly and weakly CO-positive stripes and patches. Scale bar, 1 mm.

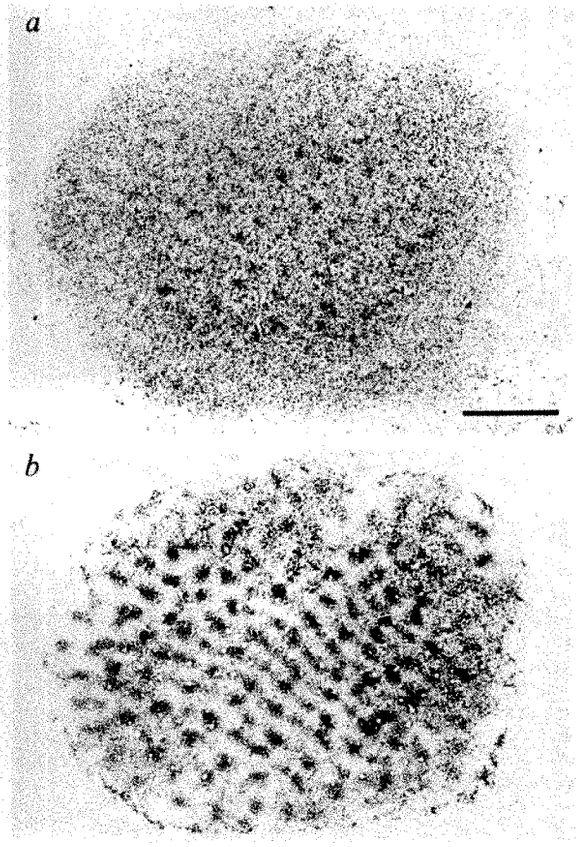


Fig. 3 Adjacent tangential sections through layer III in area 17 of a normal monkey. Patches of neurones stained immunocytochemically by CAT 301 (*a*) are aligned in rows that correspond to rows of cytochrome oxidase stained patches in the adjacent section (*b*). Many of the CAT 301-positive patches can be exactly superimposed on the CO positive densities. Scale bar, 2 mm.

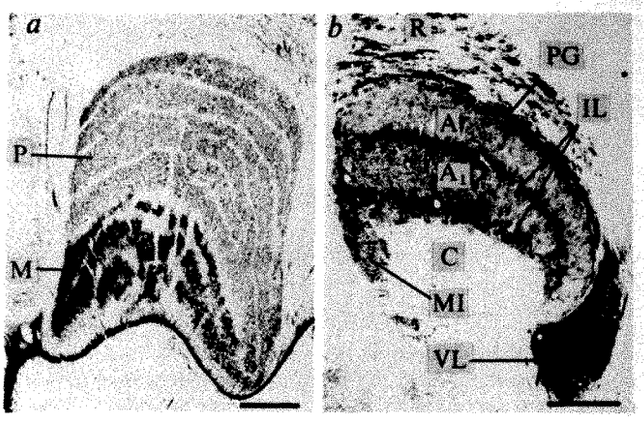


Fig. 4 Frontal sections of LGN from a monkey (*a*), and a cat (*b*), stained by CAT 301. *a*, In the monkey LGN there are many densely stained cells in the two magnocellular layers (M) and very few in the four parvocellular layers (P). The diffuse background staining of the parvocellular layers is not clearly associated with any neuronal processes. *b*, In the cat lateral geniculate complex, there is heavy staining of cells in the medial interlaminar nucleus (MI), the ventral lateral geniculate nucleus (VL), the perigeniculate nucleus (PG) and in the interlaminar plexuses (IL), with less staining of the A and A1 laminae and very little of the C laminae. Additional staining appears in the thalamic recticular nucleus (R). Scale bar, 1 mm.

cell patches are not correlated with CO-positive periodicities.

CO staining in layers III, IV and VI correlates with the termination of thalamic afferents[4]. CAT 301 patches coincide with CO staining in layers III and VI, but not in layers IVA or IVC where the heaviest thalamic terminations occur. CAT 301 is unlikely to mark afferent terminals directly because immunoelectron microscopy suggests that the surface antigen is extrasynaptic and synthesized by the cell which it outlines[10], that is, the postsynaptic cell is defined by the antigen.

The distribution of CAT 301-positive neurones in monkey visual cortex and in monkey and cat LGN may reflect the distribution of neurones responding to visual stimuli of many wavelengths (broad band cells). These are localized in the magnocellular layers of the monkey LGN[17–19] and in the A and A1 laminae and medial interlaminar nucleus[20,21] of the cat LGN regions stained by CAT 301. In the monkey visual cortex broad band cells[4] and CAT 301-positive cells are found in the CO-rich periodicities of layer III. Broad band cells may also be present in CO-rich bands of monkey area 18, which also coincide with CAT 301-positive patches (unpublished observations). CAT 301-positive cells are certainly not restricted to laminae in the LGN nor to layers in area 17 where broad band responses have been recorded, so their distributions in the visual system may be associated not only with broad band cells but also with other as yet unidentified cell colonies as well.

Previous studies with monoclonal antibodies to uncharacterized antigens have focused on classes of neurones identified by their morphology, connectivity and/or position in an organized structure[22–24]. The present and previous[9,10,25] reports demonstrate that CAT 301 recognizes surface molecules on many but not all neurones, not even those (for example, pyramidal cells) of the same morphological type. The nature of the neuronal surface molecule recognized by CAT 301 remains to be determined, as does the role of the molecule in the structure, function and development of the neurones it marks.

We thank Ms Bertha McClure for technical assistance. This study was supported by NIH USPHS grant NS10526.

Received 22 July; accepted 1 November 1983.

1. Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **158,** 267–294 (1974).
2. Hubel, D. H. & Wiesel, T. N. *Proc. R. Soc.* B **198,** 1–59 (1977).
3. Horton, J. C. & Hubel, C. H. *Nature* **292,** 762–764 (1981).
4. Livingstone, M. S. & Hubel, D. H. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6098–6101 (1982).
5. Hendrickson, A. E., Hunt, S. P. & Wu, J.-Y. *Nature* **292,** 605–607 (1981).
6. Hubel, D. H. & Livingstone, M. S. *Soc. Neurosci. Abstr.* **7,** 357 (1981).
7. Hubel, D. H. & Livingstone, M. S. *Soc. Neurosci. Abstr.* **8,** 706 (1982).
8. Humphrey, A. L. & Hendrickson, A. E. *Soc. Neurosci. Abstr.* **6,** 315 (1980).
9. Hockfield, S. & McKay, R. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6747–6751 (1982).
10. Hockfield, S. & McKay, R. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5758–5761 (1983).
11. Wong-Riley, M. *Brain Res.* **171,** 11–28 (1979).
12. Wiesel, T. N., Hubel, D. H. & Lam, D. M. K. *Brain Res.* **79,** 273–279 (1974).
13. Humphrey, A. L. & Hendrickson, A. E. *J. Neurosci.* **3,** 345–358 (1983).
14. Hendrickson, A. E. & Wilson, J. R. *Brain Res.* **170,** 353–358 (1979).
15. Graybiel, A. M. & Ragsdale, C. W. Jr *Nature* **299,** 439–442 (1982).
16. Rockland, K. S. & Lund, J. S. *J. comp. Neurol.* **216,** 303–318 (1983).
17. Schiller, P. H. & Malpeli, J. G. *J. Neurophysiol.* **40,** 788–797 (1978).
18. Wiesel, T. N. & Hubel, D. H. *J. Neurophysiol.* **35,** 1118–1139 (1966).
19. Dreher, B., Fukada, Y. & Rodieck, R. W. *J. Physiol., Lond.* **258,** 433–452 (1976).
20. Dreher, B. & Sefton, J. *J. comp. Neurol.* **183,** 47–64 (1979).
21. Wilson, P. D., Rowe, M. H. & Stone, J. *J. Neurophysiol.* **39,** 1193–1209 (1976).
22. Barnstable, C. J. *Nature* **286,** 231–235 (1980).
23. Lemmon, V. & Gottlieb, D. I. *J. Neurosci.* **2,** 531–535 (1982).
24. Giotta, G. in *Monoclonal Antibodies to Neural Antigens* (eds McKay, R., Raff, M. C. & Reichardt, L.) 51–60 (Cold Spring Harbor Laboratory, New York, 1981).
25. Hockfield, S., McKay, R., Hendry, S. & Jones, E. G. *Cold Spring Harb. Symp. quant. Biol.* (in the press).
26. Brodmann, K. J. *Psychol. Neurol.* **4,** 177–226 (1905).
27. Lund, J. S. *J. comp. Neurol.* **147,** 455–496 (1973).

Muscarinic inhibitory transmission in mammalian sympathetic ganglia mediated by increased potassium conductance

Alison E. Cole & Patricia Shinnick-Gallagher

Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77550, USA

Slow muscarinic inhibition may be a powerful influence on membrane properties in the peripheral[1–3] and central nervous system[4]. But the location of the muscarinic receptors in sympathetic ganglia, either on interneurones or on the postganglionic membrane, and the underlying mechanism of the inhibitory response, remains controversial[5–7]. In mammalian sympathetic ganglia synaptic activation of muscarinic receptors located on inhibitory interneurones was thought to release catecholamines leading to a membrane hyperpolarization called the slow inhibitory postsynaptic potential, or s.-i.p.s.p.[8]. However, the s.-i.p.s.p. in parasympathetic ganglia[3,9,10] and in amphibian sympathetic ganglia[1,2] is due to direct monosynaptic activation of muscarinic receptors, accompanied by an increased potassium conductance (but see ref. 11), and is not mediated by catecholamines. The situation is less clear in mammalian sympathetic ganglia and monosynaptic s.-i.p.s.ps observed in other ganglia could be exceptions to the hypothesis. We showed earlier that the s.-i.p.s.p. in rabbit superior cervical ganglia is not affected by catecholamine antagonists[12]. We now show that the s.-i.p.s.p. in a mammalian sympathetic ganglion is due to the monosynaptic activation of muscarinic receptors, probably by an increase in potassium conductance.

The superior cervical ganglia along with their pre- and postganglionic nerves were isolated from adult male New Zealand rabbits and prepared as described previously[12]. Glass microelectrodes (30–100 MΩ) filled with 3 M KCl were used for intracellular recording from over 150 sympathetic neurones.

In all experiments, nicotinic responses were blocked by the administration of hexamethonium (500 μM) to facilitate examination of the s.-i.p.s.p. Iontophoretic application of acetylcholine (ACh, 2 M; 50–100 MΩ) elicited a hyperpolarization that mimicked the nerve-evoked s.-i.p.s.p. (40 Hz for 250 ms). Both responses were abolished by atropine (0.1 μM) but unaffected by the adrenergic antagonist phentolamine (1 μM) (Fig. 1). We previously showed with extracellular recordings that this concentration of phentolamine blocked hyperpolarizations induced by noradrenaline, adrenaline and

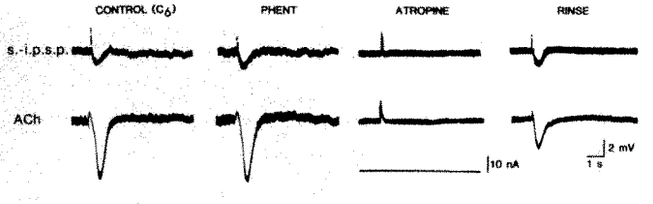


Fig. 1 Effect of antagonists on the nerve-evoked s.-i.p.s.p. and ACh hyperpolarization. Hexamethonium (C_6, 500 μM) eliminated all fast nicotinic responses leaving only the slow muscarinic responses. The s.-i.p.s.p. was elicited by orthodromic stimulation at 40 Hz for 250 ms; in the same cell, a single iontophoretic pulse of ACh was applied (50 nA, 40 ms). Phentolamine (1 μM) had no effect on either the s.-i.p.s.p. or ACh hyperpolarization, even after 30-min application. However, the muscarinic antagonist, atropine (0.1 μM) completely eliminated both responses. The responses returned after rinsing for 1 h. A representative current trace is positioned below the third ACh record. Membrane potential −62 mV.

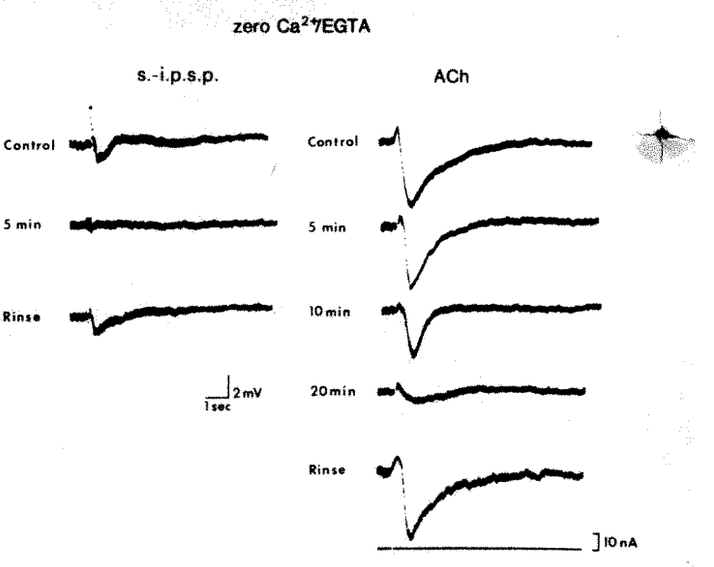


Fig. 2 Effect of blockade of synaptic transmission with 0 Ca^{2+}/EGTA solution on the nerve-evoked s.-i.p.s.p. and ACh hyperpolarization. The Ca^{2+}-free, high Mg^{2+} solution was prepared by substituting 2.5 mM $MgCl_2$ for $CaCl_2$ (3.7 mM $MgCl_2$, total); EGTA (1 mM) was also added. Both responses were recorded simultaneously from the same cell. Stimulation parameters are the same as in Fig. 1. The left-hand column shows the control s.-i.p.s.p., rapid block of the s.-i.p.s.p. in 0 Ca^{2+} solution and return of the response after rinse. The right-hand column shows that the simultaneously recorded ACh hyperpolarization was unchanged at the time when synaptic transmission was blocked (5 min). Prolonged exposure to the zero Ca^{2+} solution (20 min) eventually depressed the ACh response, possibly via a postsynaptic effect. Rinse records for both responses were recorded 20 min after return to control solution (10 and 20 min records in 0 Ca^{2+} media were omitted for the s.-i.p.s.p. as they were identical to the 5 min record). Membrane potential −60 mV.

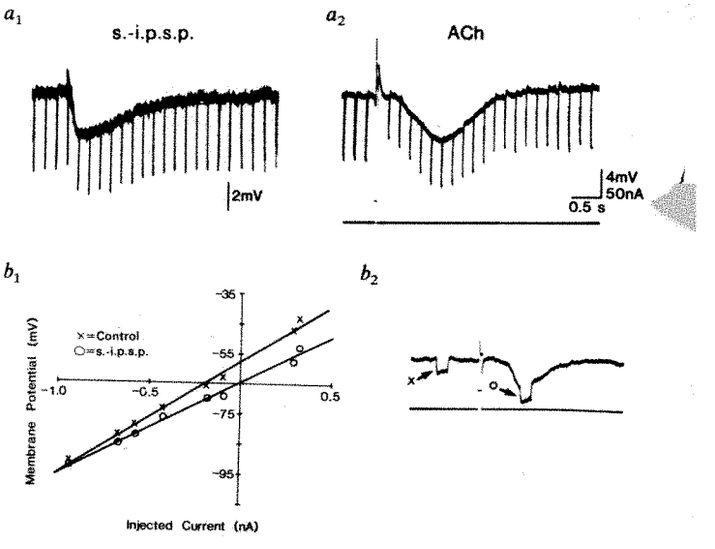


Fig. 3 Conductance changes during the s.-i.p.s.p. and ACh hyperpolarization (50 nA, 100 ms). *a*, Both responses were associated with a decrease in input resistance, reflected in the decrease in amplitude of the anodal constant current pulses. Current trace is located below the voltage trace for the ACh response. Time calibration is the same for both records. Membrane potential −55 mV. *b*, Current–voltage plot for the s.-i.p.s.p. Hyperpolarizing and depolarizing current pulses of constant duration (300 ms, see b_2) and varying amplitude were passed across the membrane before (×) and at the peak of the s.-i.p.s.p. (○). The difference in the slope of the two plots indicates a decrease in resistance during the s.-i.p.s.p. The intersection point of the lines represents the estimated reversal potential of the response, here, −94 mV. Membrane potential −58 mV.

0028–0836/84/030270—02$01.00

© 1984 Macmillan

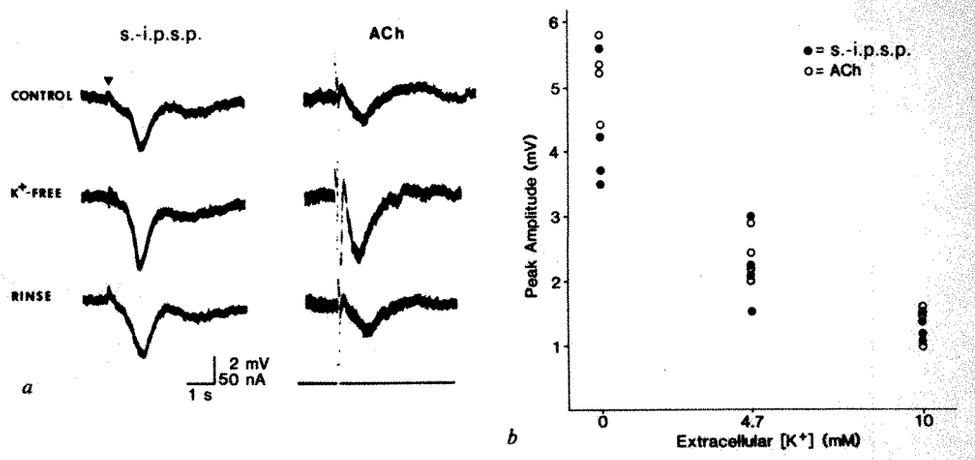


Fig. 4 Effect of extracellular K^+ concentration on the s.-i.p.s.p. and ACh hyperpolarization. *a*, Both the nerve-evoked s.-i.p.s.p. and hyperpolarization to a single iontophoretic pulse of ACh (40 nA, 100 ms) increased in amplitude when perfused with a K^+-free solution. Equimolar concentrations of NaCl were substituted for KCl in the physiological solution. Membrane potential −60 mV. *b* Illustrates the change in peak amplitude of s.-i.p.s.p. (×) and ACh hyperpolarization (○) versus extracellular K^+ concentration. Changing the K^+ concentration had no effect on the resting membrane potential of these cells.

dopamine[12]. These data suggested that catecholamines do not mediate the s.-i.p.s.p. in mammalian sympathetic ganglia.

To investigate whether the s.-i.p.s.ps are due to a disynaptic process, we tested the effect of blocking synaptic transmission on the response to iontophoretically applied ACh. Exposure to a 0 calcium/3.7 mM magnesium, EGTA (1 mM) solution quickly abolished the nerve-evoked s.-i.p.s.p. ($n = 3$, Fig. 2). In contrast, the ACh response persisted at a time when synaptic transmission was blocked, and was depressed only after prolonged exposure to the calcium-free solution (15 min) ($n = 8$). All responses returned to control levels during the rinse. Identical results were seen when synaptic release was blocked with tetrodotoxin (TTX, 1 μM; $n = 4$). After 3 min of superfusion with TTX, the s.-i.p.s.p. was eliminated while in the same four cells the ACh response was not affected. These data do not support the disynaptic hypothesis. In every case the ACh hyperpolarization persisted when synaptic transmission (that is, the s.-i.p.s.p.) was blocked. This suggests that the muscarinic action of ACh is a direct effect on the ganglion cells and the calcium-free solution may be affecting underlying ionic mechanisms at the postsynaptic membrane. This would explain the persistence and gradual depression of the ACh iontophoretic response long after transmitter release had been eliminated.

We examined the mechanism underlying the s.-i.p.s.p. and ACh hyperpolarization by passing constant current anodal pulses through the recording electrode during the responses to analyse the conductance changes (Fig. 3). In cells where both the s.-i.p.s.p. and ACh response were recorded at the same membrane potential ($n = 12$) (Fig. $3a_1$ and a_2) a small but consistent decrease in resistance was observed. These results reflect a similar increase in conductance during both the nerve-evoked s.-i.p.s.p. and the ACh hyperpolarization. In addition, current–voltage (I–V) plots were constructed by passing individual short duration current pulses across the membrane before and at the peak of the responses (Fig. $3b_2$). A series of anodal and cathodal current pulses of constant duration (200 ms) and varying amplitude (−1.5 to +1.0 nA) were passed through the recording electrode just before and at the peak of the hyperpolarization. Curves were plotted of current against absolute membrane potential. Typical plots for control resistance values (pre-response) and values at the peak of the s.-i.p.s.p. are shown in Fig. $3b_1$. A plot of the control resistance values against those values at the peak of the ACh hyperpolarization yielded similar results. The reversal potential estimated from the intersection of the current–voltage plots in the linear, non-rectifying part of the membrane[13] was 102.7 ± 10 ($n = 4$) for the s.-i.p.s.p. and 100.4 ± 11 ($n = 5$) for the ACh hyperpolarization. These experiments implied that potassium and/or chloride ions may be involved in the generation of the s.-i.p.s.p. Since the hyperpolarizations were not reversed when KCl electrodes were used, it is unlikely that chloride ions are responsible for the responses. From our evidence with zero calcium solutions, it appears that calcium ions may also be involved, although more conclusive evidence is necessary.

We compared the effects of changing the external potassium ion concentration on the ACh hyperpolarization and the s.-i.p.s.p. As shown in Fig. 4, removal of potassium from the superfusing solution increased the amplitude of both hyperpolarizing responses ($n = 9$). The response to iontophoretic ACh was increased $40 \pm 2\%$ and the s.-i.p.s.p. was increased $58 \pm 19\%$. There was no change in the duration of either potential. When the extracellular potassium concentration was elevated to 10 mM, both the ACh and s.-i.p.s.p. hyperpolarizations were depressed. The hyperpolarization to iontophoretically applied ACh decreased $25 \pm 2\%$ and the s.-i.p.s.p. by $39 \pm 4\%$. Figure 4*b* summarizes the changes in the s.-i.p.s.p. and ACh hyperpolarization as the extracellular potassium concentration was changed. This agrees with the hypothesis that the s.-i.p.s.p. and the ACh hyperpolarization which mimics it, are generated by an increased membrane conductance to potassium ions.

We report here the first evidence in a mammalian sympathetic ganglion that the s.-i.p.s.p. is mediated by an increased potassium conductance via monosynaptic activation of muscarinic receptors. Furthermore, the potassium current may be a calcium-activated potassium conductance, dependent on an influx of calcium ions, as suggested by the effect of zero calcium solution on both responses. Calcium-activated potassium conductances in a variety of nerve and muscle cells have been shown to mediate long-lasting hyperpolarizations following calcium influx[14–18]. In addition, calcium-activated potassium conductances have been linked to muscarinic responses in a number of neuronal systems[19,20]. These experiments provide further insight into the mechanisms of the muscarinic s.-i.p.s.p., a potential that may exert a powerful influence on membrane excitability and have an important role in the integration of autonomic function.

Received 14 July; accepted 6 October 1983.

1. Horn, J. P. & Dodd, J. *Nature* **292,** 625–627 (1981).
2. Dodd, J. & Horn, J. P. *J. Physiol., Lond.* **334,** 271–291 (1983).
3. Gallagher, J. P., Griffith, W. H. III & Shinnick-Gallagher, P. *J. Physiol., Lond.* **332,** 473–486 (1982).
4. Krnjevic, K. & Ropert, N. *Neuroscience* **7,** 2165–2183 (1982).
5. Gallagher, J. P., Shinnick-Gallagher, P., Cole, A. E., Griffith, W. H. & Williams, B. J. *Fedn Proc.* **39,** 3009–3015 (1980).
6. Libet, B. & Kobayashi, H. *J. Neurophysiol.* **37,** 805–814 (1974).
7. Weight, F. F. & Padjen, A. *Brain Res.* **55,** 225–228 (1973).
8. Eccles, R. & Libet, B. *J. Physiol., Lond.* **157,** 484–503 (1961).
9. Hartzell, H. C., Kuffler, S. W., Stickgold, R. & Yoshikami, D. *J. Physiol., Lond.* **271,** 817–846 (1977).
10. Griffith, W. H., Gallagher, J. P. & Shinnick-Gallagher, P. *Brain Res.* **209,** 446–451 (1981).
11. Akasu, T. & Koketsu, K. *Jap. J. Physiol.* **33,** 279–300 (1983).
12. Cole, A. E. & Shinnick-Gallagher, P. *Brain Res.* **187,** 226–230 (1980).
13. Ginsborg, B. L., House, C. R. & Silinsky, E. M. *J. Physiol., Lond.* **236,** 723–731 (1974).
14. Meech, R. W. & Standen, N. B. *J. Physiol., Lond.* **249,** 211–239 (1975).
15. Barrett, E. F. & Barrett, J. N. *J. Physiol., Lond.* **255,** 737–774 (1976).
16. Nicoll, R. A. & Alger, B. *Science* **210,** 1122–1124 (1980).
17. Hotson, J. R. & Prince, D. A. *J. Neurophysiol.* **43,** 409–419 (1980).
18. Schwartzkroin, P. A. & Stafstrom, C. E. *Science* **210,** 1125–1126 (1980).
19. Shinnick-Gallaher, P., Yoshimura, M. & Gallagher, J. P. *Neurosci. Abstr.* **8,** 555 (1982).
20. Cole, A. E. & Nicoll, R. A. *Science* (in the press).

Specific depletion of immunoreactive growth hormone-releasing factor by monosodium glutamate in rat median eminence

B. Bloch*, N. Ling, R. Benoit, W. B. Wehrenberg & R. Guillemin

Laboratories for Neuroendocrinology, Salk Institute for Biological Studies, PO Box 85800, San Diego, California 92138, USA

A potent and specific growth hormone-releasing factor (GRF) was recently isolated and characterized from a human islet cell tumour of the pancreas that caused acromegaly[1–3]. Antibodies raised against the synthetic replicate of this peptide have allowed the immunohistochemical identification of GRF-producing neurones within the primate central nervous system[4]. Such neurones are found mainly in the arcuate nucleus[4–6] in human and monkey hypothalamus, suggesting that this nucleus is a primary source of GRF. We have further investigated this hypothesis by studying the anatomical organization of GRF neurones in rat hypothalamus, using an antibody raised against the recently characterized rat hypothalamic GRF[7] in normal animals and in animals neonatally treated with monosodium glutamate (MSG), a treatment which results in the selective destruction of arcuate nucleus neurones[8–10]. We present here the results which show that GRF-producing neurones are located mainly in the arcuate nucleus of rats. MSG treatment results in the complete loss of GRF- immunoreactive cell bodies within this nucleus and provokes a selective disappearance of GRF-immunoreactive fibres in the median eminence. These results show that the arcuate nucleus is the origin of the GRF-containing fibres that project to the median eminence and establish the MSG-treated rat as an *in vivo* model for studying growth hormone secretion in the absence of neurohumoral GRF.

Antisera against rat hypothalamic GRF were prepared by immunizing rabbits with a mixture of synthetic rat GRF and methylated bovine serum albumin emulsified in Freund's adjuvant as described previously[11]. Immunohistological studies were performed on tissue sections from 19 male rats of 3 and 11 months of age. Nine animals were either untreated or injected with saline and 10 animals were injected with MSG (4 g per kg subcutaneous, s.c.) on alternate days for the first 10 days of life[9]. This dose is known to produce an 80–90% loss of neuronal perikarya in the arcuate nucleus[8–10]. To increase peptide content in cell bodies, some rats received a colchicine injection (50 μg in 50 μl saline) in the lateral ventricle 48 or 72 h before they were killed. Brains were fixed by intracardial perfusion of anaesthetized animals (chloral hydrate, 3 mg per kg) with chilled 4% paraformaldehyde in 0.12 M phosphate buffer *p*H 7.2. The brains were cut into blocks and rinsed in phosphate buffer with increasing concentrations of sucrose (12–16%). Frontal sections 10–16-μm thick were prepared as described[4], mounted on gelatin-coated slides and stored at −20 °C until used. An indirect immunofluorescence technique was performed as described previously[4]; antibodies against rat GRF were used at a final dilution of 1 : 200 to 1 : 800. In addition, we used antibodies against ovine corticotropin-releasing factor (CRF), somatostatin and luteinizing hormone-releasing factor (LHRF) for comparative topographical studies. Controls for specificity of the immunostaining consisted of a comparison of adjacent sections treated with anti-GRF serum alone or with anti-GRF serum previously incubated with rat GRF (0.5 mg ml^{-1} undiluted antibody) or PHI or vasoactive intestinal peptide (up to 10 mg ml^{-1}), two peptides having considerable structural homology to rat GRF[7]. Only preincubation with synthetic rat GRF resulted in the disappearance of specific immunostaining.

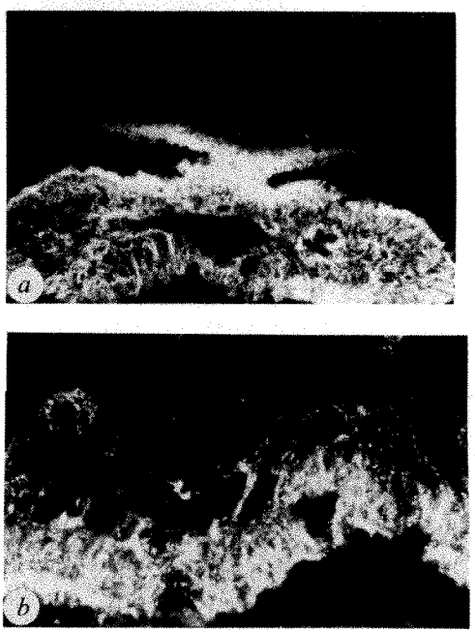


Fig. 1 Immunofluorescent staining of the median eminence of a normal adult rat with anti-rat GRF antiserum. *a*, General view of a frontal section of the posterior median eminence and the hypophyseal stalk, showing GRF-immunoreactive fibres in contact with the portal vessels. *b*, Higher magnification of a section stained with anti-rat GRF antiserum showing the course and termination of individual fibres.

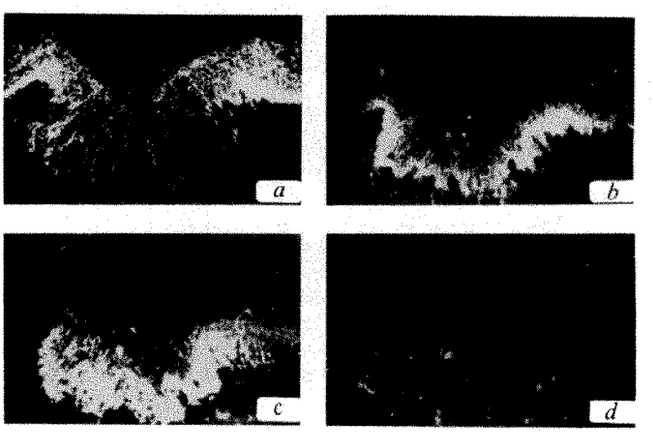


Fig. 2 Immunofluorescent staining of the median eminence of adult rats treated with monosodium glutamate (4 g per kg s.c.) on alternate days for the first 10 days of life. Immunoreactive fibres were observed in sections stained with anti-LHRF antiserum (*a*), anti-somatostatin antiserum (*b*) and anti-CRF antiserum (*c*) but were conspicuously absent from sections stained with anti-rat GRF antiserum (*d*).

In control rats, GRF immunoreactivity was constantly found in dense bundles of fibres in the median eminence, terminating in contact with portal vessels (Fig. 1), as well as in scattered fibres of the hypothalamus. No immunoreactive cell bodies were observed in animals not pretreated with colchicine. In rats pretreated with colchicine, numerous immunoreactive cells were found in the arcuate nucleus and the ventral and dorsolateral hypothalamus (Fig. 3), especially in the perifornical area and premammillary nuclei. Very few immunoreactive cell bodies were found in the ventromedial nucleus. In rats neonatally treated with MSG, the median eminence showed no GRF-immunoreactive fibres, while the distribution of LHRF-, somatostatin- and CRF-immunoreactive fibres was identical to that of control animals (Fig. 2). In the MSG-treated rats that received colchicine, GRF-immunoreactive cell bodies were only observed in the dorsolateral and ventral hypothalamus but not in the arcuate nucleus.

* Permanent address: Laboratoire d'Histologie, Faculté de Médecine, 4 Place Saint Jacques, 25000 Besançon, France.

0028–0836/84/030272—02$01.00

© 1984 Macmillan Journals Ltd

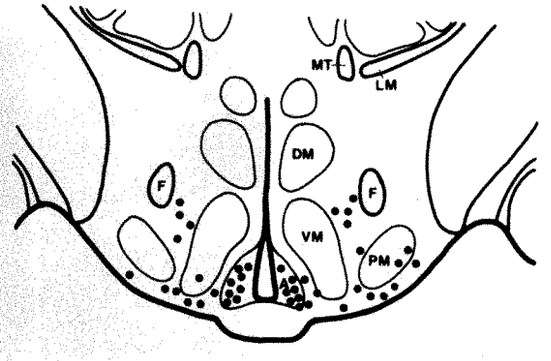


Fig. 3 Diagram of a frontal section of rat hypothalamus showing localization of GRF-immunoreactive cell bodies. A, arcuate nucleus; DM, dorsomedial nucleus; F, fornix; LM, medial lemniscus; MT, mamillothalamic tract; PM, ventral premamillary nucleus; VM, ventromedial nucleus.

Thus, the results obtained in normal rats show that the arcuate nucleus is the main source of GRF as has been found in man and monkey[4–6]. A very low abundance of neurones containing GRF immunoreactivity in the ventromedial nucleus of rats as well as primates suggests that this nucleus has a minor role in GRF production. This is in contrast to what has been hypothesized on the basis of electrical stimulation[12] and lesion experiments[13]. Since most of the GRF cell bodies are located in areas contiguous to the ventromedial nucleus, it is probable that the effects observed by destruction or stimulation of the ventromedial nucleus were due to extension of the lesion or diffusion of the stimulation to adjacent areas. Nevertheless, it cannot be excluded that neurones of the ventromedial nucleus might exert a direct stimulatory effect on GRF neuronal activity in the arcuate nucleus.

The disappearance of GRF immunoreactivity in the arcuate nucleus and median eminence after MSG treatment demonstrates that the arcuate nucleus is the source of the GRF fibres in the median eminence ending in contact with portal vessels. These results correlate well with the dramatic decrease of GRF bioactivity in the median eminence of MSG-treated rats reported by Acs *et al.*[14]. Actual quantification of this decrease must await the development of a radioimmunoassay for rat GRF. Although MSG treatment has been reported to result in a decrease in total pituitary growth hormone content in rats[15,16], pituitaries from such animals seem to have normal growth hormone secretion *in vitro*[17] and have a normal response to GRF *in vivo*[18]. Thus, the dramatic impairment of growth observed in MSG-treated rats must be related to the selective destructuion of the GRF neurones projecting in the median eminence, a conclusion supported by previous physiological studies[15]. The specificity of MSG in destroying GRF-containing neurones within the arcuate nucleus suggests that MSG-treated rats may serve as a useful *in vivo* model for the study of selective growth hormone deficiency of hypothalamic origin, an entity also observed in human neuroendocrinology[19].

This research was supported by NIH grants HD-09690 and AM-18811, the Robert J. and Helen C. Kleberg Foundation and the Andrew W. Mellon Foundation. B.B. was supported by fellowships from the Fondation pour la Recherche Médicale and the Philippe Foundation. We thank Drs F. Bloom and B. Gonzales for providing animals and facilities and B. Phillips for technical assistance.

Received 15 August; accepted 21 November 1983.

1. Esch, F. S. *et al. Biochem. biophys. Res. Commun.* **109,** 152–158 (1982).
2. Guillemin, R. *et al. Science* **218,** 585–587 (1982).
3. Rivier, J., Spiess, J., Thorner, M. & Vale, W. *Nature* **300,** 276–278 (1982).
4. Bloch, B. *et al. Nature* **301,** 607–608 (1983).
5. Bloch, B., Brazeau, P., Bloom, F. & Ling, N. *Neurosci. Lett.* **37,** 23–28 (1983).
6. Bloch, B., Gaillard, R. C., Brazeau, P., Lin, H. D. & Ling, N. *Regul. Peptides* (in the press).
7. Spiess, J., Rivier, J. & Vale, W. *Nature* **303,** 532–535 (1983).
8. Kizer, J. S., Nemeroff, C. B. & Youngblood, W. W. *Pharmac. Rev.* **29,** 301–318 (1978).
9. Olney, J. W. *Science* **164,** 719–721 (1969).
10. Simson, E. L., Gold, R. M., Standish, L. J. & Pellett, P. L. *Science* **198,** 515–517 (1977).
11. Benoit, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 917–921 (1982).
12. Frohman, L. & Bernardis, L. *Endocrinology* **82,** 1125–1132 (1968).
13. Frohman, L., Bernardis, L. & Kant, K. *Science* **162,** 580–582 (1968).
14. Acs, Z., Antoni, F. A. & Makara, G. B. *J. Endocr.* **93,** 239–245 (1982).
15. Millard, W. J., Martin, J. B. Jr, Audet, J., Sagar, S. M. & Martin, J. B. *Endocrinology* **110,** 540–550 (1982).
16. Terry, L. C., Epelbaum, J. & Martin, J. B. *Brain Res.* **217,** 129–142 (1981).
17. Antoni, F. A., Kanyicska, B. & Makara, G. B. *J. Endocr.* **96,** 427–432 (1983).
18. Wehrenberg, W. B. *et al. Regul. Peptides* (in the press).
19. Bierich, J. R. in *Proc. 2nd Int. Symp. on Growth Hormone* (eds Pecile, A. & Muller, E. (408 (Excerpta Medica, Amsterdam, 1972).

Astrocytes present myelin basic protein to encephalitogenic T-cell lines

Adriano Fontana*, Walter Fierz & Hartmut Wekerle

Max-Planck-Gesellschaft, Clinical Research Unit for Multiple Sclerosis, Hermann- and Lilly-Schilling Foundation, Box 6120, D-8700 Würzburg, FRG

Astrocyte proliferation and perivascular lymphocyte infiltration are conspicuous among the cellular changes in the active brain lesions of multiple sclerosis patients[1]. Recent observations have indicated that most of the perivascular lymphocytes are T cells[2–4] which may be actively involved in the generation of the brain lesions. Much less is known about the significance of the proliferative astrocytes, although the fact that they produce an interleukin-1 (IL-1)-like factor that enhances the release of interleukin-2 by T lymphocytes[5,6], may provide a clue. We show here that rat astrocytes are able to present antigen to T lymphocytes in a specific manner which is restricted by the major histocompatibility complex (MHC) and that they can in particular activate myelin basic protein (BP)-specific, encephalitogenic T-cell lines. Only on such interaction do astrocytes express Ia antigens in easily detectable amounts. Antigen presentation by astrocytes may have a central role in the generation of immune responses in the brain.

T lymphocytes cannot recognize free antigen but are dependent on antigen presentation by accessory cells. This function, which is MHC-restricted and requires IL-1, has been attributed to macrophages[7–11] and more recently to dendritic cells[12–14]. In several studies accessory cell function has been found to be mediated also by other Ia-positive cells such as Langerhans cells of the skin and endothelial cells[15]. Here we evaluate astrocytes for their ability to present antigen.

Immune cytochemical studies using rabbit antisera against glial fibrillary acidic protein (GFAP), a defined astrocyte marker[16], revealed that astrocytes derived from newborn rats could be cultured to almost complete purity[17]. Such glial cell cultures were trypsinized after 3–4 weeks *in vitro*, and the cells plated on coverslips. Practically all of these cultured brain cells were GFAP-positive. In fact, if after trypsinization of glial cell cultures, the cells were plated on coverslips at a density of 2×10^5 cells, less than 20 cells per coverslip were found not to react with anti-GFAP antisera of three different sources (M. Schachner[18], A. F.[5] and Dakopatts Ltd). These results were considered to reflect the cell composition of the original cultures, as in non-confluent monolayers quantification of GFAP-negative cells was possible and during the staining procedure with anti-GFAP antibodies no loss of cells was observed. The cultures were also studied by indirect immunofluorescence using antibodies against galactocerebroside[19], the major glycolipid in myelin, which is thought to be a specific cell-surface marker for oligodendrocytes[20,21]. No galactocerebroside-positive cells were seen in our cultures. To exclude contamination by microglia cells/macrophages, the amount of cells with receptors for the Fc portion of immunoglobulin was determined using the techniques of Raff *et al.*[20]. A small amount of positive cells with a vacuolar labelling pattern due to ingested fluorescein isothiocyanate (FITC)-conjugated immunoglobulin–anti-immunoglobulin complexes became detectable in primary

* Permanent address: Section of Clinical Immunology, Department of Internal Medicine, University Hospital, Häldeliweg 4, CH-8044 Zürich, Switzerland.

© 1984 Macmillan Journals Ltd

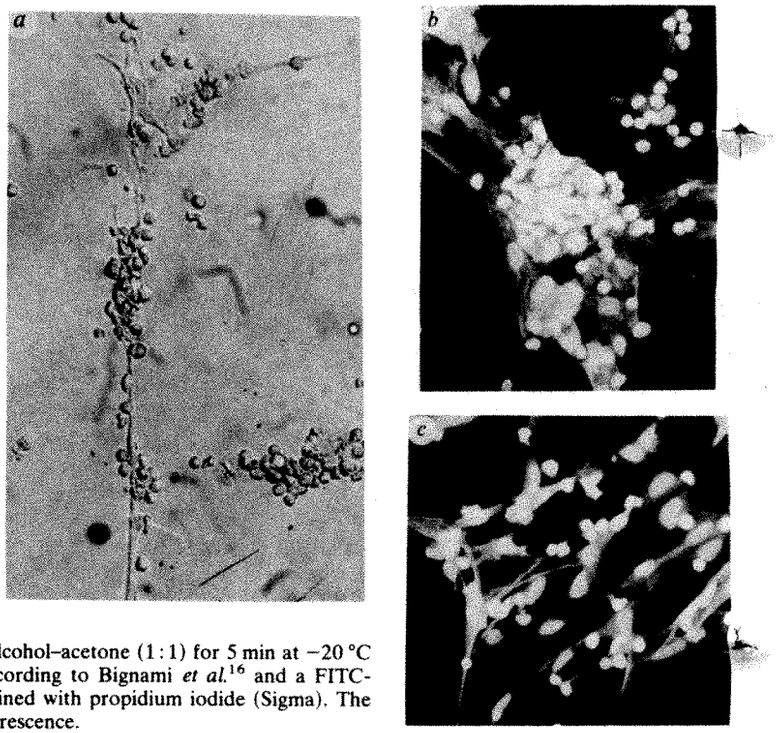


Fig. 1 *a*, The attachment of BP-specific Lewis T cells to Lewis astrocytes in the presence of soluble BP. *b*, *c*, Astrocytes with large, oval nuclei and GFAP-positive processes and cytoplasmic filaments; in contrast, the nuclei of T cells appear smaller, round and intensely fluorescent. *b* Shows tight clusters of BP-specific Lewis, T cells on Lewis astrocytes in the presence of BP. In control cultures of astrocytes of the MHC-congenic L.BN origin treated with BP, the T cells are randomly distributed and unblasted (*c*). **Methods:** For astrocyte cultures, brains of newborn rats (Lewis, L.BN or (Lewis × BN)F_1) were dissociated according to the method of Manthorpe *et al.*[17]. After trypsinization, 10^6 brain cells were plated in 5 ml glial culture medium (Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 300 μg ml^{-1} L-glutamine) in 25-cm^2 tissue culture flasks. Medium was changed on alternate days. After 3–4 weeks, the flasks were vigorously shaken by hand (see ref. 34), the detached cells discarded and the monolayer was washed three times. After this procedure the monolayer was trypsinized, the cells were reseeded on coverslips and allowed to adhere and spread for 2 days. Then BP- or OVA-specific Lewis cells and the corresponding antigen (BP or OVA) were added and cultured for 3–6 days. Direct interactions between T cells and astrocytes were studied by bright-field microscopy. To demonstrate the astrocyte-specific glial fibrillary acidic protein (GFAP), the cells on the glass slides were immersed in phosphate-buffered saline, dried, fixed in alcohol–acetone (1 : 1) for 5 min at −20 °C and stained with rabbit antisera against GFAP prepared according to Bignami *et al.*[16] and a FITC-conjugated goat anti-rabbit IgG. Cell nuclei were counterstained with propidium iodide (Sigma). The slides were examined for green (GFAP) and red (nuclei) fluorescence.

cultures but disappeared completely after passaging by trypsinization and reseeding of the astrocytes. The same picture emerged when testing a monoclonal antibody (W3/25) which stains both rat helper T cells and macrophages[22]. Cells of passaged astrocyte cultures did not label with anti-W3/25.

To test antigen presentation, graded amounts of passaged astrocytes were co-cultivated with cells of permanent BP-specific T-lymphocyte lines. These T-cell lines were originally derived from lymph node cells of rats immunized with BP[23]. They are kept in long-term culture by repeated restimulation with BP in the presence of irradiated normal syngeneic thymus cells as accessory cells and by T-cell growth factor-dependent expansion between the restimulations. They express the 'helper' cell phenotype (W3/25+)[24]. Within 24 h of culture in the presence of BP, BP-specific T cells of Lewis rat origin (L-BP) adhered tightly to the syngeneic astrocytes and formed aggregates of 10 and more lymphocytes per astrocyte (Fig. 1*a*). Within 48–72 h of culture, the adherent T cells transformed to large activated blast cells. This interaction was immune-specific: when ovalbumin (OVA) instead of BP was added to L-BP–astrocyte cultures, the T cells were randomly distributed, with no signs of physical contact with the astrocytes during observation periods of up to 72 h. Moreover, T-cell–astrocyte complexes were not formed when L-BP T cells were cultured in the presence of BP with MHC-congeneic L.BN astrocytes. The population of brain cells binding antigen-specific syngeneic T cells was identified as astrocytes by using GFAP as a marker (Fig. 1*b*, *c*). In either antigen or MHC-incompatible cell systems T lymphocytes remained in the state of small resting cells.

Table 1 Ia expression by astrocytes after specific interaction with T cells

Astrocyte treatment	Anti-Ia staining pattern of astrocytes of Lewis rats	L.BN rats
Con A	–	–
L-BP + BP	OX6, OX3 HL	–
L-BP + Con A	OX6, OX3 HL	OX6

BP-specific cells of Lewis rat origin (L-BP) were co-cultured with astrocytes and BP or Con A (5 μg ml^{-1}). Astrocyte cultures were double stained with anti-Ia monoclonal antibodies and anti-GFAP antiserum as described in Fig. 3 legend. Two anti-Ia antisera were used, the OX6 being an anti-Ia common determinant and the OX3 HL an anti-polymorphic determinant (Lewis-specific)[33]. –, No reactivity; OX6 or OX3 HL, positive staining of astrocytes with the anti-Ia antiserum OX6 or OX3 HL (Sera-Labs).

Morphological activation of astrocyte-associated T-cell lines resulted in their rapid proliferation. This was quantified by culturing T cells in the presence of both antigen and irradiated astrocytes and measuring ^{3}H-thymidine uptake. As a positive control, thymus was used as an optimal source of accessory cells. The results confirmed the microscopic observation. As shown in Fig. 2*a*, when L-BP T cells were cultured with astrocytes of Lewis rat origin in the presence of BP, ^{3}H-thymidine incorporation was highly elevated. The response was dependent on the number of astrocytes, with astrocyte-to-responder ratios lower than one. The response of L-BP T cells was dependent on the specific antigen BP, as no proliferation was observed when, as an irrelevant antigen, OVA was added to the T cells instead of BP (Fig. 2*a*). On the other hand, OVA was highly immunogenic to an OVA-specific Lewis T-cell line, when presented by Lewis astrocytes. The incorporation of ^{3}H-thymidine by 10^4 OVA-treated T cells was 33 ± 7 c.p.m. and in the presence of 10^4 irradiated syngeneic astrocytes 9,859 ± 2,469 c.p.m. These experiments demonstrate antigen specificity of astrocyte-dependent T-cell proliferation.

Antigen presentation by astrocytes was restricted by the MHC. In the presence of BP, significant incorporation of ^{3}H-thymidine by 10^4 responder L-BP T cells was produced by syngeneic Lewis rat astrocytes and astrocytes from (Lewis × BN)F_1 hybrids (Fig. 2*b*). No response was observed in the presence of MHC-congeneic L.BN astrocytes. These L.BN astrocytes, however, functioned perfectly well as accessory cells when added to a BP-specific T-cell line from a (Lewis × BN)F_1 rat (data not shown).

There is ample evidence that Ia antigens expressed by antigen-presenting cells regulate immune responsiveness in concert with the Ia-restricted T-cell receptor[25]. So far, Ia antigens have been found on cells having accessory function such as macrophages and dendritic cells. The MHC-restricted astrocyte-mediated activation of T helper lymphocytes would require the presence of Ia antigens on astrocytes also. It has been shown that the antigen recognition by our T-cell lines is only possible in the context of Ia antigen on the presenting cells, as evidenced by restriction experiments (E. Günther *et al.*, in preparation),

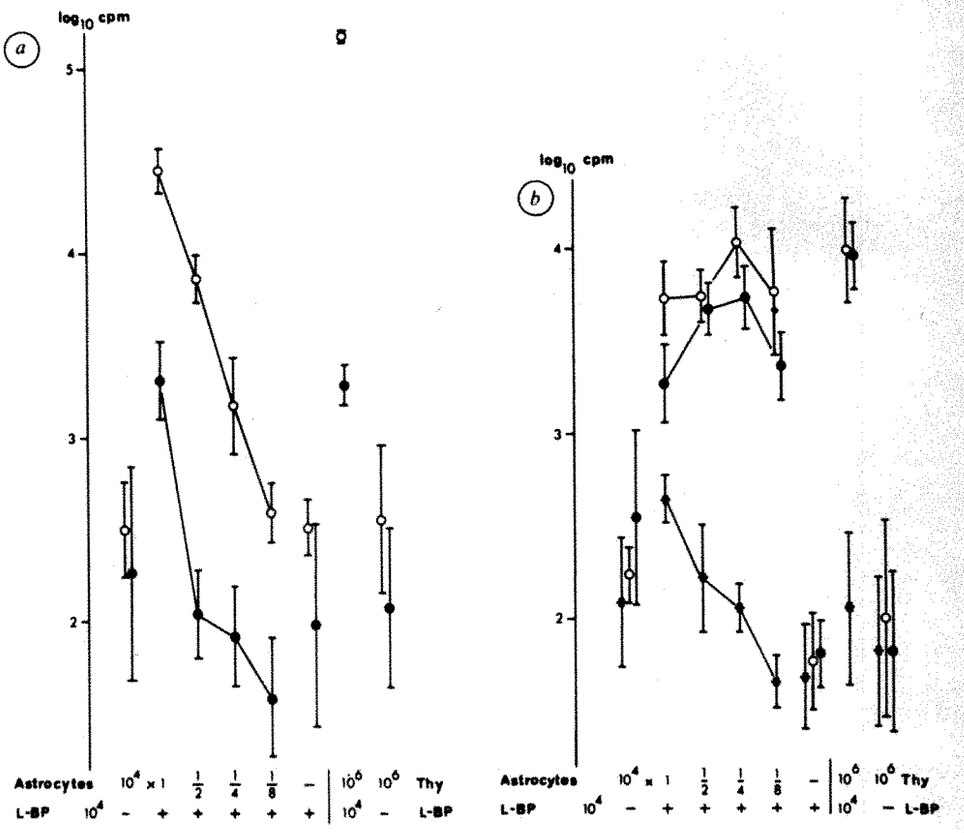


Fig. 2 *a*, L-BP T cells co-cultured with Lewis astrocytes or Lewis thymus cells in the presence of BP (○) or OVA (●). *b*, L-BP T cells added in the presence of BP to astrocytes or thymus cells of Lewis (○), L.BN (*) or (Lewis×BN)F_1 (●) origin. Data are given as $\log_{10}$ c.p.m. of ^{3}H-thymidine incorporation ±95% confidence limits.
Methods: BP-specific Lewis T-cell lines (L-BP) were prepared according to the method of Ben-Nun *et al.*[23]. Astrocytes were prepared as described in Fig. 1 legend, irradiated (3,000 rad) and co-cultured in quadruplicate in round-bottomed microtitre wells (0.2 ml) with L-BP T cells in the indicated concentrations. As a positive control, 10^6 irradiated thymus cells were used instead of astrocytes. The medium consisted of DMEM supplemented with 1% Lewis rat serum L-glutamine (300 μg ml^{-1}), L-asparagine (36 μg ml^{-1}) and indomethacin (1 μg ml^{-1}). ^{3}H-thymidine was added (0.2 μCi per well, specific activity 2 Ci mmol^{-1}) after 48 h of culture and cells were collected after another 14 h.

blocking experiments and adaptation of Ia from presenter cells by T-cell line during activation (H.W., in preparation). We have been unable to demonstrate Ia antigens on cultured naive astrocytes with anti-Ia antisera, but this does not exclude the possibility that a low concentration of functionally active Ia molecules is present. More important, however, we could demonstrate the expression of Ia antigens by astrocytes after specific contact with T lymphocytes (Table 1, Fig. 3). The induced Ia antigens were expressed by astrocytes as shown by double staining with anti-Ia and anti-GFAP antisera (Fig. 3) and by the phenotype of the expressed Ia following the genotype of the astrocytes rather than that of the lymphocytes. The allogeneic activation of the Lewis T-cell line L-BP by L.BN astrocytes and concanavalin A (Con A), which overrules MHC restriction, induced Ia antigens on GFAP-positive cells, which carried the non-Lewis phenotype (Table 1). The induction of Ia expression by astrocytes after specific contact with T lymphocytes, and perhaps also after other stimuli, could explain the controversial results of recent immune histological studies on Ia expression in mouse brain[26,27]. On human glioblastoma cells binding assays clearly revealed HLA-DR determinants[28].

Other investigators have shown that an Ia-positive subpopulation of macrophages and/or the dendritic cells are most active in antigen presentation[7–14]. Contamination of astrocyte cultures with dendritic cells is unlikely, as, in contrast to astrocytes, the rat dendritic cell is a nonadherent cell. Furthermore, it has been demonstrated in the rat that dendritic cells are widely distributed in the interstitial connective tissues (for example spleen, liver, skin) but have not been found in brain. At least a proportion of GFAP-negative cells detected in our cultures (0.01%) could represent macrophages. Using different techniques it was found that the non-passaged primary brain cell cultures contained small amounts of macrophages, while contamination with macrophages was not observed in the passaged cultures used for the T-cell proliferation assay. However, trace amounts of undetected contaminating macrophages or dendritic cells could be a possible source of error in our experiments. We regard this theoretical possibility as very unlikely. Evidence supporting the antigen-presenting function of astrocytes are both the finding of a MHC-restricted and antigen-specific physical contact observed between the astrocytes and the T cells, as well as the induction of Ia antigen expression by astrocytes exposed to activated T cells. In addition, previous studies showing the release of IL-1-like factors by mouse astrocytes stimulated with endotoxin[5], rat C6 glioma cells[6], rat primary astrocyte cultures (A.F., unpublished observation) and human glioblastoma cells[29] further substantiate the hypothesis that astrocytes are active accessory cells.



Fig. 3 To demonstrate Ia antigens, Lewis astrocytes were co-cultured with syngeneic BP-specific T cells and BP in round-bottomed microtitre wells as described in Fig. 2 legend. After 3 or 6 days of culture, cells were removed from the microtitre wells and stained with monoclonal anti-Ia antibodies[33] and rhodamine-conjugated rabbit anti-mouse immunoglobulin. Smears of stained cells were dried, fixed and stained for GFAP as described in Fig. 1 legend. The cells were examined for red fluorescence demonstrating cell-surface Ia antigens (*a*) and green fluorescence demonstrating intracellular GFAP (*b*). The relatively globular form of the astrocyte without long processes results from the incubation condition in the round-bottomed microtitre well where astrocytes in the cell pellet are prevented from adherence and spreading.

The role of astrocytes as accessory cells could be of primary importance in presenting cerebral antigens to infiltrating lymphocytes within inflammatory lesions of brains from patients with multiple sclerosis. This, and the finding that astrocytes themselves can be activated by factors released from stimulated lymphocytes[30], may connect two morphological hallmarks of the active multiple sclerosis lesion, that is, T-cell infiltration and astrocyte proliferation[1]. More generally, astrocytes may have a previously unrecognized physiological cell function pivotal to the generation of immune responses within the brain tissue. The central nervous system has been considered to be an immunologically privileged site as it lacks a lymphatic drainage and is shielded from the blood by the blood–brain barrier. At the site of this barrier an intense contact has been observed between astrocytes and the brain vascular tissue[1,31], and therefore an active contact between the immune system and the brain may directly depend on astrocyte function. Whether the accessory cell function of astrocytes is applicable to all or only a subpopulation of astrocytes such as process bearing type II cells (see ref. 32) remains to be determined.

We thank Miss P. Bourquin, Mrs R. Röhrig and Mrs E. Weber for technical assistance, Dr M. Schachner for gifts of anti-GFAP antisera and Dr Bologa-Sandru for anti-galactocerebroside antisera. The project was in part supported by the Swiss NSF (project no. 3.938–0.82) (to A.F.).

Received 26 September; accepted 8 November 1983.

1. Peters, G. *Handb. spez. path. Anat. Histol.* **13,** Pt 2A, 531–566 (1958).
2. Nyland, H., Matre, R., Mork, S., Bjerke, J. R. & Naess, A. *New Engl. J. Med.* **307,** 1643–1644 (1982).
3. Brinkman, C. J. J., Ter Laak, H. J., Hommes, O. R., Poppema, S. & Delmotte, P. *New Engl. J. Med.* **307,** 1644–1645 (1982).
4. Traugott, U., Reinherz, E. L. & Raine, C. S. *Science* **219,** 308–310 (1983).
5. Fontana, A., Kristensen, F., Dubs, R., Gemsa, D. & Weber, E. *J. Immun.* **129,** 2413–2419 (1982).
6. Fontana, A., McAdam, K. P. W. J., Kristensen, F. & Weber, E. *Eur. J. Immun.* **13,** 685–689 (1983).
7. Rosenthal, A. S. & Shevach, E. M. *J. exp. Med.* **138,** 1194–1197 (1973).
8. Rosenstreich, D. L., Farrar, J. J. & Dougherty, S. *J. Immun.* **116,** 131–139 (1976).
9. Persson, U., Hammarstrom, L., Möller, E., Möller, G. & Smith, C. I. *Immun. Rev.* **40,** 78–101 (1978).
10. Erb, P. *et al. J. Immun.* **125,** 2504–2508 (1980).
11. Habu, S. & Raff, M. C. *Eur. J. Immun.* **7,** 451–457 (1977).
12. Steinmann, R. A. & Cohen, Z. A. *J. exp. Med.* **137,** 1196–1212 (1973).
13. Klinkert, W. E. F., LaBadie, J. H., O'Brien, J. A., Beyer, C. F. & Bowes, W. E. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5414–5418 (1980).
14. Klinkert, W. E. F., LaBadie, J. H. & Bowers, W. E. *J. exp. Med.* **156,** 1–19 (1982).
15. Lipsky, P. E. & Kettman, J. R. *Immun. Today* **3,** 36–42 (1982).
16. Bignami, A., Eng, L. F., Dahl, D. & Uyeda, C. T. *Brain Res.* **43,** 429–435 (1972).
17. Manthorpe, M., Adler, R. & Varon, S. *J. Neurocytol.* **8,** 605–621 (1979).
18. Schachner, M., Smith, Ch. & Schoonmaker, G. *Devl Neurosci.* **1,** 1–14 (1978).
19. Bologa-Sandru, L. *et al. Brain Res.* **210,** 217–229 (1981).
20. Raff, M. C. *et al. Brain Res.* **174,** 283–308 (1979).
21. Raff, M. C. *et al. Nature* **274,** 813–816 (1978).
22. Brideau, R. J., Carter, Ph.B., MacMaster, W. R., Mason, D. W. & Williams, A. F. *Eur. J. Immun.* **10,** 609–615 (1980).
23. Ben-Nun, A., Wekerle, H. & Cohen, I. R. *Eur. J. Immun.* **11,** 195–199 (1981).
24. Ben-Nun, A. & Cohen, I. R. *J. Immun.* **129,** 305–308 (1982).
25. Benacerraf, B. *J. Immun.* **120,** 1809–1812 (1978).
26. Hart, D. N. J. & Fabre, J. W. *J. exp. Med.* **153,** 347–361 (1981).
27. Ting, J. P., Shigekawa, B. L., Linthicum, D. S. & Weiner, L. P. *Proc. natn. Acad. Sci. U.S.A.* **78,** 3170–3173 (1981).
28. Carrel, St., de Tribolet, N. & Gross, N. *Eur. J. Immun.* **12,** 354–357 (1982).
29. Fontana, A., Hengartner, H., de Tribolet, N. & Weber, E. *J. Immun.* (in the press).
30. Fontana, A., Otz, U., de Weck, A. L. & Grob, P. J. *J. Neuroimmun.* **2,** 73–81 (1981).
31. White, F. P., Dutton, G. R. & Norenberg, M. D. *J. Neurochem.* **36,** 328–332 (1981).
32. Raff, M. C., Miller, R. H. & Noble, M. *Nature* **303,** 390–396 (1983).
33. McMaster, W. R. & Williams, A. F. *Eur. J. Immun.* **9,** 426–433 (1979).
34. McCarthy, K. D. & de Vellis, J. *J. Cell Biol.* **85,** 891–902 (1980).

Hydroxyl radical scavengers inhibit human natural killer cell activity

Manikkam Suthanthiran, Scott D. Solomon, Perry S. Williams, Albert L. Rubin, Abraham Novogrodsky & Kurt H. Stenzel

Departments of Biochemistry and Medicine, The Rogosin Kidney Center, Cornell University Medical College, 1300 York Avenue, New York, New York 10021, USA

As natural killer (NK) cell activity is an essential constituent of host defence systems[1] and reactive oxygen intermediates participate in such defence[2–4], the effect of scavengers of oxygen radicals on NK cell activity was investigated. Hydroxyl radical ($OH^{\cdot}$) scavengers[5] (dimethyl sulphoxide (DMSO), thiourea, dimethylurea, tetramethylurea, benzoic acid, ethanol, methanol and ethylene glycol) inhibited NK cell activity. Catalase, a scavenger of H_2O_2, and superoxide dismutase (SOD), a scavenger of O_2^-, either alone or in combination, did not inhibit NK cell activity. Inhibition of the lipoxygenase pathway of arachidonic acid metabolism, a potential source of cellular $OH^{\cdot}$, with nordihydroguaiaretic acid[6] and 5,8,11,14-eicosatetraynoic acid (ETYA)[7] resulted in marked inhibition of NK cell activity. Inhibition of the cyclooxygenase pathway[7,8] with acetylsalicylic acid or indomethacin had minimal effects on NK cell activity. Taken together, these findings suggest that $OH^{\cdot}$, possibly generated via the lipoxygenase pathway of arachidonic acid metabolism, is critical for NK cell cytotoxicity.

NK cell activity is inhibited by a variety of structurally unrelated compounds that all share the property of being able to scavenge $OH^{\cdot}$ (Fig. 1). Statistically significant inhibition of NK cell activity mediated by large granular lymphocytes (LGL), which are the principal NK cells in peripheral blood, with MOLT-4 as target cells is shown in Fig. 1*a*, and with K-562 as target cells in Fig. 1*b*. Urea, which is structurally related to thiourea, tetramethylurea and dimethylurea, but is not an $OH^{\cdot}$ scavenger, did not inhibit NK cell activity even at 50 mM concentration. Other $OH^{\cdot}$ scavengers, such as ethanol, methanol and ethylene glycol, also mediated inhibition of NK cell activity. The alcohols are relatively less efficient scavengers[5] of $OH^{\cdot}$ and inhibition of NK cell activity required higher concentrations than for the other agents. Fifty per cent inhibition of NK cell activity was observed with LGL as effector cells and MOLT-4 or K-562 as target cells with the addition of methanol (315 mM), ethanol (80 mM) and ethylene glycol (320 mM) to the NK test system. None of the $OH^{\cdot}$ scavengers affected the binding capacity of LGL to target cells at concentrations that resulted in significant inhibition of NK cell activity as tested in the target cell-binding assay[9]. The target cell-binding assay was performed by incubating LGL with MOLT-4 or K562 at an effector to target ratio of 1:1. LGL (1×10^6 cells ml^{-1}) were incubated with target cells (1×10^6 cells ml^{-1}) for 30 min at 4 °C with or without various $OH^{\cdot}$ scavengers. The cells were then resuspended and a minimum of 200 effector cells were counted in a Neubaur Counting Chamber covered with an acridine orange-coated coverslip with a simultaneous UV light and phase contrast illumination to determine the number of effector–target conjugates. The use of acridine orange-coated coverslips and UV light[10] allowed us to clearly distinguish between effector and target cells.

The addition of Me_2SO (75 mM), thiourea (10 mM), tetramethylurea (10 mM), dimethylurea (25 mM), benzoic acid (10 mM), ethylene glycol (320 mM), ethanol (80 mM) and methanol (315 mM) to the NK test system did not result in significant alterations in the number of effector–target conjugates formed. Twenty-five–40% of LGL formed conjugates with the target cells in the presence or absence of $OH^{\cdot}$ scavengers.

Nonspecific toxicity of agents used as $OH^{\cdot}$ scavengers may reasonably be excluded as an explanation for their inhibition of NK cell activity as target cells released less ^{51}Cr in the presence of the $OH^{\cdot}$ scavengers. Several additional experiments were performed to exclude further the possibility that nonspecific toxic effects of $OH^{\cdot}$ scavengers were responsible for the inhibition. First, none of the $OH^{\cdot}$ scavengers affected the viability of LGL, as determined by Trypan blue exclusion at concentrations that mediated significant inhibition of NK cell activity (Table 1, protocol 1). Second, LGL pretreated with DMSO (150 mM), thiourea (50 mM) and tetramethylurea (25 mM) and subsequently washed before being used as effector cells, exhibited normal NK cell activity (Table 1, protocol 2). The third and perhaps most compelling evidence against nonspecific toxicity

0028–0836/84/030276—03$01.00

Table 1 Lack of nonspecific toxicity of OH· scavengers

Protocol	DMSO (150)	Thiourea (50)	OH· scavenger (mM) Ethylene glycol (320)	Methanol (630)	Tetramethylurea (25)
(1) Cell viability	100 (3)	100 (3)	100 (3)	100 (3)	100 (3)
(2) Pretreatment	130 ± 10 (4)	113 ± 9 (4)	ND	ND	125 ± 13 (4)
(3) Antibody-dependent cell-mediated cytotoxicity	103 ± 6 (6)	87 ± 9 (10)	81 ± 8 (3)	86 ± 5 (3)	ND

Protocol 1: Cell viability was determined using a Trypan blue exclusion assay. Per cent of viable cells found in three experiments is shown. *Protocol 2*: Large granular lymphocytes were incubated with OH· scavengers for 3 h at 37 °C, washed three times with PBS, then used as effector cells with MOLT-4 as target cells. Results are expressed as per cent specific ^{51}Cr release found with untreated LGL as effector cells. *Protocol 3*: Antibody-dependent cell-mediated cytotoxicity (ADCC) was determined with peripheral blood mononuclear cells as effector cells, and antibody-coated chicken red blood cells as target cells in a 3-h ^{51}Cr release assay[23] (effector:target ratio is 5:1). Results are expressed as per cent specific ^{51}Cr release found in OH· scavenger-free ADCC. Values shown are % of control value (mean ± s.e.) with the number of experiments given in parentheses. ND, not determined.

of OH· scavengers is our finding of intact antibody-dependent cell-mediated cytotoxicity (ADCC) activity in the presence of DMSO (150 mM), thiourea (50 mM), ethylene glycol (320 mM) or methanol (630 mM) (Table 1, protocol 3). The alterations in NK cell activity found with preincubation of LGL with OH· scavengers (protocol 2) and the effect of OH· scavengers on ADCC (protocol 3) were not significant by Student's *t*-test for two means. Another argument against a nonspecific toxicity of OH· scavengers is our earlier finding of intact phytohaemagglutinin (PHA) and concanavalin A (Con A)-induced mitogenesis of human peripheral blood mononuclear cells in the presence of OH· scavengers at concentrations that resulted in the inhibition of NK cell activity[11].

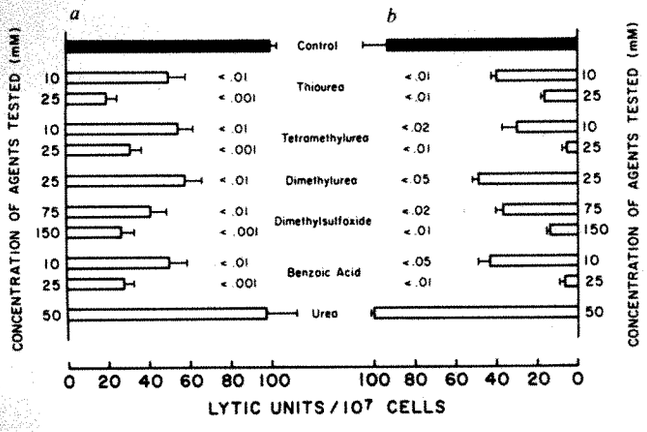


Fig. 1 Inhibition of NK cell activity by OH· scavengers. Large granular lymphocytes (LGL) isolated from the non-adherent fractions of peripheral blood mononuclear cells by centrifugation on a discontinuous Percoll density gradient by the method of Timonen and Saksela[24] were used as effector cells. Adherent cell contaminating peripheral blood mononuclear cells were removed by passage over a Sephadex G-10 column as described previously[25]. The NK cell preparations contained 0.1% monocytes or polymorphonuclear leukocytes as determined by nonspecific esterase staining[26] or by scoring LGL in a Neubaur Counting Chamber covered with an acridine orange-coated coverslip with simultaneous UV light and phase contrast illumination[10]. Cell lines, MOLT-4 or K-562, maintained as suspension cultures, were labelled with ^{51}Cr and used as target cells in a 3-h ^{51}Cr release assay[23]. The per cent specific release (SCR) was calculated by the formula:

$$\frac{\text{experimental counts} - \text{spontaneous counts}}{\text{maximum counts}} \times 100$$

The cytotoxicity assay was established at multiple effector:target ratios and dose–response curves were generated by plotting per cent SCR versus the number of effector cells to compute lytic units. The number of effector cells needed to lyse 30% of the target cells was designated as one lytic unit. The effect of OH· scavengers on NK cell activity was determined by adding various agents, at concentrations shown, to the cytotoxicity assay. Results are mean (±s.e.) of four experiments with MOLT-4 as target cells (*a*) and results of three experiments with K-562 as target cells (*b*). Statistical significance of inhibition mediated by OH· scavengers was determined using Student's *t*-test for two means.

A potential source of OH· is the univalent reduction of molecular oxygen. In the monovalent pathway of oxygen reduction, superoxide anion (O_2^-) is produced first, followed by the formation of hydrogen peroxide[12]. O_2^- can interact with H_2O_2 to form OH·, as originally proposed by Haber and Weiss[13]. As O_2^- and H_2O_2 can serve as precursors of OH·, we examined the effect of SOD, a scavenger of O_2^-, and catalase, a scavenger of H_2O_2, on NK cell activity. The results obtained by adding catalase (1,000 and 5,000 units ml^{-1}) or SOD (50 and 250 μg ml^{-1}), or catalase (5,000 units ml^{-1}) plus SOD (250 μg ml^{-1}) to the NK test system are summarized in Fig. 2. Catalase and SOD did not inhibit NK activity either alone or in combination. The inability of catalase to inhibit NK activity agrees with previous reports[14]. The lack of inhibitory effect of SOD on NK activity, however, does not support the findings of Roder *et al.*[14], that SOD inhibited NK activity mediated by LGL using K-562 as target cells. In multiple experiments, we found that SOD, over a wide range of concentrations (50–250 μg ml^{-1}), failed to inhibit NK cell activity mediated by peripheral blood mononuclear cells, the non-adherent fraction of peripheral blood mononuclear cells or LGL, using either K-562 or MOLT-4 as target cells, in our experimental conditions.

The finding that OH· scavengers inhibit NK cell activity, while scavengers of O_2^- and H_2O_2 (potential precursors of OH·) do not, suggests several possibilities. First, OH· might be generated at the interplasma membrane space between the effector and target cells and catalase or SOD might not gain access to this space. Second, OH· might be generated at an intracellular site;

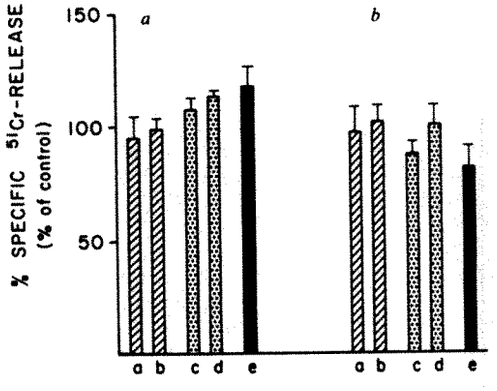


Fig. 2 Effect of catalase or SOD on human NK cell activity. *a*, Results of three experiments (mean ± s.e.) with LGL as effector cells and MOLT-4 as target cells. *b*, Results of five experiments with K-562 target cells. Effector:target ratio 25:1. a and b: catalase 1,000 and 5,000 units ml^{-1} respectively; c and d: SOD 50 and 250 μg/ml respectively; e: catalase 5,000 units ml^{-1} + SOD 250 μg ml^{-1}.) Per cent of SCR without catalase or SOD (100% values) was 43 ± 2% with MOLT-4 as target cells and 38 ± 4% with K-562 as target cells.

Table 2 Effect of inhibitors of arachidonic acid metabolism on NK cell activity

Agent tested	Concentration (μm)	Target cells: K-562 Lytic units per 10^7 cells	P	Target cells: Molt-4 Lytic units per 10^7 cells	P
None		174±18	P	96±6	P
NDGA	10	46±2	<0.01	23±3	<0.01
NDGA	25	15±4	<0.01	9±2	<0.01
ETYA	10	130±15	NS	65±13	<0.02
ETYA	25	72±13	<0.02	33±3	<0.02
ETYA	50	38±6	<0.01	ND	
Indomethacin	1	143±16	NS	81±10	NS
Indomethacin	10	138±19	NS	71±7	NS
Acetylsalicylic acid	100	149±16	NS	85±12	NS
Acetylsalicylic acid	1,000	135±17	NS	73±9	NS

LGL functioned as effector cells and ^{51}Cr-labelled K-562 or MOLT-4 were used as target cells. Inhibitors of arachidonic acid metabolism were added, at concentrations shown, to the NK test system. Lytic units were calculated as described in Fig. 1 legend. Results (mean±s.e.) of three experiments. *P* determined by Student's *t*-test for two means. NS, not significant; ND, not determined.

a site that is also inaccessible to catalase or SOD. Third, $OH^{\cdot}$ might also be generated during arachidonic acid metabolism[15]. Besides being the source of $OH^{\cdot}$, additional interactions between $OH^{\cdot}$ and arachidonic acid can also be envisioned. Specifically, arachidonate may be oxidized by $OH^{\cdot}$ and the resulting metabolic products might be responsible for the cytolytic activity of NK cells. The findings that phospholipase A_2 is activated during human NK cell cytotoxicity[16] and that horse eosinophil degranulation might depend on calcium-induced activation of phospholipase A_2 and generation of lipoxygenase products of arachidonic acid metabolism[17], lend credence to the latter hypothesis. As arachidonic acid metabolism might be involved in NK cell activity either as a source of oxygen-centred radicals or of their metabolic products, either directly or indirectly, the effect of various inhibitors of arachidonic acid metabolism on NK cell activity was investigated. Nondihydroguaiaretic acid (NDGA), an inhibitor of the lipoxygenase pathway of arachidonic acid metabolism, and ETYA which inhibits both cyclooxygenase and lipoxygenase pathways, mediated significant inhibition of NK cell activity (Table 2). In contrast, acetylsalicylic acid and indomethacin, which inhibit the cyclooxygenase pathway, failed to mediate significant inhibition of NK activity.

How might $OH^{\cdot}$ participate in NK cell activity of LGL? The possibility that $OH^{\cdot}$ contributes directly to target cell lysis by being generated at the interplasma membrane space between target cells and effector cells cannot be rigorously excluded. However, involvement of $OH^{\cdot}$ in earlier steps in the sequence of events leading to target cell lysis seems more likely. This latter notion is supported by the findings of defective NK cell activity in Chediak-Higashi patients, whose cells generate reactive oxygen intermediates in response to NK-susceptible targets[14] but are inefficient as NK cells, suggesting that free radical generation alone might not be sufficient to mediate cytotoxicity. In the context of a 'stimulus–secretion' model for NK cell activity[9,18,19], and in view of evidence that lysosomal enzymes may be the actual lytic moiety[1,9,18], $OH^{\cdot}$ participation may be via release of potent lysosomal enzymes either by direct lysosomal membrane peroxidation[20] or by a cyclic nucleotide-regulated mechanism[21,22]. Alternatively, $OH^{\cdot}$ might also participate in NK cell cytotoxicity via lipoxygenase pathway products. Clearly, further work is required to elucidate the role of $OH^{\cdot}$ in the complex biochemical mechanisms leading to target cell lysis in the NK cell system. It is already clear, however, that $OH^{\cdot}$ or a closely related species of free radical is involved in the metabolic programme pertinent to acquisition of lytic potential by NK cells.

We thank Ms Monica Evelyn and Ms Regina Vogel for technical assistance and Ms Ann Seton and Ms Jennifer Stenzel for secretarial help in the preparation of this manuscript. This work was supported in part by Sylvan League Fund and by Helena Rubinstein and Talisman Foundation.

Received 12 May; accepted 25 October 1983.

1. Herberman, R. B. *NK Cells and Other Natural Effector Cells* (Academic, New York, 1982).
2. Babior, B. M. *New Engl. J. Med.* **298,** 659–668, 721–725 (1978).
3. Klebanoff, S. J. *Semin. Hemat.* **12,** 117–142 (1975).
4. Badwey, J. A. & Karnovsky, M. L. *A. Rev. Biochem.* **49,** 695–726 (1980).
5. Dorfman, L. M. & Adams, G. E. *Natn. Stand. Ref. Data Serv. U.S.A.* **46,** 1–59 (1973).
6. Tappel, A. L., Lundberg, W. & Boyer, P. D. *Am. Biochem. Biophys.* **42,** 293–304 (1953).
7. Ahern, D. G. & Downing, D. T. *Biochim. biophys. Acta* **210,** 456–461 (1970).
8. Flower, R. J. *Pharmac. Rev.* **26,** 33–67 (1974).
9. Roder, J. C. *et al. Immunology* **40,** 107–116 (1980).
10. Suthanthiran, M. *et al. Regulatory Mechanisms in Lymphocyte Activation* (ed. Lucus, D. O.) 362–364 (Academic, New York, 1976).
11. Novogrodsky, A., Ravid, A., Rubin, A. L. & Stenzel, K. H. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1171–1174 (1982).
12. Fridovich, I. *Science* **201,** 875–880 (1978).
13. Haber, F. & Weiss, J. *Proc. R. Soc.* **A147,** 332–351 (1934).
14. Roder, J. C. *et al. Nature* **298,** 569–572 (1982).
15. Kuehl, F. A. Jr & Egan, R. W. *Science* **210,** 978–984 (1980).
16. Hoffman, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 3839–3843 (1981).
17. Henderson, W. R., Chi, E. Y., Jorg, A. & Klebanoff, S. J. *Am. J. Path.* **111,** 341–349 (1983).
18. Carpen, O., Virtanen, I. & Sakesla, E. *Cell Immun.* **58,** 97–106 (1981).
19. Quan, P. C., Ishizaka, I. & Bloom, B. R. *J. Immun.* **12B,** 1786–1791 (1982).
20. Fong, K. L., McCay, P. B., Poyer, J. L., Keele, B. B. & Misra, H. *J. biol. Chem.* **248,** 7792–7797 (1973).
21. Mittal, C. K. & Murad, F. *Proc. natn. Acad. Sci. U.S.A.* **74,** 4360–4364 (1977).
22. Haddox, M. K., Stephenson, J. H., Moser, M. E. & Goldberg, N. D. *J. biol. Chem.* **253,** 3143–3152 (1978).
23. Suthanthiran, M., Stenzel, K. H., Rubin, A. L. & Novogrodsky, A. *Cell Immun.* **50,** 379–391 (1980).
24. Timonen, T. & Saksela, E. *J. immunol. Meth.* **36,** 285–291 (1980).
25. Novogrodsky, A., Rubin, A. L. & Stenzel, K. H. *J. Immun.* **122,** 1–7 (1979).
26. Li, C. Y., Lam, K. W. & Yam, L. T. *J. histochem. Cytochem.* **21,** 1–12 (1973).

Viral perturbation of endocrine function: disordered cell function leads to disturbed homeostasis and disease

Michael B. A. Oldstone*, Moses Rodriguez†, William H. Daughaday‡ & Peter W. Lampert†

* Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037, USA
† Department of Pathology, University of California, San Diego, La Jolla, California 92093, USA
‡ Metabolism Division, Washington University School of Medicine, St Louis, Missouri 63110, USA

Virus-induced disease occurs both through direct destruction of cells by viruses and/or secondarily through lysis of infected cells by immunological assault[1–5]. We asked whether viruses could also cause injury and disease by altering the cell's normal or expected functions without destroying the cells. Here we show that a relatively noncytopathic virus can perturb endocrine functions by disordering the synthesis of a hormone needed for growth and glucose regulation, while replicating in a specialized cell making that hormone. Yet, despite viral replication and alteration in synthesis of the specialized cell's homeostatic product, the infected cell remains free from structural injury.

0028–0836/84/030278—04$01.00

Newborn C3H/St, C3H/HeJ, C57Br/cdj, CBA/N, AKR/J and BALB/kae nude mice infected with 60 plaque-forming units (PFU) of lymphocytic choriomeningitis virus (LCMV) Armstrong strain 1371 had clear-cut developmental retardation manifested as decreases in body weight and length[6] that became evident as early as 5 to 7 days after viral inoculation. Pituitaries from infected mice had viral antigens but were structurally normal (Fig. 1*A*). Despite the presence of viruses in these cells, there was no evidence of altered anatomy, cell lysis or inflammatory infiltrates by low power microscopy; high resolution microscopy showed normal cell morphology and structure[7,8].

Using monoclonal antibodies[9] directed against LCMV polypeptides, we demonstrated the restriction of viral replication to the pituitary glands' anterior lobe (Fig. 1*B*). Application of a two-fluorochrome system[10] designed to stain LCMV nucleoprotein with one dye and either growth hormone (GH) or prolactin (PL) with the other revealed LCMV replicating primarily in those cells that made GH and not in cells making PL (Fig. 1*C–G*). Other studies showed that LCMV did not replicate in cells making thyroid stimulating hormone or adrenocorticotrophic hormone[7,8].

The two major activities of GH are regulation of growth and glucose metabolism[11,12]. Accordingly, mice persistently infected with LCMV ARM 1371 underwent significant decreases in body length and weight and became defective in glucose metabolism (Table 1, Fig. 2). Simultaneously, GH levels diminished in pituitary glands of infected mice, but PL levels remained normal. Serum contains insulin-like growth factors (IGFs) at concentrations dependent on GH[13]. Measurement of IGF-I[14] in serum from 16 individual LCMV-infected C3H/St mice yielded a concentration of 0.15 ± 0.02 U ml^{-1} (mean ± 1 s.e.) as compared with mean values from 20 uninfected matched controls of 0.45 ± 0.04 U ml^{-1} (d.f. 34, $P < 0.001$). These results further support a generalized GH deficiency. In the sera, cortisol and insulin levels were normal, but blood glucose decreased markedly (Table 1). The rise in blood sugar after glucose administration in LCMV-infected mice was greater than in uninfected mice (Fig. 2). Histopathological analysis of the pancreas, specifically islets of Langerhans cells, showed normal cell structure and morphology (Fig. 1*H*) by both light and electron microscopy in over 98% of the 500 islets studied. Approximately 2% of islets had cellular infiltrates, but with minimal necrosis. Viral antigen appeared both in accinar cells of the pancreatic ductal tissue and in the β cells of the islets of Langerhans. The amount of insulin in pancreases of LCMV-infected mice ($n = 5$, mean ± 1 s.e.: 0.33 ± 0.02 μU of insulin per g of pancreas) was equivalent to that found in uninfected matched 15-day-old C3H/St controls (0.34 ± 0.04).

To demonstrate definitively the role of GH deficiency in the metabolic disorder of mice infected with LCMV, cloned GH-producing cells (GH-3)[15] were transplanted into 6-day-old nude mice infected at birth with 100 PFU of LCMV. Figure 3 shows that the LCMV-infected recipients of GH grew normally and survived at the rate expected for uninfected age- and sex-matched controls. In contrast, infected littermates without GH transplants died from severe hypoglycaemia associated with their GH deficiency. Mice persistently infected with LCMV and with replaced GH had normal blood glucose levels (infected mice with replaced GH: blood sugar (mg per dl: mean ± 1 s.e.), 128 ± 5, uninfected matched controls 134 ± 8, $P = 0.4$, d.f. $= 14$), and normal glucose tolerance tests (Fig. 4). Hence, the severe disease was caused by GH deficiency associated with LCMV replication in GH-producing cells and was corrected by replacing GH in the virus-infected mice.

There are three primary ways in which viruses could alter cellular function. First, as a result of infection, the cell might release a product like interferon (IFN) that would modulate its function. However, IFN does not have a role in the GH deficiency due to LCMV infection. In our experiments, antibody to mouse IFN that significantly blocked IFN-associated glomerular membrane and liver disease associated with LCMV infection[16–19] (F. J. Dutko and F. Chisari, manuscript in prepar-

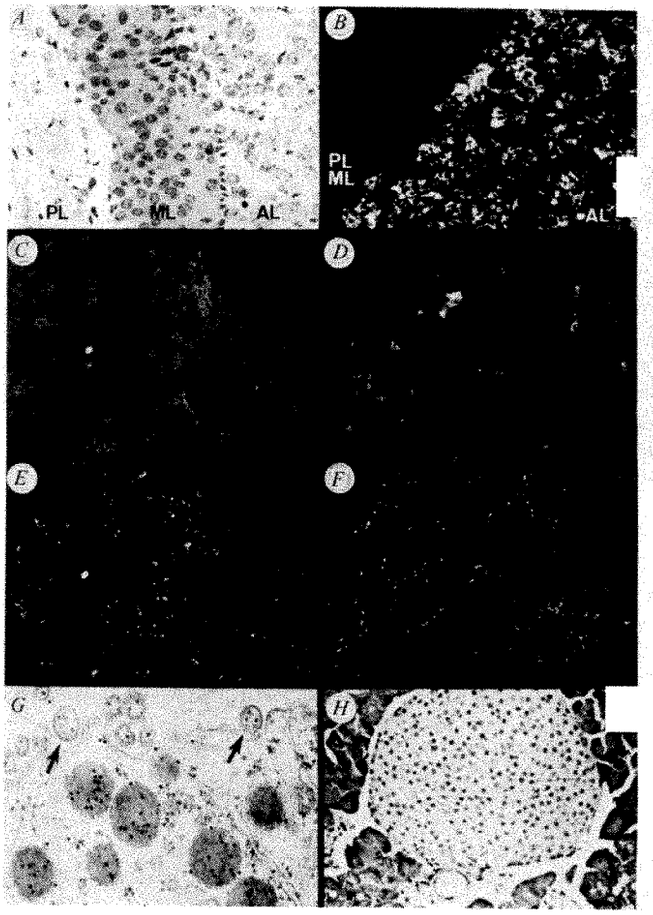


Fig. 1 Histopathological and immunochemical studies of endocrine tissues and cells taken from 15–18-day-old C3H/St mice persistently infected by injection at birth with 60 PFU of LCMV intracerebrally. Intraperitoneal inoculations gave corresponding findings. All mice carried infectious virus as determined by either PFU assay or intracerebral transfer of their sera into 4-week-old susceptible mice. *A*, Normal cell structure and relationships of anterior (AL), middle (ML) and posterior (PL) pituitary lobes. No cell destruction or inflammatory infiltrates are present. *B*, LCMV antigen expression is restricted to cells of the anterior lobe (AL) of the pituitary gland and not to the middle or posterior lobes. Four-micrometre cryostat-cut sections of the pituitary gland were stained with monoclonal antibody to LCMV nucleoprotein and fluorescein isothiocyanate (FITC) dye. Results were similar in over 50 pituitaries from individual LCMV-infected mice examined with these reagents or with a guinea pig polyclonal antibody to LCMV. *C*, *E* represent an identical tissue section. In *C*, tissue is stained with a monospecific antibody to growth hormone (GH) and FITC, while in *E*, tissue is stained with a monoclonal antibody to LCMV nucleoprotein and a rhodamine dye. Results were similar when a monoclonal antibody to mouse GH–FITC was substituted for the monkey antibody to mouse GH. Note similarity of staining pattern indicating that LCMV is replicating in cells making GH. *D*, *F* represent a tissue section stained either with monospecific antibody to prolactin (PL) and FITC or a monoclonal antibody to LCMV nucleoprotein and rhodamine dye (*F*). Note dissimilarity of staining pattern indicating that LCMV is not replicating in PL-containing cells. *G* Demonstrates the replication of LCMV in GH-containing cells of the anterior lobe of the pituitary gland. GH in secretory granules was labelled using monkey antibody to mouse GH and goat antibody to monkey IgG conjugated to gold particles (×17,500). Of over 100 cells studied in the pituitary gland, LCMV (arrows) budded from only those cells with GH-containing vesicles. *H* Illustrates the normal cytomorphology of islet of Langerhans in the pancreas and absence of cell lysis or inflammatory infiltrates. (For methods for plaquing virus, conjugation using two fluorochrome dyes, immunofluorescence, immunoelectron microscopy and light microscopy see refs 7, 8, 10, 25.)

Table 1 Perturbation of growth and glucose metabolism associated with persistent LCMV infection of cells producing growth hormone in the anterior pituitary

	No. of mice†	C3H/St mice* at Day 5 Uninf.	Day 5 Inf.	Day 5 P§	Day 15 Uninf.	Day 15 Inf.	Day 15 P
Growth							
Length (cm)	20	2.8±0.03‡	2.5±0.02	<0.01	4.2±0.05	3.3±0.08	<0.001
Weight (g)	50	3.3±0.1	3.2±0.1	NS	7.7±0.1	4.1±0.1	<0.001
Pituitary gland							
Growth hormone	12	5.3±1	6.0±1	NS	16.2±2	7.0±1	<0.001
Prolactin	6	0.3±0.02	0.3±0.03	NS	0.3±0.04	0.3±0.05	NS
Blood							
Glucose (ng dl^{-1})	34	ND	ND	ND	144±20	43±5	<0.001
Insulin (μU ml^{-1})	20	ND	ND	ND	28±2	35±5	NS
Insulin-like growth factor (U ml^{-1})	16	ND	ND	ND	0.45±0.04	0.15±0.02	<0.001
Cortisol (ng ml^{-1})	20	ND	ND	ND	11±1	9±1	NS
Glucose tolerance test (abnormal)	10	ND	ND	ND	0/12	10/10	<0.001
Sodium (mequiv. l^{-1})	15	ND	ND	ND	141±6	142±6	NS
Potassium (mequiv. l^{-1})	15	ND	ND	ND	5.6±0.1	5.1±0.2	NS

Results from the pituitary gland are in μg pituitary hormone per mg of pituitary gland. Pituitary hormones were isolated, using 10% acrylamide base gel and quantitated by radioimmune assay as described by Sinha[22,23]. Mean values for individual pituitary glands were found. Blood samples from individual mice were assayed. Glucose, sodium and potassium concentrations were determined in 5 to 20 μl of serum using microautomatic Beckman Astra-8. Cortisol and insulin determinations were by radioimmune assay and a competitive inhibition technique. For glucose tolerance tests each mouse received 2 mg glucose per g of body weight, intraperitoneally[24]. Samples obtained from each mouse before glucose challenge and 1 h afterward were tested for glucose and, when sufficient material was left, for insulin and cortisol levels. The numerator represents the number of mice showing a twofold or greater increment in glucose levels 1 h after a measured glucose challenge. The denominator shows the total number of mice studied. Radioimmune assay was used for insulin-like growth factor[20]. NS, not significant; ND, not done.

* Within 18 h of birth, mice were inoculated with 60 PFU of LCMV Armstrong 1371.

† Minimal number of mice per group. ‡ Mean ± 1 s.e.m. § *P* value, NS, not significant difference.

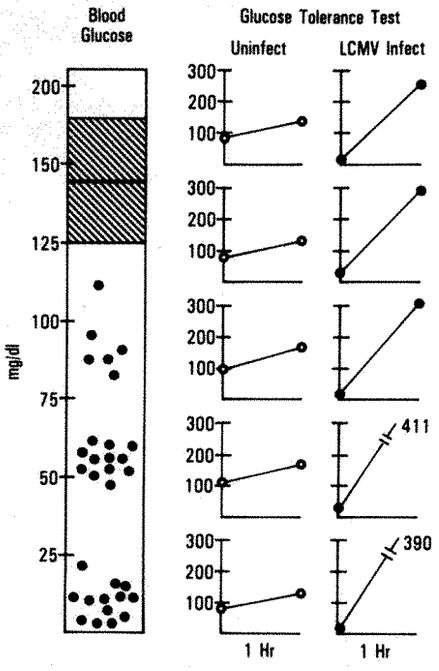


Fig. 2 Perturbation of glucose metabolism in 15-day-old C3H/St mice persistently infected with LCMV. On the left is the mean glucose value of 144 mg dl^{-1} (heavy horizontal line) and 2 s.e. above and below mean (stripped areas) in 48 age- and sex-matched uninfected controls. The blood glucose values for individual C3H/St mice persistently infected with LCMV (●) are shown. On the right are 1-h glucose tolerance tests (GTT) performed on uninfected and LCMV infected mice. Of the 12 uninfected mice studied, none developed twofold increases by 1 or 2 h after receiving 2 mg glucose per g body weight. In contrast, all 10 LCMV-infected mice showed far greater than twofold enhancement in similar conditions. Values for five other LCMV-infected mice not diagrammed were: pre-glucose challenge–post-glucose challenge: 48–570; 60–356; 52–184; 56–334; 52–314.

ation) failed to alleviate deficiencies in either growth or glucose metabolism. Further, IFN levels in the pituitary gland were similar in virus-infected and uninfected matched controls (less than 1.2 units of IFN per g pituitary tissue). Second, the virus might alter cell function by changing the structure of GH. Peptide maps of GH developed by using trypsin or chymotrypsin digests failed to show any structural differences in GH isolated from pituitary glands of infected or uninfected mice (data not shown). Thus, LCMV does not appear to modify the GH polypeptide structurally, although there are no data as yet on the functional activity of these molecules. Third, and most likely, viruses may affect the synthesis of GH at transcriptional or translational levels. Thus, the cells' capacity for membrane translocation and synthesis may be limited so that production of viral gene products competes with manufacture of GH, thereby overloading the system. Alternatively, the virus may induce a specific alteration in an enzyme or transport system needed for the production and release of GH. Work is continuing to investigate these and other possibilities.

Considering the enormous variety of human diseases of which aetiologies are still unknown, it is interesting to speculate that many of these could be caused by failure of the specialized or differentiated function of a cell due to a virus infection. This would include disorders involving faulty manufacture of immune regulators, insulin or other hormones, or neurotransmitters. Others reported[20,21] that viruses can lyse differentiated cells, thereby causing disease. In contrast, we show here that virus causes pathology without cytocidal injury. This may signal a search for viruses in a variety of disorders even in the absence of defined anatomic or morphological changes.

This is Publication No. 3116-IMM from the Scripps Clinic and Research Foundation and was supported by USPHS grants NS-12428, AI-09484 and AG-04342. We thank Drs Yogi Sinha for performing the radioimmune assay of prolactin and Ion Gresser for providing antibody to mouse interferon, Roxanne Fisher, Antoinette Tishon, Paul Blount, Gary McDaniel, Janet Anderson and Robert Garret for technical contributions, and Phyllis Minick and Sally Booz for aid in manuscript preparation.

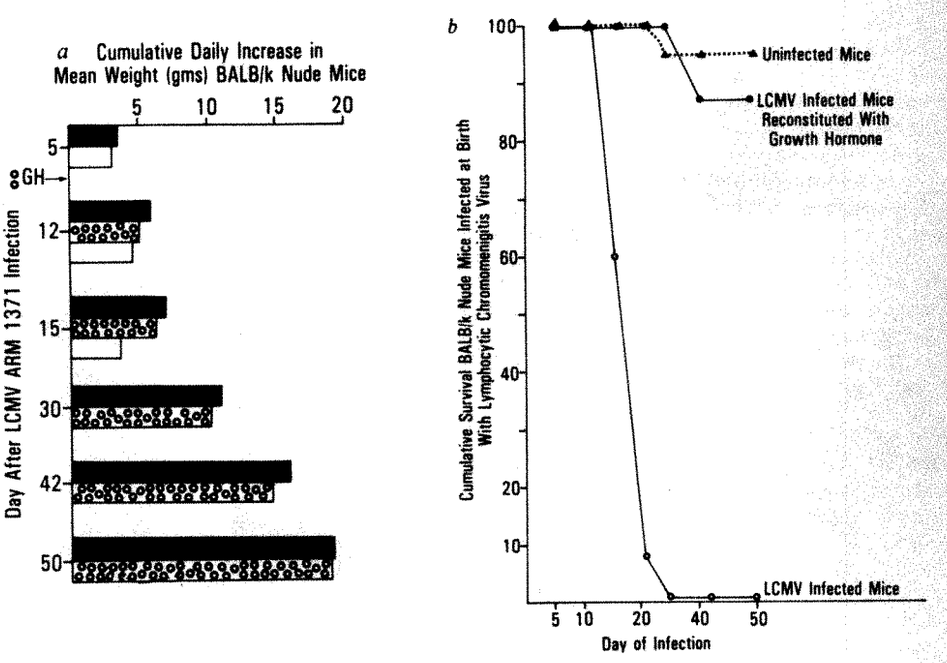


Fig. 3 Transplantation with GH-producing cells corrected the growth deficiency of mice persistently infected with LCMV and allowed a survival rate comparable with that of uninfected age- and sex-matched controls. For these experiments, littermates receiving LCMV at birth were segregated, at random, into three groups. One group received 1×10^7 GH-3 cells (making about 7,800 ng of GH per 24 h in culture) intraperitoneally, initially and twice weekly thereafter. A second group received intraperitoneally, either phosphate-buffered saline or cells not making GH. The third group was not inoculated at all. Each group contained a minimum of eight mice and was handled identically. ■, Uninfected control; □, LCMV-infected but not GH replaced; ⊠, LCMV-infected and GH replaced. Cumulative survival of such 50-day-old uninfected mice and LCMV-infected mice reconstituted with GH exceeded 80%. In contrast, the survival rate of LCMV-infected mice not without replaced GH was <5%.

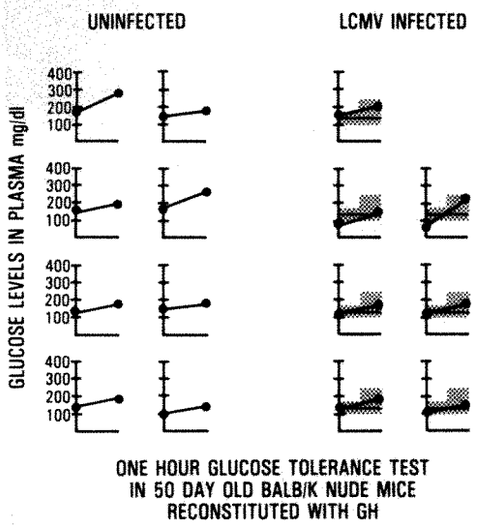


Fig. 4 Replacement with GH normalized the glucose metabolism of mice persistently infected with LCMV. Results of the glucose tolerance test consisted of pre-test samples compared with serum samples taken 1 h after challenge with 2 mg of glucose per g of body weight, intraperitoneally. LCMV-infected mice: horizontal bar represents the mean blood glucose levels of 10 uninfected age matched controls (2 not shown); the shaded area represents 2 s.e.m.

Received 2 August; accepted 4 October 1983.

1. Fenner, F., McAuslan, B., Mims, C., Sambrook, J. & White, D. *The Biology of Animal Viruses*, 2nd edn (Academic, New York, 1974).
2. Notkins, A. (ed.) *Viral Immunology and Immunopathology* (Academic, New York, 1975).
3. Bablanian, R. *Prog. med. Virol.* **19**, 40–83, 1975.
4. Blackwood, W. & Cornsellis, J. (eds) *Neuropathology* (Edward Arnold, London, 1976).
5. Robbins, S., Angell, M. & Kumar, V. *Basic Pathology* (Saunders, Philadelphia, 1981).
6. Doyle, L. B., Doyle, M. V. & Oldstone, M. B. A. *Immunology* **40**, 589–596 (1980).
7. Rodriguez, M., von Wedel, R. J., Garrett, R. S., Lampert, P. W. & Oldstone, M. B. A. *Lab. Invest.* (in the press).
8. Oldstone, M. B. A. *et al. Science* **218**, 1125–1127 (1982).
9. Buchmeier, M. J., Lewicki, H. A., Tomori, O. & Oldstone, M. B. A. *Virology* **113**, 73–85 (1981).
10. Oldstone, M. B. A. & Buchmeier, M. J. *Nature* **300**, 360–362 (1982).
11. Guyton, A. *Textbook of Medical Physiology*, 6th edn (Saunders, Philadelphia, 1981).
12. Bondy, P. & Rosenberg, L. *Metabolic Control and Disease*, 8th edn (Saunders, Philadelphia, 1980).
13. Rinderknecht, E. & Humbel, R. *J. biol. Chem.* **253**, 2769–2776 (1978).
14. Daughaday, W., Parker, K., Borowsky, S., Trevedi, B. & Kapadia, M. *Endocrinology* **110**, 575–581 (1982).
15. Tashjian, A., Yasumura, Y., Levine, L., Sato, G. & Parker, M. *Endocrinology* **82**, 342–352 (1968).
16. Gresser, I., Morel-Maroger, L., Maury, C., Tovey, M. & Pontillon, F. *Nature* **263**, 420–422 (1976).
17. Gresser, I., Morel-Maroger, L., Verroust, P., Riviere, Y. & Guillon, J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3413–3916 (1978).
18. Riviere, Y. *et al. J. exp. Med.* **152**, 663–640 (1980).
19. Dutko, F. J. & Oldstone, M. B. A. *J. gen. Virol.* (in the press).
20. Notkins, A. L. *Scient. Am.* **241**, 62–73 (1979).
21. Onodera, T. *et al. J. exp. Med.* **153**, 1451–1473 (1981).
22. Sinha, Y., Lewis, J. & Vanderlaan, W. *J. Endocr.* **55**, 1–9 (1972).
23. Sinha, Y., Selby, F., Lewis, U. & Vanderlaan, W. *Endocrinology* **91**, 784–792 (1972).
24. Onodera, T., Jenson, A., Yoon, J. & Notkins, A. *Science* **201**, 529–531 (1978).
25. Buchmeier, M. J., Welsh, R. M., Dutko, F. J. & Oldstone, M. B. A. *Adv. Immun.* **30**, 275–331 (1980).

Homologous cell-derived oncogenes in avian carcinoma virus MH2 and murine sarcoma virus 3611

Hans W. Jansen, Rudi Lurz & Klaus Bister*

Max-Planck-Institut für Molekulare Genetik, D-1000 Berlin 33 (Dahlem), FRG

Tom I. Bonner, George E. Mark & Ulf R. Rapp

Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701, USA

Retroviral oncogenes (v-*onc*) are derived from cellular genes (c-*onc*) which are highly conserved among different species[1–3]. Retrovirus-transduced oncogenes are most commonly associated with the induction of haematopoietic tumours and sarcomas[4]. The avian retrovirus Mill Hill no. 2 (MH2) was isolated from a spontaneous ovarian tumour of a chicken and is distinguished by the predominant induction of liver and kidney carcinomas in fowl[4–6]. MH2 also induces transformation of fibroblasts, macrophages and epithelial cells in culture[2,6,7]. The genome of MH2 contains two unrelated and independently expressed cell-derived oncogenes, v-*mil* and v-*myc*[8,9]. Three other viral isolates among avian acute transforming retroviruses

* To whom correspondence should be addressed.

© 1984 Macmillan Journals Ltd

contain the v-*myc* oncogene[2,10], but only MH2 contains both v-*myc* and v-*mil*. Hence, some of the pathogenic specificities of MH2 may be due to the simultaneous expression of two oncogenes. The murine sarcoma virus 3611 (3611-MSV) isolated from a mouse carrying lung carcinoma and peritoneal tumours[11], induces fibrosarcomas in newborn mice and the transformation of fibroblasts and epithelial cells in culture[11,12]. The oncogenic properties of 3611-MSV are due to the presence in its genome of a cell-derived oncogene termed v-*raf*[13]. We report here that the two independently transduced oncogenes v-*mil* and v-*raf* are closely related and that they were apparently derived from homologous cellular genes of avian and mammalian species.

The first indication of a possible sequence relationship between cloned v-*mil* and v-*raf* DNAs came from a comparison of their restriction site patterns (Fig. 1). Both genes have a complexity of ~1.2 kilobases (kb) and share *Sph*I and *Stu*I sites in their central regions. Relative to these sites, the 5′ and 3′ borders of these genes seem to be non-overlapping. Figure 2 provides direct demonstration of sequence homology. Cloned MH2 DNA was digested with appropriate restriction enzymes, and blotted DNA fragments were hybridized to both v-*mil* and v-*raf* probes (Fig. 2*a–c*). All *mil*-containing fragments strongly hybridized with the v-*mil*-specific probe, including fragments resulting from partial digestion and undigested 8.0-kb DNA (Fig. 2*b*). Using the v-*raf*-specific probe, hybridization was observed with basically the same fragments (Fig. 2*c*). The differential intensity of hybridization of specific fragments in the relatively stringent conditions used allowed us to make some conclusions about the regions of strong homology; especially informative was the hybridization of partially and completely digested fragments obtained from digestion with *Stu*I. The 4.60-kb and 3.84-kb fragments of integrated MH2 DNA resulting from incomplete digestion with *Stu*I revealed stronger hybridization to the v-*mil* probe than the fragments of 4.16 and 3.40 kb resulting from complete digestion (Fig. 2*b*), because the larger fragments contain more sequences homologous to the v-*mil* probe. This effect was much more pronounced, however, when the v-*raf* probe was used (Fig. 2*c*), and the 5′ 4.16-kb fragment did not hybridize at all in the conditions used. This implies that the central region of v-*mil*, around the *Sph*I site, contains sequences closely homologous to v-*raf*, while the 5′ and 3′ regions are less homologous, in good agreement with the restriction patterns shown in Fig. 1. Complete confirmation of these conclusions was obtained by digestion of cloned 3611-MSV DNA with appropriate restriction enzymes and hybridization of blotted DNA fragments with both the v-*raf* and v-*mil* probes (Fig. 2*d–f*). Again, fragments containing the central region of v-*raf* around the *Sph*I site, like the 0.50-kb *Hpa*I/*Sst*II fragment, showed the strongest hybridization with the v-*mil* probe (Fig. 2*f*).

Figure 3 shows a heteroduplex analysis between cloned integrated MH2 DNA and a v-*raf*-containing segment of 3611-MSV DNA subcloned in pBR322. Reannealing of denatured DNAs was done in conditions of low stringency and for short periods of time to allow isolation of heteroduplex structures formed between sequences of moderate homology; v-*raf* and v-*mil* sequences annealed over a contiguous segment of ~0.9 kb. The v-*raf*-containing DNA fragment contained about 0.27 kb of pBR322 sequences 5′ from the *Xho*I site of v-*raf* (compare with Fig. 1); since the 5′ single-stranded region (d) of the v-*raf*-containing DNA in the heteroduplex structure was approximately of that length, it seems that homology to v-*mil* starts near the *Xho*I site of v-*raf* and continues for ~0.9 kb. This is in agreement with the alignment of the two genes relative to the conserved *Sph*I site (Fig. 1) and with the pattern of hybridization obtained between cloned v-*mil* and v-*raf* DNAs in Southern blots (Fig. 2).

Chicken and human genetic loci related to v-*mil* and v-*raf*, respectively, have been molecularly cloned and characterized (refs 9, 14 and H.W.J., K.B., T.I.B. and U.R.R., unpublished data). The relationship of v-*mil* and v-*raf* to the chicken and human genes is shown in Fig. 4. DNA of the λ phage clone c-*mil*-4, containing the single-copy chicken locus related to v-*mil*[9], and of a pBR322 clone containing the single active human homologue of v-*raf*, hu c-*raf*-1 [14], were digested with appropriate restriction enzymes. Blotted DNA fragments were hybridized to the v-*mil* and the v-*raf* probe in conditions of high and low stringency (Fig. 4*b–e*). From an *Eco*RI/*Bam*HI digest of c-*mil*-4 DNA, three fragments of 10.0, 2.2 and 1.0 kb hybridized with the v-*mil*-specific probe to a similar extent at high and low stringency, confirming the close homology[9]. In contrast, the v-*raf* probe hybridized only to the 10.0-kb fragment at high and low stringency conditions, and in addition very weakly to the 1.0 kb fragment in low stringency conditions. The 2.2-kb fragment, containing the c-*mil* exon homologous to the extreme 5′ sequences of v-*mil*, did not hybridize at all with v-*raf*, in agreement with the data shown in Figs 1–3. From an *Eco*RI/*Sal*I digest of hu c-*raf*-1 cloned in pBR322, three fragments of 4.8, 3.0 and 0.7 kb hybridized with the v-*raf*-specific probe equally well in high and low stringency conditions, and an additional 2.8-kb fragment hybridized weakly in the relaxed conditions (the 3.7-kb *Eco*RI/*Sal*I fragment of pBR322 hybridized to the pBR322 sequences in the v-*raf* probe). With the v-*mil*-specific probe, the 4.8- and 3.0-kb fragments hybridized in stringent conditions and even stronger in relaxed conditions, and the 0.7-kb fragment hybridized only in low stringency conditions. In general, the data in Fig. 4 confirm the relationship between v-*mil* and v-*raf* demonstrated above, and strongly suggest that these two viral oncogenes were derived from homologous genetic loci. This is also supported by the observation that the same fragments of total digested genomic DNA either of chicken or of human origin hybridized to both the v-*mil* and the v-*raf* probe (not shown). Furthermore, the exon–intron arrangements of the human c-*raf* and the chicken c-*mil* loci seem very similar (refs 9, 14 and T.I.B., U.R.R., H.W.J. and K.B., unpublished data).

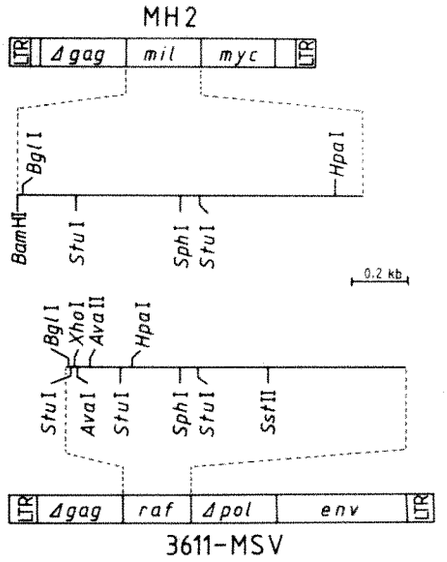


Fig. 1 Comparison of the restriction site patterns of the avian oncogene v-*mil* and the murine oncogene v-*raf*. The location of these genes on the genetic maps of cloned proviral DNAs of MH2 [8,9] and 3611-MSV[13], is shown at the top and bottom, respectively. Both genes are resistant to the following restriction endonucleases: *Bgl*II, *Cla*I, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I, *Mlu*I, *Pvu*I, *Pvu*II, *Sst*I, *Sal*I, *Sma*I and *Xba*I.

The extensive sequence homology reported here for the avian oncogene v-*mil* and the murine oncogene v-*raf* strongly supports the view that these two genes were derived from homologous genetic loci of avian and mammalian species. The totally independent transductions of these genes in two highly oncogenic retroviruses of different species directly indicates that the *mil*/*raf* gene family is associated with potential oncogenic

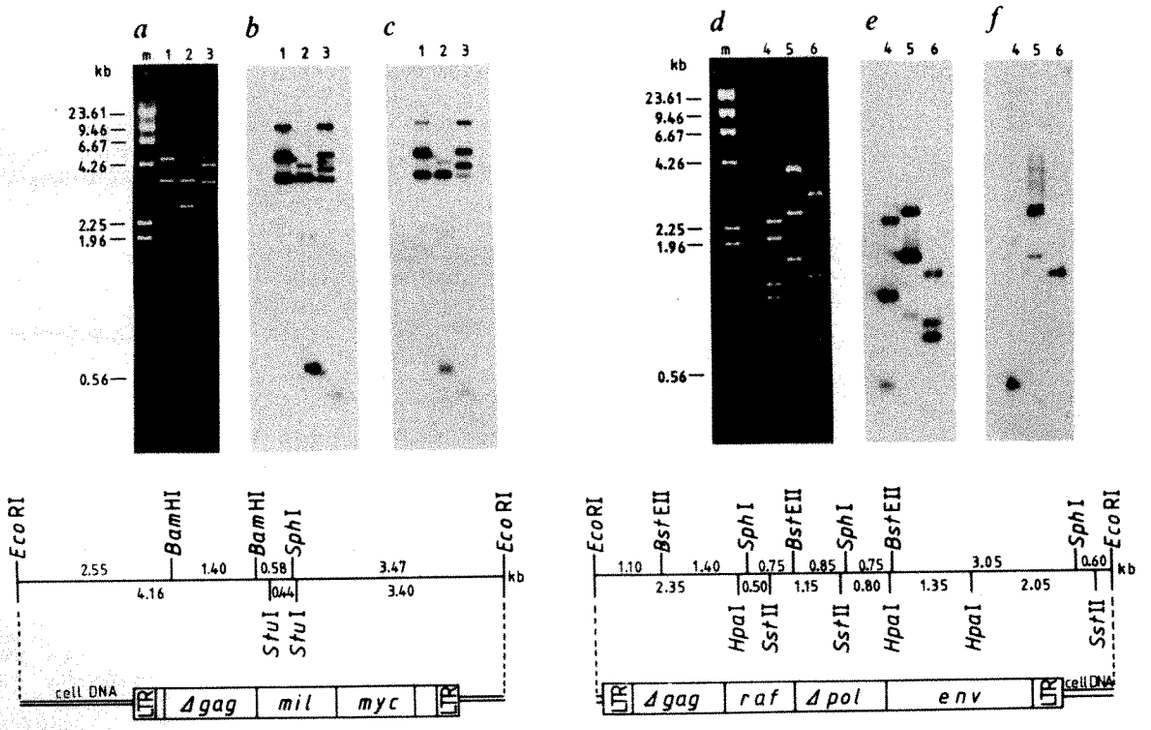


Fig. 2 Detection of sequence homology between v-*mil* and v-*raf*. ***a***, The purified 8.0-kb *Eco*RI fragment of phage clone λ MH2-1 (refs 8, 9), containing the entire MH2 provirus flanked by quail cellular sequences, was digested with *Sph*I (lane 1), *Sph*I and *Bam*HI (lane 2), or *Stu*I (lane 3). The digests were separated in two parallel sets by electrophoresis through a 1.25% agarose gel using a *Hin*dIII digest of λ *c*I857S7 as molecular weight standard (m). Faint bands visible in the ethidium bromide stain result from incomplete digestion of the 8.0-kb fragment. The location and complexities (in kb) of the fragments obtained by these digestions are shown below in the physical map of MH2 DNA. DNA was transferred to nitrocellulose sheets and hybridized with either ***b***, a v-*mil*-specific probe, represented by the gel-purified 1.1-kb *Bam*HI/*Hpa*I fragment of cloned MH2 DNA[8,9] (compare with Fig. 1); or ***c***, a v-*raf*-specific probe, represented by the gel-purified 1.8-kb *Bgl*I fragment of a pBR322 plasmid containing in its *Sal*I and *Hin*dIII sites a 1.9-kb *Xho*I/*Hin*dIII fragment of cloned 3611-MSV DNA extending from the 5′ end of v-*raf* into the Δ*pol* sequences[13] (compare with Fig. 1). The purified 1.8-kb *Bgl*I fragment used as a probe contains 0.27 kb of pBR322, all the v-*raf* sequences 3′ of the *Xho*I site and 0.5 kb of Δ*pol* sequences. Probes were radiolabelled by nick-translation using [α-^{32}P]dCTP to a specific activity of ~5×10^7 c.p.m. μg^{-1}. Hybridization was at 42 °C for 18 h in a solution containing 50% (v/v) formamide, 5×SSC (1×SSC is 150 mM NaCl, 15 mM sodium citrate, *p*H 7.0), 10×Denhardt's solution (0.2% Ficoll, 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone), 0.1% SDS, 100 $\mu g\ ml^{-1}$ denatured DNA from herring testes, and 1.5×10^7 c.p.m. of the corresponding probes. Filters were washed three times for 45 min each at 65 °C in a solution containing 2×SSC and 0.1% SDS. ***d***, The purified 8.5-kb *Eco*RI fragment of a pBR322 plasmid containing the entire 3611-MSV provirus flanked by mouse cellular sequences[13] was digested with *Hpa*I and *Sst*II (lane 4), *Sph*I (lane 5), or *Sph*I and *Bst*EII (lane 6). The location and complexities of the fragments obtained by these digestions are shown below on the physical map of 3611-MSV DNA. Electrophoresis of the digests, transfer of DNA, and hybridization with: ***e***, the v-*raf* probe; or ***f***, the v-*mil* probe, was as described above.

function. A similar close relationship has been reported between the transforming genes of three different isolates of feline sarcoma viruses and the oncogenes of avian Fujinami sarcoma virus, simian sarcoma virus, or Abelson murine leukaemia virus, respectively[15–17]. Some of these genes belong to a family of oncogenes specifying tyrosine-specific protein kinases[1]. In contrast, no enzymatic activity has been demonstrated yet for the products of the v-*mil* and v-*raf* genes, which are both expressed as *gag*-related hybrid proteins[8,9,11,13]. The oncogenic properties of MH2 and 3611-MSV suggest that the *mil*/*raf* gene family may be involved in epithelial cell transformation. Both viruses transform such cells in culture, and MH2 induces a high

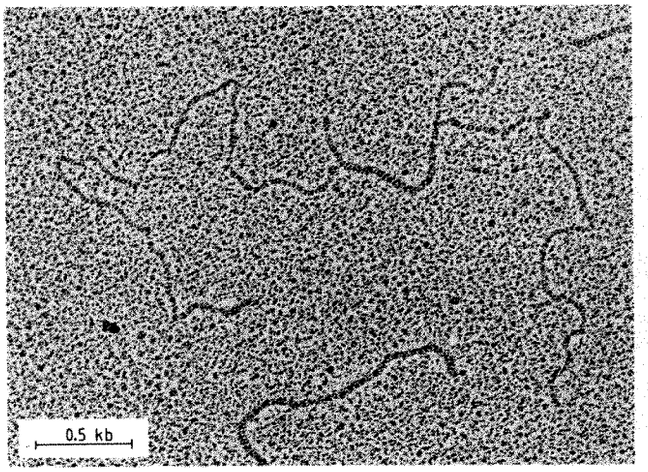


Fig. 3 Homology between v-*mil* and v-*raf* demonstrated by heteroduplex analysis. The purified 8.0-kb *Eco*RI fragment of λ MH2-1 DNA containing the entire MH2 provirus (compare with Fig. 2) and the purified 1.8-kb *Bgl*I fragment containing most of the v-*raf* sequences (see Fig. 2 legend) were used for the analysis, which was done essentially as described previously[9] except that the following parameters were modified: after denaturation of the DNAs, 2 M sodium perchlorate was added to a final concentration of 400 mM and renaturation was for 3 min only. The contour lengths (in kb) of single- and double-stranded regions and their standard deviations were determined: *a*, 4.22±0.31; *b*, 0.92±0.08; *c*, 3.03±0.32; *d*, 0.23±0.04; *e*, 0.59±0.08. From 44 to 53 heteroduplex structures of this type were analysed for the individual length measurements.

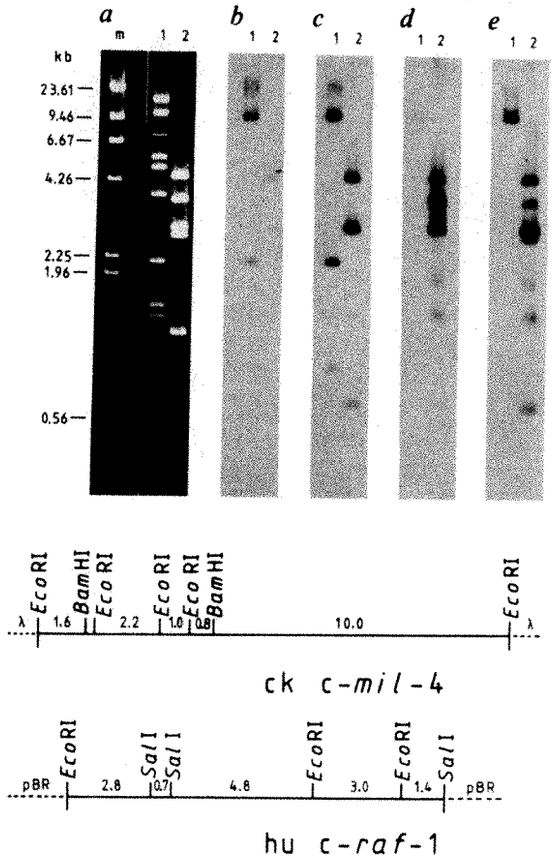


Fig. 4 Sequence relationship of the chicken locus c-*mil* and the human locus c-*raf* to both v-*mil* and v-*raf*. *a*, DNA of λ phage clone ck c-*mil*-4, carrying a 15.9-kb segment of the chicken genome including sequences homologous to all of v-*mil*[9], was digested with *Eco*RI and *Bam*HI (lane 1). DNA of a pBR322 plasmid carrying a 12.7-kb fragment of the human genome including sequences homologous to all of v-*raf* (hu c-*raf*-1)[14] was digested with *Eco*RI and *Sal*I (lane 2). The digests were separated in four parallel sets by electrophoresis through a 1% agarose gel using molecular weight standards (m) as described in Fig. 2 legend. The locations and complexities (in kb) of the fragments obtained are shown on the simple restriction maps of the two cloned loci. DNA was transferred to nitrocellulose sheets and hybridized with the v-*mil* (*b*, *c*) or the v-*raf* (*d*, *e*) probes described in Fig. 2 legend. Hybridization with 2×10^6 c.p.m. of the respective probes was for 4 h in conditions of higher (*b*, *d*) or lower (*c*, *e*) stringency. For higher stringency, conditions were like those described in Fig. 2 legend. For lower stringency, hybridization was at 60 °C in a solution containing 3×SSC, 10×Denhardt's solution, 0.1% SDS and 100 μg ml^{-1} denatured DNA from herring testes. Filters were washed at 60 °C in 2×SSC (*b*, *d*) or 3×SSC (*c*, *e*) containing 0.1% SDS. The autoradiographs were exposed for 30 min using intensifying screens.

incidence of liver and kidney carcinomas *in vivo*. Also, the human homologue of the *mil/raf* gene family is located on chromosome 3 (ref. 14) which is specifically rearranged in small-cell lung carcinoma, certain cases of familial renal carcinoma, and mixed parotid gland tumours[18–22]. We can now analyse whether the human *mil/raf* locus is involved in such specific rearrangements.

We have shown previously that the chicken cellular homologues of the two oncogenes of MH2, c-*mil* and c-*myc*, do not map in close proximity to each other on the chicken genome[9]. We now know that the human c-*mil/raf* gene is located on chromosome 3 (ref. 14), whereas the human c-*myc* gene maps to chromosome 8 (ref. 22). Hence, two unrelated and unlinked cellular genes have been brought together in the MH2 genome. The mechanism of such a dual transduction is unknown, but it is possible that before transduction, the *mil* and *myc* loci had been brought together by chromosomal translocations[22] in the primary tumour from which the virus was isolated. Alternatively, two independent recombinational events between the transducing virus and the cellular genome could have occurred. The presence of two oncogenes on one retroviral genome opens the possibility that these genes may function cooperatively in the induction of specific tumours. Such a synergistic action between oncogenes has been suggested for the cell transformation by another avian retrovirus carrying two oncogenes, avian erythroblastosis virus[23], and for the transformation of primary cell cultures by transfection with pairs of oncogenes belonging to different complementation groups[24,25].

We thank Tilo Patschinsky for useful discussions and Christiane Trachmann for technical assistance.

Received 19 September; accepted 21 November 1983.

1. Bishop, J. M. *A. Rev. Biochem.* **52,** 301–354 (1983).
2. Bister, K. in *Mechanisms of Viral Leukaemogenesis* (eds Goldman, J. M. & Jarrett, J. O.) 38–63 (Churchill-Livingstone, Edinburgh, 1984).
3. Weinberg, R. A. *Cell* **30,** 3–4 (1982).
4. Beard, J. W in *Viral Oncology* (ed. Klein, G.) 55–87 (Raven, New York, 1980).
5. Carr, J. G. *Br. J. Cancer* **14,** 77–82 (1960).
6. Alexander, R. W., Moscovici, C. & Vogt, P. K. *J. natn. Cancer Inst.* **62,** 359–366 (1979).
7. Graf, T. & Beug, H. *Biochim. biophys. Acta* **516,** 269–299 (1978).
8. Jansen, H. W., Patschinsky, T. & Bister, K. *J. Virol.* **48,** 61–73 (1983).
9. Jansen, H. W., Rückert, B., Lurz, R. & Bister, K. *EMBO J.* **2,** 1969–1975 (1983).
10. Bister, K. & Duesberg, P. H. *Cold Spring Harb. Symp. quant. Biol.* **44,** 801–822 (1980).
11. Rapp, U. R., Reynolds, F. H. & Stephenson, J. R. *J. Virol.* **45,** 914–924 (1983).
12. Keski-Oja, J., Rapp, U. R. & Vaheri, A. *J. cell. Biochem.* **20,** 139–148 (1982).
13. Rapp, U. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 4218–4222 (1983).
14. Bonner, T. I. *et al. Science* (in the press).
15. Shibuya, M. & Hanafusa, H. *Cell* **30,** 787–795 (1982).
16. Besmer, P., Snyder, H. W., Murphy, J. E., Hardy, W. D. & Parodi, A. *J. Virol.* **46,** 606–613 (1983).
17. Besmer, P. *et al. Nature* **303,** 825–828 (1983).
18. Whang-Peng, J. *et al. Science* **215,** 181–182 (1982).
19. Pathak, S., Strong, L. C., Ferrell, R. E. & Trindade, A. *Science* **217,** 939–941 (1982).
20. Cohen, A. J. *et al. New Engl. J. Med.* **301,** 592–594 (1979).
21. Mark, J., Dahlenfors, R., Ekedahl, C. & Stenman, G. *Cancer Genet. Cytogenet.* **2,** 231–241 (1980).
22. Klein, G. *Cell* **32,** 311–315 (1983).
23. Frykberg, L. *et al. Cell* **32,** 227–238 (1983).
24. Land, H., Parada, L. F. & Weinberg, R. A. *Nature* **304,** 596–602 (1983).
25. Ruley, H. E. *Nature* **304,** 602–606 (1983).

Cell lineage-specific undermethylation of mouse repetitive DNA

Verne Chapman*, Lesley Forrester*, Janet Sanford*, Nicholas Hastie† & Janet Rossant‡

* Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, New York 14263, USA
† MRC Clinical and Population Genetics Unit, Western General Hospital, Edinburgh EH4 2XU, UK
‡ Department of Biological Sciences, Brock University, St Catharines, Ontario, Canada L2S 3A1

Several distinct cell lineages are established during mouse embryogenesis. The trophectoderm and primitive endoderm give rise to extraembryonic structures alone, while the primitive ectoderm becomes the fetus proper[1–3]. Recent studies suggest that the levels of DNA modification are lower in inactive X chromosomes from extraembryonic tissues than in embryonic and adult somatic tissues. Using *Hpa*II/*Msp*I isoschizomers, Southern blots and cloned probes, we show here that repetitive DNA sequences from all derivatives of the two extraembryonic lineages, trophectoderm and primitive endoderm, are substantially undermethylated compared with primitive ectoderm derivatives. This contrasts with the highly methylated state of these repetitive elements observed in adult somatic tissues. Specific demethylation or inhibition of *de novo* methylation, or a combination of both mechanisms, may be involved. These findings suggest that elements of gene regulation dependent on DNA modification may be different in extraembryonic cell lineages.

0028–0836/84/030284—03$01.00

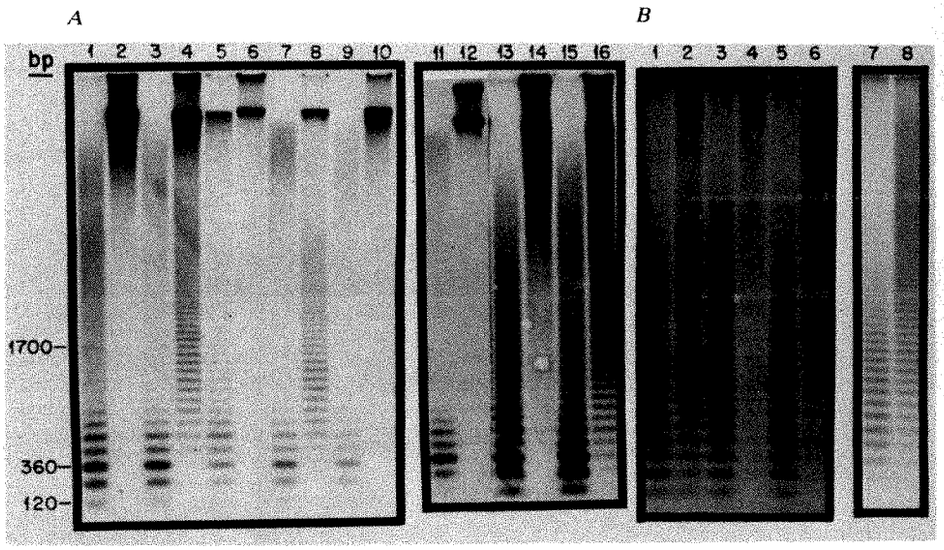


Fig. 1 Southern blot[25] probed with minor satellite sequence (pMR150) for DNA from various sources. Odd-numbered lanes, *Msp*I; even-numbered lanes, *Hpa*II digestion. *A*: lanes 1, 2, liver; 3, 4, sperm; 5, 6, day 7.5 embryonic tissue; 7, 8, day 7.5 ectoplacental cone (epc); 9, 10, day 7.5 decidual cells; 11, 12, day 7.5 embryonic tissue; 13, 14, day 15 yolk sac mesoderm; 15, 16, day 15 yolk sac endoderm. *B*: lanes 1, 2, day 7.5 extraembryonic ectoderm (ex); 3, 4, day 7.5 embryonic tissue; 5, 6, day 7.5 ectoplacental cone (epc); 7, 8, day 12 parietal endoderm. All tissues are from Ha/ICR randomly bred mice. Derivatives of the trophectoderm were obtained from 7.5-day conceptuses, at which stage these tissues can still be isolated free of contaminating ICM-derived tissues. Conceptuses at 7.5 days post-coitum were dissected into embryonic, extraembryonic and ectoplacental cone fractions in phosphate-buffered saline. The extraembryonic regions were further treated with pancreatin (2.5%; Sigma) and trypsin (0.5% in Ca^{2+}, Mg^{2+}-free saline) at 4 °C for 10 min to isolate the extraembryonic ectoderm[26]. Decidual tissue from the day 7.5 conceptuses was also retained for DNA extraction. Derivatives of the primitive endoderm were separated from 12–15-day conceptuses. Parietal endoderm and Reichert's membrane were peeled away from the overlying giant cells[4]. Visceral endoderm was obtained by enzyme separation from the visceral yolk sac[9]. The remaining mesoderm of the yolk sac was also retained. Mature sperm were isolated from cauda epididymides. All tissues were centrifuged, and the saline was replaced by Pronase buffer (50 mM Tris-HCl, *p*H 10, 0.15 M NaCl, 0.1 M EDTA). Liver was Dounce-homogenized in 5% sucrose–TKM (50 mM Tris-HCl, *p*H 7.5, 24 mM KCl, 5 mM $MgCl_2$) buffer, and nuclei were isolated by centrifugation through a 20% sucrose–TKM cushion (5,000 r.p.m., 10 min). The pellet was resuspended in Pronase buffer. Tissue samples were incubated at 37 °C for 30 min in Pronase buffer with 1 mM dithioerythritol (DTE; Sigma). SDS (0.2% final concentration) and preboiled RNase A (150 μg ml^{-1} final concentration; Boehringer Mannheim) were added and cells incubated at 37 °C for 1.5 h. The solution was made up to 0.8% SDS and 150 μg ml^{-1} Pronase (Calbiochem) and incubated for 4 h at 37 °C. The digests were extracted twice with phenol (BRL ultrapure) and three times with chloroform/isoamyl alcohol (24:1). DNA was ethanol-precipitated, desiccated and resuspended in sterile water. DNA yield estimates are 6, 15 and 14 μg from ex, epc and embryo populations respectively for 30 7.5-day conceptuses and 200 μg parietal endoderm DNA from 68 12-day conceptuses. Yolk sac DNA yields are as previously reported[13]. Sperm DNA (800 μg) was extracted from free-swimming sperm released from four minced cauda epididymides. DNA was cut with restriction endonucleases *Msp*I (NE Biolabs) and *Hpa*II (BRL) in conditions recommended by the suppliers. Following overnight incubation, λ DNA was added and incubated for a further 6 h. Restricted DNA (0.5 μg per lane) was subjected to electrophoresis in 0.8% SeaKem Agarose gels using 36 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA *p*H 7.4 buffer. To confirm complete digestion, we visualized limit-digest λ DNA fragments on the ethidium bromide-stained gel. The DNA was denatured, transferred to nitrocellulose (Schleicher and Schuell 85) and prehybridized in 4×SSC, 5×Denhardt's solution[27], 0.1% SDS, 0.1% Na pyrophosphate and 150 μg ml^{-1} denatured herring sperm DNA for 4 h at 65 °C. ^{32}P (Amersham)-labelled nick-translated[28] pMR150 (specific activity 2×10^8 c.p.m. μg^{-1}, 20×10^6 c.p.m. per blot) was added and hybridized overnight. Filters were washed in 0.5×SSC, 0.1% SDS at 65 °C and radiolabelled bands were visualized after 4 days by autoradiography (XAR-5 Kodak film).

The early stages of mouse embryogenesis are characterized by the establishment of several distinct cell lineages. By 4.5 days of development, two specialized lineages, trophectoderm and primitive endoderm, have been set aside from the pluripotent primitive ectoderm[4], and it is clear that cells are irrevocably committed to separate cell lineages by the late blastocyst stage[5–7]. Studies of X-inactivation suggest that processes controlling gene expression in the specialized trophectoderm and primitive endoderm lineages may differ from those found in pluripotent primitive ectoderm. Derivatives of trophectoderm and primitive endoderm in the developing conceptus share the feature of preferential expression of the maternal X chromosome, whereas primitive ectoderm derivatives undergo random X-inactivation[8–11]. Recent studies using DNA-mediated gene transfer have demonstrated that the DNA of the inactive X chromosome of adult somatic tissues seems to be modified and relatively inefficient in DNA transformation[12]. By contrast, the inactive paternal X chromosome DNA of the visceral yolk sac endoderm (a primitive endoderm derivative) is relatively efficient in transformation and, therefore, is presumed to be less modified[13]. One possible explanation for these findings is that the general levels of DNA modification are lower in extraembryonic tissues than in embryonic and later adult somatic tissues. We have examined the levels of methylation at CpG sites at CCGG tetramers in the DNA of derivatives of different early cell lineages using probes for dispersed and centromeric repetitive DNA families. Comparison of the restriction patterns generated by the restriction endonuclease *Msp*I and the methylcytosine-sensitive isoschizomer, *Hpa*II, reveals that repetitive DNA from all derivatives of the two extraembryonic lineages, trophectoderm and primitive endoderm, was substantially undermethylated compared with primitive ectoderm derivatives and adult somatic tissues.

Several families of repetitive DNA have been identified from a library of cloned mouse (*Mus musculus*) middle repetitive DNA sequences[14]. Two of these cloned probes, pMR150 and pMR134 (pMR = plasmid mouse repetitive), identify families which are characterized by well defined patterns in Southern blots following restriction with *Msp*I. The sequences identified by pMR150 (minor satellite) (Fig. 1) are centromeric and are present in the genome as tandemly repeating elements of 120 base pairs (bp) with $\sim5\times10^4$ copies. By contrast, pMR134 (*Mus* interspersed family, MIF) sequences (Fig. 2) are dispersed throughout the genome with ~30,000 copies of a 5–7-kilobase (kb) element[15–18]. Both of these sequences seem to be fully methylated at CpG sites in adult liver (Fig. 1, lane 2; Fig. 2, lane 2).

The restriction patterns of *Msp*I and *Hpa*II digested DNA from ectoplacental cone (epc) and extraembryonic ectoderm (ex), which are post-implantation derivatives of trophectoderm, are shown for minor satellite sequences in Fig. 1*A*, lanes 7, 8 and *B*, lanes 1, 2. These patterns indicate that a portion of the CpG sites of minor satellites are undermethylated relative to the amount observed in adult liver. By contrast, the DNA from the embryonic portion of the 7.5 day conceptus (Fig. 1, lanes 5, 6, 11, 12) does not seem to be digested by *Hpa*II and produces

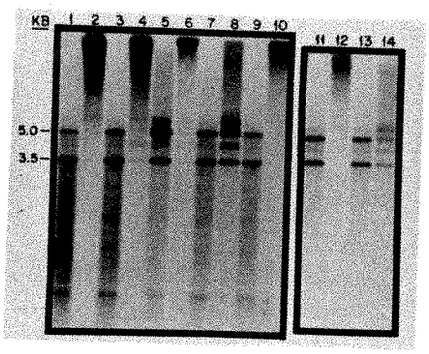


Fig. 2 Southern blot[25] probed with pMR134 (MIF) for DNA from various sources. Odd-numbered lanes, *Msp*I; even-numbered lanes, *Hpa*II digestion. 1, 2, liver; 3, 4, sperm; 5, 6, 7.5-day embryo; 7, 8, ectoplacental cone (epc); 9, 10, decidual cells; 11, 12, yolk sac mesoderm; 13, 14, yolk sac endoderm. All procedures and suppliers were as described in Fig. 1 legend.

a pattern comparable with that observed in adult liver (Fig. 1*A*, lane 2). An unusual *Msp*I pattern of the minor satellite sequences was observed in our original experiments with embryonic DNA (Fig. 1*A*, lane 5). Reanalysis of *Msp*I restriction of embryonic minor satellite sequences suggests that the pattern seen in the first experiment was due to incomplete digestion (Fig. 1*A*, lane 11). This finding suggests that our assessment of the relative *Hpa*II digestion of minor satellite sequences in liver and embryonic DNAs is valid. Maternal decidual cells, which surround the embryo in the uterus, also show extensive methylation of the minor satellite sequences (Fig. 1*A*, lanes 9, 10). *Hpa*II digestion of genomic DNA probed with MIF indicates that the undermethylation observed in epc and ex is not confined to the minor satellite sequences. The level of *Hpa*II digestion of DNA probed with MIF in epc is almost comparable with that produced by *Msp*I (Fig. 2, lanes 7, 8). Embryonic DNA from the 7.5 day conceptus shows no discernible restriction with the methyl-sensitive *Hpa*II (Fig. 2, lanes 5, 6).

We also examined the relative methylation of minor satellite and MIF CpG sites in parietal endoderm from day 12 conceptuses and visceral endoderm dissected from day 15 conceptuses. These two tissues are the later derivatives of the primitive endoderm[4,19]. The Southern blot patterns for minor satellite sequences of *Hpa*II-restricted DNA from these tissues are similar to those observed for epc and ex (Fig. 1*B*, lanes 7, 8 and *A*, lanes 15, 16). The relative digestion of MIF sequences by *Hpa*II also shows significant undermethylation but the levels may be different from those observed in trophectoderm derivatives (Fig. 2, lanes 13, 14). By contrast, the DNA of the yolk sac mesoderm, which is a primitive ectoderm derivative, shows levels of methylation comparable with those seen in embryonic DNA (Fig. 1*A*, lanes 13, 14; Fig. 2, lanes 11, 12). Some *Hpa*II digestion of minor satellite sequences is detectable in the mesodermal extracts (Fig. 1*A*, lane 14). The degree of *Hpa*II digestion is less than that seen in visceral endoderm DNA and could be due to either contamination of mesodermal extracts with endoderm cells during enzyme separation of the two cell layers from yolk sac, or the fact that minor satellite sequences are partially undermethylated compared with embryonic DNA. Previous studies with X-chromosome expression in yolk sac derivatives[9] suggest the first explanation but we cannot rule out the second possibility.

These studies indicate that both centromeric and dispersed repetitive DNA sequences of the mouse are undermethylated in the derivatives of cell lineages differentiated early in embryogenesis, that is, the trophectoderm and the primitive endoderm. By contrast, these same sequences appear to be fully methylated at 7.5 days of development in the primitive ectoderm cell lineage, which gives rise to the embryo. A previous study of methylation levels in total DNA of rabbit embryos also reported undermethylation in trophectoderm[20]. This was attributed to specific demethylation as the trophoblast DNA had the same degree of methylation as adult tissues whereas the embryonic ectoderm DNA was heavily methylated. Whether undermethylation of repetitive sequences in the extraembryonic lineages in the mouse embryo is due to specific demethylation or failure of *de novo* methylation can only be determined unequivocally by examining the levels of methylation in DNA from earlier stages of embryogenesis. However, the levels of methylation of these sequences in oocytes and sperm suggest that both mechanisms may be operating. Both sperm DNA (Fig. 1*A*, lanes 3, 4) and oocyte DNA (J.S., L.F., V.C., A. Chandley and N.H., in preparation) show undermethylation of minor satellite sequences similar to that found in extraembryonic tissues, suggesting that the *de novo* methylase activity known to occur in early embryos[21] may be confined to the pluripotent primitive ectoderm lineage. However, comparison of the levels of methylation of MIF sequences in extraembryonic tissues and sperm suggests that there may also be specific demethylation of some CpG sites in extraembryonic lineages.

The observation that both minor satellite and MIF sequences are undermethylated in certain extraembryonic lineages argues that the undermethylation is not limited to either a particular class of repetitive sequences or a particular chromosomal localization. These data further suggest that the general levels of DNA methylation may be reduced for the entire genome and that elements of gene regulation dependent on DNA modification may differ in these extraembryonic cell lineages. Such differences could conceivably account for the differences in DNA transfer of inactive X-chromosome genes from yolk sac[13] and the undermethylation of human globin genes in placental DNA[22]. It is also conceivable that the presence of some cellular oncogene transcripts in extraembryonic lineages[23] and the expression of endogenous retroviruses in placenta[24] are a function of the undermethylation of DNA in these tissues. Study of the levels of methylation of these and other structural genes in the extraembryonic lineages will determine whether undermethylation is a widespread phenomenon in these tissues. These studies will have considerable implications for the role of DNA modifications in cell lineage development and differentiation.

We thank Dr K. Bennett for the pMR probes and assistance in developing these studies and L. D. Siracusa for helpful discussions and reading the manuscript. This work was supported in part by grants GM24125 from the NIH, USPHS and the Canadian Natural Sciences and Engineering Research Council.

Received 22 August; accepted 21 October 1983.

1. Gardner, R. L., Papaioannou, V. E. & Barton, S. C. *J. Embryol. exp. Morph.* **30,** 561–572 (1973).
2. Papaioannou, V. E. *J. Embryol. exp. Morph.* **68,** 199–209 (1982).
3. Gardner, R. L. *J. Embryol. exp. Morph.* **68,** 175–198 (1982).
4. Rossant, J. & Papaioannou, V. E. in *Concepts in Early Mammalian Embryogenesis* (ed. Sherman, M. D.) 1–35 (MIT Press, Boston, 1977).
5. Rossant, J. *J. Embryol. exp. Morph.* **33,** 991–1001 (1975).
6. Hogan, B. & Tilly, R. *J. Embryol. exp. Morph.* **45,** 107–121 (1978).
7. Pederson, R. A., Spindle, A. I. & Wiley, L. M. *Nature* **270,** 435–437 (1977).
8. Takagi, N. & Sasaki, M. *Nature* **256,** 640–642 (1975).
9. West, J. D., Frels, W. I., Chapman, V. M. & Papaioannou, V. E. *Cell* **12,** 873–882 (1977).
10. Frels, W. I., Rossant, J. & Chapman, V. M. *Devl Genet.* **1,** 123–132 (1979).
11. Harper, M. I., Fosten, M. & Monk, M. *J. Embryol. exp. Morph.* **67,** 127–135 (1982).
12. Chapman, V. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 5357–5361 (1982).
13. Kratzer, P. G., Chapman, V. M., Lambert, H., Evans, R. E. & Liskay, R. M. *Cell* **33,** 37–42 (1983).
14. Pietras, D. F. *et al. Nucleic Acids Res.* **11,** 6965–6983 (1983).
15. Cheng, S.-M. & Schildkraut, C. L. *Nucleic Acids Res.* **8,** 4075–4091 (1980).
16. Heller, R. & Arnheim, N. *Nucleic Acids Res.* **8,** 5031–5042 (1980).
17. Brown, S. D. M. & Dover, G. *J. molec. Biol.* **150,** 441–466 (1981).
18. Bennett, K. L. thesis, State Univ. New York, Buffalo (1983).
19. Gardner, R. L. & Rossant, J. *J. Embryol. exp. Morph.* **52,** 141–152 (1979).
20. Manes, C. & Menzel, P. *Nature* **293,** 589–590 (1981).
21. Jähner, D. *et al. Nature* **298,** 623–628 (1982).
22. van der Ploeg, L. H. T. & Flavell, R. A. *Cell* **19,** 947–958 (1980).
23. Müller, R., Slamon, D. J., Tremblay, J. M., Cline, M. J. & Verma, I. M. *Nature* **299,** 640–644 (1982).
24. Sawyer, M. H., Nachlas, N. E. & Parem, S. *Nature* **275,** 62–64 (1978).
25. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
26. Rossant, J. & Ofer, L. *J. Embryol. exp. Morph.* **39,** 183–194 (1977).
27. Denhardt, D. T. *Biochem. biophys. Res. Commun.* **23,** 641–646 (1966).
28. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72,** 1184–1188 (1975).

Regulation of *Antennapedia* transcript distribution by the *bithorax* complex in *Drosophila*

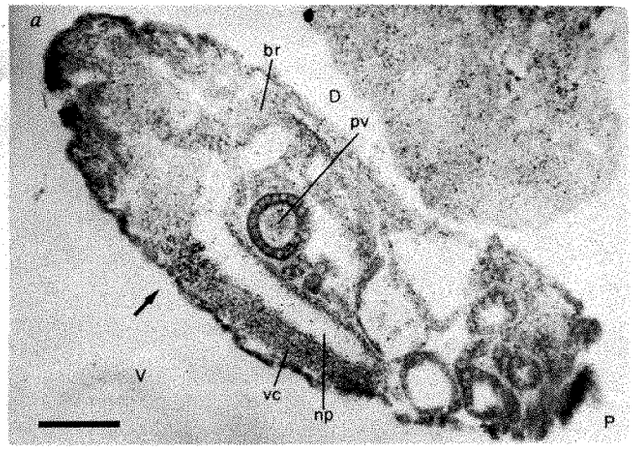
Ernst Hafen, Michael Levine & Walter J. Gehring

Department of Cell Biology, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

The homoeotic genes of *Drosophila* seem to be involved in the establishment of developmental pathways and the specification of different anatomical segment identities[1,2]. For example, it has been proposed that a function of the *Antennapedia*$^+$ (*Antp*$^+$) locus is to direct embryonic cells to follow a mesothoracic developmental pathway[3–5]. Using cloned cDNA probes of the *Antp*$^+$ (ref. 6) locus and an improved *in situ* hybridization method[7], we found that the neural cells of the embryonic and larval mesothorax possess higher levels of *Antp*$^+$ transcripts than the neural tissue of the other segments[7,8]. Here we present further evidence that *Antp*$^+$ products are associated with formation of the mesothorax, obtained by analysing the distribution of *Antp*$^+$ transcripts in *bithorax* mutants. Embryos homozygous for any one of several *bithorax* mutations display a transformation of the epidermis of the more posterior segments into the homologous tissue of the mesothorax[1]. For example, the metathorax and first seven abdominal segments of embryos that completely lack the *bithorax* gene complex show a cuticular phenotype characteristic of the normal mesothorax[1,9]. By *in situ* hybridization of an *Antp*$^+$ cDNA probe to tissue sections of these embryos, we have found that the neural cells of the transformed segments accumulate *Antp*$^+$ transcripts to a level characteristic of the normal mesothorax.

Drosophila neurogenesis involves the formation of a ventral nerve cord. On its initial establishment during early embryogenesis, the ventral cord is a composite of discrete, repeating ganglia[10]. The epidermis of each of the three thoracic and eight abdominal segments of the embryo is innervated by a corresponding ganglion of the ventral cord. Figure 1*a* shows an autoradiograph of a tissue section through an 18-h wild-type embryo after hybridization to a tritium-labelled *Antp*$^+$ cDNA probe. The same tissue section is shown in Fig. 1*b* after dark-field photomicroscopy. At this advanced stage of embryogenesis, condensation of the ventral nerve cord has resulted in the loss of overt segmentation. Nonetheless, from similar examination of younger embryos[7,8], it is clear that the highest accumulation of silver grains corresponds to the ganglion cells of the mesothoracic segment (arrow). It is possible that the strong hybridization signal includes some of the ganglion cells in the posterior portion of the prothoracic segment. Comparisons of tissue sections through different embryos of the same stage indicate that the orientation of these strongly labelled cells is roughly constant with respect to the proventriculus (pv), which therefore serves as an indicator of the mesothoracic ganglion. The other ganglia of the ventral cord display much weaker labelling.

The chromosomal deficiency *Df(3R)P9* deletes all known genes of the *bithorax* complex and embryos homozygous for this allele die during the terminal stages of embryogenesis. Inspection of the cuticle of such embryos indicates that the metathorax and the first seven abdominal segments acquire features characteristic of both the posterior portion of the normal prothoracic segment and the anterior portion of the mesothorax[1,4,9]. Figure 2*a* shows an autoradiograph of an embryo homozygous for the *P9* deficiency after hybridization to the *Antp*$^+$ cDNA probe. The corresponding dark-field photomicrograph of this tissue section is shown in Fig. 2*b*. As compared with a wild-type embryo of similar age (Fig. 1), a much larger proportion of the ventral ganglion cells is strongly labelled in the *P9* embryo. This labelling extends uniformly posteriorly from the ganglion cells of the mesothoracic segment through those of the first seven abdominal segments. As in wild-type embryos, the ventral ganglion cells of the anterior portion of the prothoracic segment and of the 8th abdominal segment show considerably less labelling at this stage.



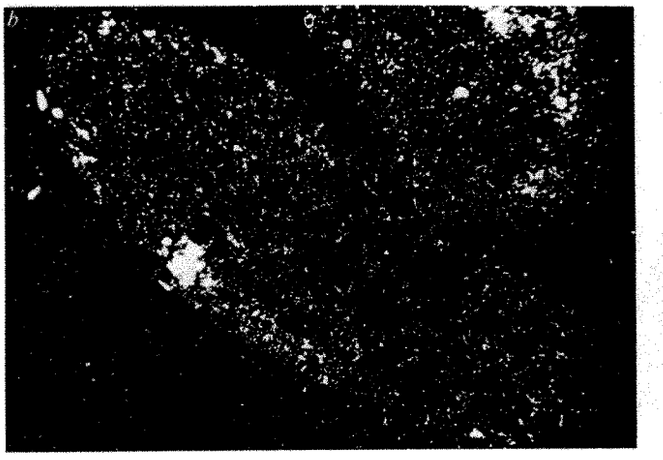


Fig. 1 Localization of *Antennapedia*$^+$ (*Antp*$^+$) transcripts in a sagittal section through an 18-h wild-type embryo. *a*, A bright-field photomicrograph of the embryo section; *b*, a dark-field photomicrograph of the same embryo section. The diagram in *a* is a schematic representation of the segmentation pattern of the embryonic cuticle and the nervous system. A, anterior aspect of the embryos; A1–A8, eight abdominal ganglia of the ventral nervous system; br, brain; D, dorsal aspect of the embryo; np, neuropile; P, posterior aspect of the embryo; pv, proventriculus; T1–T3, three thoracic ganglia of the ventral nervous system; V, ventral aspect of the embryo; vc, ventral cord. Scale bar, 0.1 mm. The section was hybridized to the 903 *Antp*$^+$ cDNA probe and autoradiographed for 15 days. 903 is a pBR322 recombinant containing a 2.2-kilobase (kb) cDNA insert that is the reverse transcription product of poly(A)$^+$ RNA extracted from 0–8 h wild-type embryos. This cDNA segment was shown to share homology with four non-contiguous chromosomal DNA sequences encompassing 100-kb cloned genomic DNA derived from the *Antp*$^+$ locus[6]. The 903 recombinant was tritium-labelled and hybridized to frozen tissue sections of staged wild-type embryos as previously described[7]. The arrow indicates strong hybridization of the 903 cDNA probe to the mesothoracic ganglion cells.

© 1984 Macmillan Journals Ltd

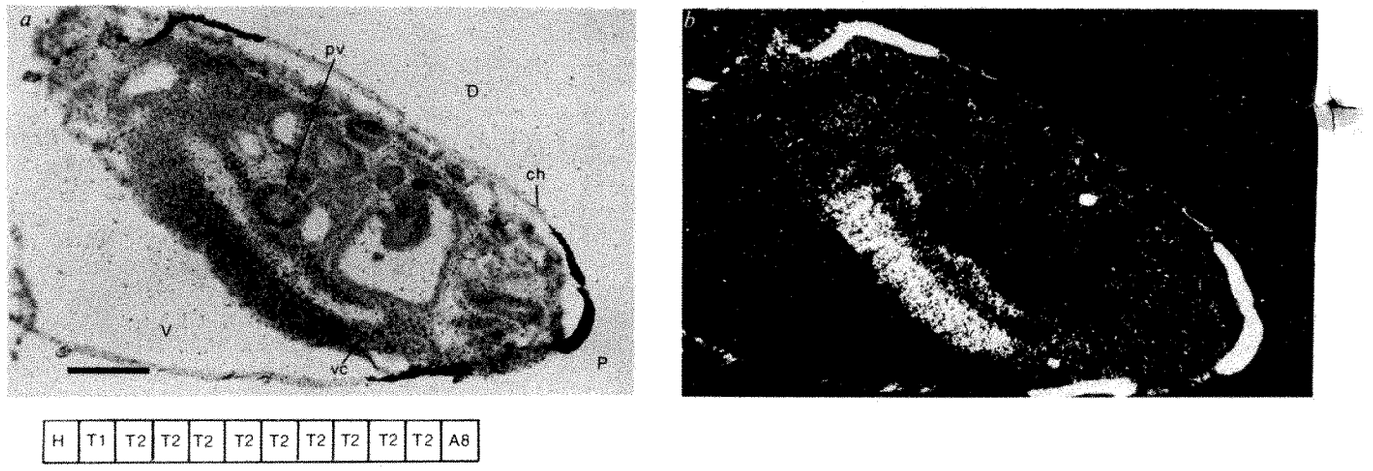


Fig. 2 Localization of *Antennapedia*$^+$ (*Antp*$^+$) transcripts in an embryo homozygous for the *P9* deficiency of the *bithorax* complex. A dark-field photomicrograph of the section shown in *a* is presented in *b*. The diagram in *a* is a schematic representation of the cuticular segmentation pattern of the embryos homozygous for the *P9* deficiency. Abbreviations as in Fig. 1 and ch, chorion shell. Scale bar, 0.1 mm. Embryos were collected from the mutant strain *Dp(3.3)P100/Df(3R)P9* (kindly provided by E. B. Lewis) for 4 h and the embryos were allowed to develop at 25 °C for an additional 26 h, so that all the viable embryos were able to hatch. The unhatched embryos were collected and sections were prepared as described previously[7] except that the embryos were embedded without removal of the chorion shell and without prefixation in paraformaldehyde. *a* Shows a sagittal section through a 26-h embryo homozygous for the *P9* deficiency after hybridization to the 903 *Antp*$^+$ cDNA probe and autoradiography for 24 days.

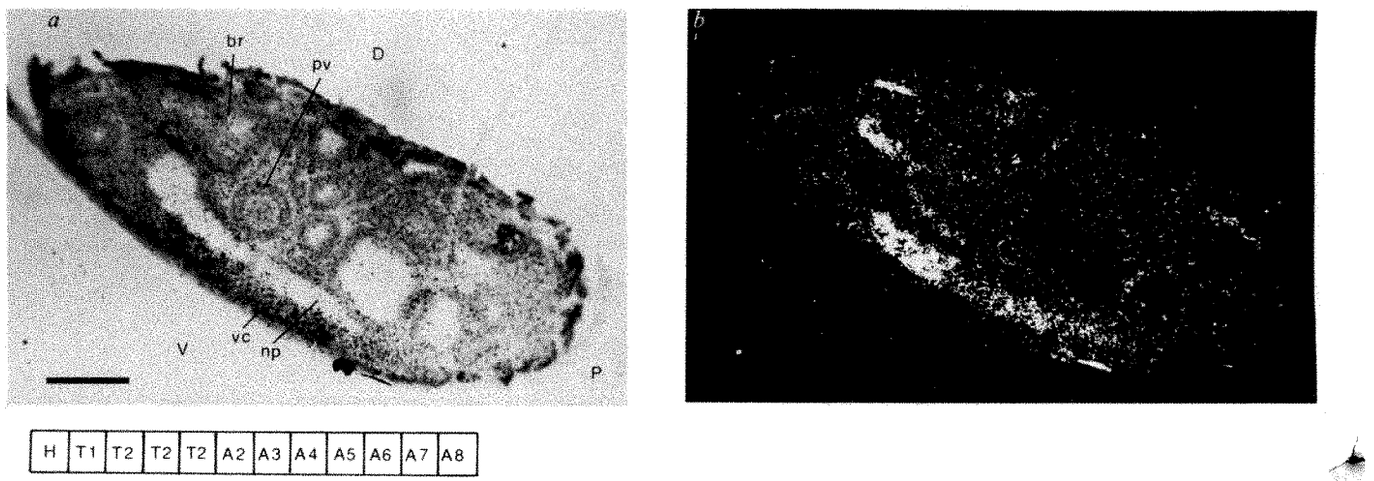


Fig. 3 Localization of *Antennapedia*$^+$ (*Antp*$^+$) transcripts in a homozygous *Ubx*^{105} embryo. Embryos were collected from four winged flies heterozygous for *Ubx*^{105} and two recessive *bithorax* alleles, *bx* and *pbx*. *a*, A sagittal section through a 20-h embryo homozygous for *Ubx*^{105} after hybridization to the 903 *Antp*$^+$ cDNA probe and autoradiography for 24 days; the diagram is a schematic representation of the cuticular segmentation pattern of embryos homozygous for *Ubx*^{105}. The accumulation of silver grains at the presumptive mouthhooks does not appear to be due to specific interaction of the probe DNA with a cognate RNA, since the same pattern was occasionally observed with control hybridization probes not derived from the *Antp*$^+$ locus. *b*, Dark-field photomicrograph of the same section as in *a*. Abbreviations as in Fig. 1. Scale bar, 0.1 mm.

In addition to the homoeotic transformation of the metathoracic and abdominal epidermis in *P9* embryos, it has been shown that the ganglia of these more posterior segments possess some of the morphological features characteristic of a normal thoracic ganglion[11]. During the latter periods of normal embryogenesis the ganglion cells of the mesothorax possess substantially higher concentrations of *Antp*$^+$ RNAs than do the ganglion cells of the other segments[7,8]. Therefore, strong hybridization signals with the *Antp*$^+$ cDNA probe serve to indicate the identity of mesothoracic ganglion cells. As a result, the strong labelling of the metathoracic and first seven abdominal ganglia of *P9* embryos is a further indication that, in addition to the epidermis, there is also a corresponding mesothoracic transformation of the neural tissue.

The distribution of *Antp*$^+$ transcripts was also examined in embryos homozygous for the *Ubx*^{105} allele of the *bithorax* gene complex. Such individuals die either during late larval development or during pupation. The terminal cuticular phenotype of *Ubx*^{105} homozygotes involves the transformation of the metathorax and first abdominal segment into a mesothorax (ref. 1 and E. B. Lewis, personal communication). Figure 3*a* shows an autoradiograph of a 20-h embryo homozygous for *Ubx*^{105} after hybridization to the *Antp*$^+$ cDNA probe. The corresponding dark-field photomicrograph is shown in Fig. 3*b*. As compared with the wild-type pattern shown in Fig. 1, a larger proportion of ventral ganglion cells display strong hybridization signals. The anterior-most labelled ganglion cells are located in a similar position with respect to the proventriculus as are the mesothoracic ganglion cells of a wild-type embryo of similar age (Fig. 1). However, in contrast to the wild-type hybridization pattern, an increased accumulation of silver grains is also observed in more posterior ganglion cells. Based on estimates of the number of ganglion cells within each segment of the ventral cord, the extent of the labelling is sufficient to encompass the metathoracic and first abdominal ganglia. Thus, in the absence of normal *Ubx*^{105} function, there appears to be a trans-

formation of both the neural and epidermal tissue of the metathorax and first abdominal segment to the homologous tissue of the mesothorax.

The results presented here indicate that *bithorax* mutants which are wild type with respect to the $Antp^+$ gene exhibit an altered pattern of $Antp^+$ expression. In such individuals a close correlation has been established between the phenotypic transformation of the cuticular portion of the metathoracic and abdominal segments to a mesothorax and an augmented steady-state level of $Antp^+$ RNA present in the ganglion cells of the affected segments. Evidence supporting a role for $Antp^+$ products in the specification of the mesothorax had been previously obtained by examination of the pattern of $Antp^+$ expression in wild-type embryos by *in situ* hybridization[7,8]. Even during early periods of normal embryonic development the mesothoracic segment in the ventral nervous system is associated with relatively high levels of $Antp^+$ RNAs[8]. The present study substantially extends this observation. In the absence of normal *bithorax* function, $Antp^+$ RNAs accumulate in the progenitors of more posterior ventral ganglia where they might function to impose a mesothoracic developmental pathway.

During early periods of normal embryonic development, $Antp^+$ transcripts are detected in the neural progenitors of each of the thoracic and abdominal ganglia. However, during subsequent development, the RNAs that are initially detected in the pro-, metathoracic and abdominal ganglia gradually diminish such that by mid-embryogenesis the mesothoracic ganglion cells contain the highest concentrations of $Antp^+$ transcripts[8]. Once established, this segment-specific pattern of $Antp^+$ expression is maintained through the remaining periods of embryonic development and subsequent larval development. It has been proposed that expression of the *bithorax* gene complex is confined to the metathoracic and abdominal segments[1]. Consistent with this proposal is the recent demonstration that *Ultrabithorax*$^+$ (Ubx^+) transcripts are distributed along the ventral cord of developing wild-type embryos as observed for $Antp^+$ transcripts, except that by late embryogenesis the ganglion cells of parts of the metathorax and the first abdominal segment possess the highest concentrations of Ubx^+ RNAs[12]. $Antp^+$ transcripts might be prevented from reaching high steady-state levels in the posterior ganglia of wild-type embryos by the *bithorax* products which are also present in these cells. In the mesothorax, $Antp^+$ RNAs stably accumulate since *bithorax* products are largely absent from this segment. Thus, one possible explanation for the increased levels of $Antp^+$ transcripts in the metathoracic and abdominal ganglia of *P9* embryos is the absence of *bithorax* products from these positions. This interpretation of the results agrees with previous genetic findings which suggest a direct or indirect regulatory influence of products from the *bithorax* complex on the proper expression of genes in the *Antp* complex[5].

We thank Bill McGinnis for discussing and reviewing the manuscript and Erika Wenger-Marquardt for assistance in preparing the manuscript. This work was supported by a grant from the Swiss NSF and the Kanton Basel-Stadt. E.H. was supported by the Emil Barell-Stiftung and M.L. by a Jane Coffin Childs fellowship.

Received 18 August; accepted 25 October 1983.

1. Lewis, E. B. *Nature* **276**, 565–570 (1978).
2. Kaufman, T. C., Lewis, R. & Wakimoto, B. *Genetics* **94**, 115–133 (1980).
3. Struhl, G. *Nature* **292**, 635–638 (1980).
4. Denell, R. E., Hummels, K. R., Wakimoto, B. T. & Kaufman, T. C. *Devl Biol.* **81**, 43–50 (1981).
5. Struhl, G. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7380–7384 (1982).
6. Garber, R. L., Kuroiwa, A. & Gehring, W. J. *EMBO J.* **2**, 2027–2036 (1983).
7. Hafen, E., Levine, M., Garber, R. L. & Gehring, W. J. *EMBO J.* **2**, 617–623 (1983).
8. Levine, M., Hafen, E., Garber, R. L. & Gehring, W. J. *EMBO J.* **2**, 2037–2046 (1983).
9. Duncan, I. *Genetics* **100**, 520 (1981).
10. Poulson, D. F. in *Biology of* Drosophila (ed. Demerec, M.) 168–274 (Wiley, New York, 1950).
11. Jiménez, F. & Campos-Ortega, J. A. *Wilhelm Roux's Arch. dev. Biol.* **190**, 370–373 (1981).
12. Akam, M. *EMBO J.* **2**, 2075–2084 (1983).

Activation of extra copies of genes coding for nitrogenase in *Rhodopseudomonas capsulata*

Pablo A. Scolnik & Robert Haselkorn

Department of Biophysics and Theoretical Biology, The University of Chicago, Chicago, Illinois 60637, USA

Biological nitrogen fixation requires the nitrogenase enzyme complex, ATP, and a strong reductant[1]. *Klebsiella pneumoniae* contains 15 linked nitrogen fixation (*nif*) genes[2,3], three of which, *nifH*, *nifD* and *nifK* have been sufficiently conserved in evolution that cloned *K. pneumoniae nifHDK* DNA[4] will hybridize to DNA sequences from every nitrogen-fixing bacterium examined to date, including the purple, non-sulphur bacterium *Rhodopseudomonas capsulata*[5], in which one complete *nifHDK* operon has been mapped[6]. Using cloned *K. pneumoniae nifHDK* DNA we report here that *R. capsulata* contains multiple copies of the genes for nitrogenase components. Two regions containing sequences homologous to all three *nif* structural genes have been identified, and mutations in one region produced a Nif$^-$ phenotype. Nif$^+$ pseudorevertants were derived from these mutants, some of which retained the original mutation suggesting that some of the extra *nif* gene sequences can be functionally activated.

Hybridization of pSA30 to a *Hin*dIII digest of total DNA from *R. capsulata* shows two strong bands of 11.8 kilobasepairs (kbp) and 4.9 kbp, as well as additional bands of lower intensity (and presumably lower homology) (Fig. 1, lane *a*). A *nifH* gene-specific probe hybridized only to the 11.8 and 4.9-kbp fragments (lane *b*), while *nifD* and *nifK* probes annealed to several additional bands including two at 3.6 and 1.9 kbp (lanes *c*, *d*). The 11.8-kbp *Hin*dIII fragment contains the functional *nifHDK* operon previously mapped[6], referred to as 'copy I' (Fig. 3, for strain SB1003). The results in Fig. 1 show that there is at least one additional *nifH*-related sequence in the 4.9-kbp *Hin*dIII fragment and several additional *nifD*- and *nifK*-related sequences. Other related sequences appear to be present on two larger fragments as well.

Multiple copies of *nif* gene-related sequences are also seen in the *Eco*RI digest, particularly with the single gene probes. The two bands that are identified by pSA30 at 7.6 and 5.0 kbp (lane *e*) derive from copy I; an *Eco*RI site is near the C-terminal end of the *nifD* gene (Fig. 3). Three additional bands, 11.0, 6.5 and 6.0 kbp, are identified by all three single gene probes (lanes *f*–*h*), enabling us to conclude that *R. capsulata* contains at least four copies of sequences related to the structural genes for nitrogenase components.

To clone the *nif*-related sequences for detailed study, we first isolated a fragment of copy I DNA by screening a library of recombinant λ containing *Eco*RI fragments of *R. capsulata* DNA in λgt7-*ara*6 (R. W. Davis, unpublished). One such recombinant phage contained the 7.6-kbp *Eco*RI fragment from copy I, which contains all of *nifH* and most of *nifD* (Fig. 1, lanes *e*–*h*, and Fig. 3). That fragment was purified and used to probe a cosmid library of *Hin*dIII fragments (Fig. 1 legend). Two cosmids were found to contain related sequences. Plasmid p103 contains the 11.8-kbp *Hin*dIII fragment and an additional 32 kbp of adjacent chromosomal DNA, while plasmid p108 contains the 4.9-kbp *Hin*dIII fragment and an additional 40 kbp of chromosomal DNA. The two cosmids have no restriction fragments in common.

Further study of the relationships among the *nif*-related sequences was facilitated by subcloning the 11.8- and 4.9-kbp *Hin*dIII fragments from p103 and p108, respectively, into the mobilizable vector pRK292 (ref. 7). The results of hybridizing each of these plasmids back to total chromosomal DNA of *R. capsulata* are shown in Fig. 1, lanes *j* and *l*. These results indicate that the extra copies are more closely related to each other than any of them is to copy I.

© 1984 Macmillan Journals Ltd

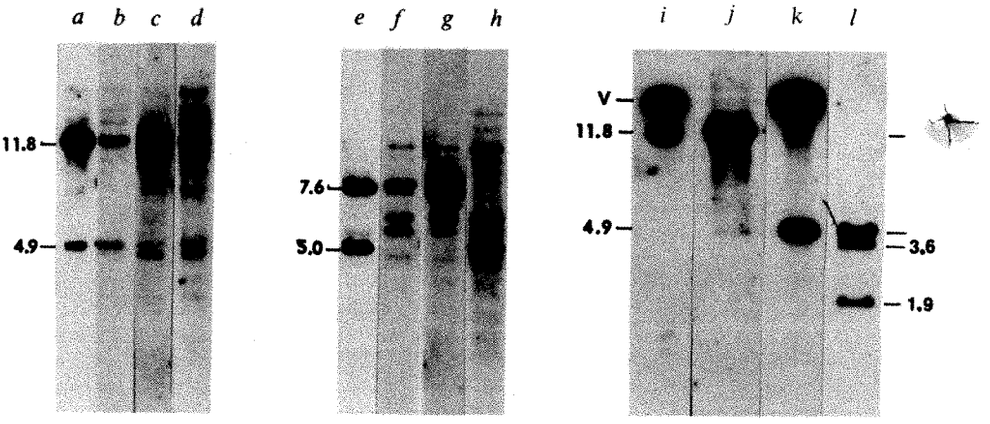


Fig. 1 Multiple copies of *nif* gene-related sequences in *R. capsulata* DNA revealed by Southern hybridization with heterologous probes. Lanes *a–d* contained *R. capsulata* DNA digested with *Hin*dIII; lanes *e–h* the same DNA digested with *Eco*RI. Radioactive probes contained *Klebsiella nifHDK* DNA (lanes *a, e*), *Klebsiella nifH* (lanes *b, f*), *Anabaena nifD* (lanes *c, g*) and *Klebsiella nifK* (lanes *d, h*). The 11.8-kbp *Hin*dIII fragment and two major *Eco*RI fragments of 7.6 and 5.0 kbp are derived from copy I of the *nifHDK* operon (see map in Fig. 3). The 11.8-kbp and 4.9-kbp *Hin*dIII fragments were isolated from a cosmid library and subcloned in pRK292 to yield plasmids pRPA5 and pRPA7, respectively. pRPA5 was used as probe in Southern hybridization to *Hin*dIII cut pRPA5 (lane *i*) or total *R. capsulata* DNA (lane *j*). pRPA7 was the probe with pRPA7 (lane *k*) or total *R. capsulata* (lane *l*). V indicates the vector DNA band. The extra copy probe, pRPA7, identifies copies which were less visible in lanes *c* and *d*.

Methods: Total DNA from *R. capsulata* was isolated by the method of Bendich *et al.*[18]. Approximately 2 μg were digested with *Hin*dIII or *Eco*RI and the resulting fragments were separated on 0.7% agarose gels. DNA was transferred to nitrocellulose by the method of Southern[19] and annealed with $1–2\times10^6$ d.p.m. of ^{32}P-labelled nick-translated probe. Hybridization was for 20 h at 65 °C in 5×SSPE, (0.15 M NaCl, 0.01 M NaH_2PO_4, 1 mM Na_2EDTA, *p*H 7.0) followed by three 45-min washes in 3×SSPE. The *Klebsiella nifHDK* probe pSA30 has been described[4]. The internal *nifH* probe was prepared by subcloning an *Eco*RI–*Bgl*II fragment[4] from pSA30 into pBR322 cut with *Eco*RI and *Bam*HI. The *nifD* probe was pAn256, which contains most of the *nifD* gene of *Anabaena* 7120[15]. The internal *nifK* probe was prepared by subcloning a *Sal*I fragment[4] from pSA30 into the *Sal*I site of pBR322. The cosmid library of *R. capsulata* DNA was prepared from a size-fractionated *Hin*dIII partial digest ligated into the cosmid vector pDPT5Cm (ref. 20) and maintained in *E. coli* HB101. Screening the library with the 7.6-kbp *Eco*RI fragment (lane *e*), which had been isolated from a λ phage library, yielded cosmids p103 and p108. Each cosmid contained over 40 kbp *R. capsulata* DNA. The 11.8-kbp *Hin*dIII fragment (copy I) was subcloned from p103 into the mobilizable plasmid pRK292 (ref. 7) to yield pRPA5 (lanes *i, j*). The 4.9-kbp fragment was subcloned from p108 into pRK292 to yield pRPA7 (lanes *k, l*).

We next wished to study the functional properties of the cloned *nif* gene-containing fragments. For this purpose, we designed a method for site-specific mutagenesis of *R. capsulata* using the gene transfer agent (GTA)[8], a defective transducing phage that packages 4.6-kbp of linear, double-stranded chromosomal DNA fragments[9], to make insertions or deletions. The method is illustrated in Fig. 2. Briefly, a 2.4-kbp *Xho*I fragment (the 'cartridge'), carrying the gene for kanamycin resistance from Tn*5* (ref. 10) (which is expressed in *R. capsulata*) is ligated *in vitro* into a *Xho*I, *Sal*I or *Ava*I site in a cloned target fragment. The plasmid so constructed is used to transform *Escherichia coli* and then mobilized into a GTA-overproducing strain of *R. capsulata*. Finally, GTA from this strain is used to transfer Km^r to a recipient *R. capsulata*. Km^r transductants are produced by a double recombination event, one in each of the sequences flanking the Km^r gene. Although this method can only be used in *R. capsulata*, it has the advantages over previously described procedures[11] that it does not depend on transposition to generate insertions and it does not leave a plasmid behind after the insertion or deletion is created in the recipient chromosome.

The results of hybridization to *Hin*dIII digests of total DNA from wild-type *R. capsulata* and from strain 1051, in which the Km^r cartridge has been inserted in the *Sal*I site of the *nifH* gene of copy I, are shown in Fig. 2, *e* and *f*, respectively. The probe in this case was pRPA5, which contains the cloned 11.8-kbp fragment. The 2.4-kbp Km^r cartridge contains a single *Hin*dIII site (Fig. 2), so inserting it into the *nifH* gene introduces a new *Hin*dIII site into the 11.8-kbp fragment. The two fragments produced, 9.9 and 4.3 kbp, correspond to the sum of 11.8 and 2.4 kbp.

Maps of one insertion and two deletions produced in copy I by this method are shown in Fig. 3, with details of the strain constructions. Southern hybridizations confirming the map positions of the insertions and deletions are shown in Fig. 4. The hybridization of DNA from strain 1051 was described above. In strain DN51, the 750-bp *Xho*I fragment in *nifD* was deleted and replaced with the 2.4-kbp Km^r cartridge. Thus, the 11.8-kbp *Hin*dIII fragment is replaced by two new fragments (Fig. 4, lane *d*) that total 13.4 kbp, while the 7.6-kbp *Eco*RI fragment is replaced by one of $7.6+2.4-0.75=9.25$ kbp (Fig. 4, lane *h*). The latter result can be compared with that for strain 1051 (lane *f*) in which the insertion was made without deletion; the new band in that case is 10.0 kbp. Finally, in strain 306, the large *Ava*I fragment entirely within the 7.6-kbp *Eco*RI fragment was deleted and replaced with the Km^r cartridge. In Fig. 4, lane *c*, the two new *Hin*dIII fragments total 7.2-kbp and in lane *g* the new *Eco*RI fragment is 3.0 kbp, consistent with the map shown in Fig. 3. The blots in Figs 2 and 4 also show that only the target sequence is affected; the extra copies are unaltered in strains in which the Km^r marker was inserted in copy I. All of the strains in which the Km^r marker was inserted in copy I are Nif$^-$, indicating that, in the conditions used in the laboratory, only copy I *nifHDK* genes are functional. This conclusion has also been reached on the basis of complementation of independently isolated Nif$^-$ point mutants by the cloned copy I fragment[6].

The insertions and deletions created in copy I *nif* genes should not revert. However, Nif$^+$ pseudorevertants were obtained from both types of mutations. We obtained Km^r, Nif$^+$ strains from each of the mutants shown in Fig. 3. Such strains grew slowly in nitrogen-free media and had about 10% of the wild-type acetylene reduction activity. These strains retained the Km^r cartridge in the copy I genes, shown by hybridization to total DNA prepared from the revertants (data not shown). Some Nif$^+$ revertants of strain 1051 were found to be Km^s. These strains had wild-type level nitrogenase activity and their DNA showed restoration of the wild-type restriction pattern (*Hin*dIII and *Eco*RI) in the copy I genes. We think that, in these strains, extra copy *nifH* sequences were used to convert (delete) the inserted cartridge at copy I. Precise excision of the cartridge is less likely because the only region of homology at the ends of the insert is the *Xho*I site.

These results indicate that sequences homologous to all three structural genes for nitrogenase are present in several copies in the *R. capsulata* genome, but differences in the restriction pattern and in the intensity of hybridization with the specific probes indicate that the multiple sequences are not identical. Do the extra copies of the *nif* genes have any physiological meaning? At present we know too little about them to offer an

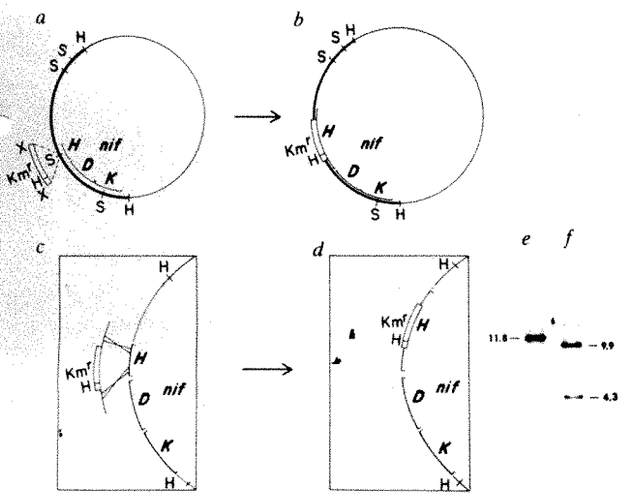


Fig. 2 Site-directed mutagenesis of *Rhodopseudomonas capsulata* with a kanamycin resistance gene cartridge. ***a***, Plasmid pRPA5 DNA (Tet^r) was digested with *Sal*I in the presence of ethidium bromide[21] to generate one cut per molecule. A 2.4-kbp *Xho*I fragment carrying Km^r from Tn*5* was ligated in and the resulting plasmid population was used to transform *E. coli* HB101, selecting for tetracycline and kanamycin resistance, to yield *b*. Plasmid DNA from individual clones was extracted and digested with *Hin*dIII and *Eco*RI. The Km^r cartridge contains one *Hin*dIII site and no *Eco*RI sites, so the original 11.8-kbp fragment is replaced in the insertions by two *Hin*dIII segments (9.9 and 4.3 kbp for the insert in *nifH* oriented as shown), and the 7.6-kbp *Eco*RI fragment containing *nifH* and most of *nifD* (see map in Fig. 3) is replaced by a 10-kbp fragment. Insertions in several *Sal*I sites were obtained. The insert in the *nifH* site was identified by hybridizing the *Hin*dIII cut plasmid fragments with ^{32}P-labelled pSA30. Since this probe is homologous only to the *nifHDK* region and both *Hin*dIII fragments showed homology, the insertion has to be in the *nifH Sal*I site shown. ***b***, This plasmid was then mobilized into *R. capsulata* Y262, a gene transfer agent (GTA) overproducer[9], using the helper plasmid pRK2013. The conjugational cross was done on RCVB minimal medium, selecting for tetracycline resistance (5 μg ml^{-1}). *E. coli* HB101 does not grow in this medium. GTA was prepared from these cells[9] and used to transduce wild-type *R. capsulata* SB1003, selecting for Km^r. Since GTA transduces linear, double-stranded segments of DNA 4.6 kbp in size, any fragment containing genomic DNA on both sides of the Km^r cartridge can recombine with the target sequences in the chromosome of the recipient cell (**c**) generating a Km^r mutant (strain 1051) (**d**). Total genomic DNA from strains SB1003 (**e**) and 1051 (**f**) was digested with *Hin*dIII, and the fragments separated in a 0.7% agarose gel, blotted onto nitrocellulose and hybridized with ^{32}P-labelled pRPA5 DNA. The *Hin*dIII site is 750 bp from one end of the *Xho*I Km^r fragment. The *Sal*I site is 3.55 kbp from the right end of the 11.8-kbp *Hin*dIII fragment of wild-type DNA. Thus, the wild-type 11.8-kbp band in **e** has been replaced by two fragments (9.9 and 4.3 kbp) in strain 1051 (**f**), corresponding to 8.25+1.65 kbp and 0.75+3.55 kbp, respectively. This method may also be used to generate deletions (Figs 3, 4) by removing a restriction fragment at the time of cartridge insertion *in vitro*.

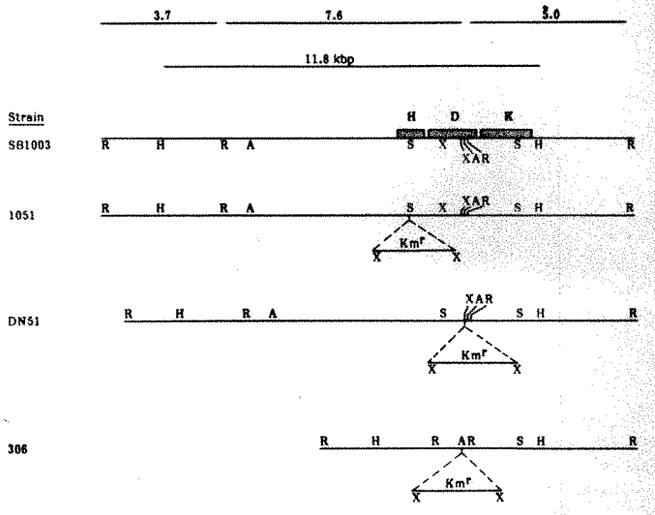


Fig. 3 Physical map of the copy I *nifHDK* region in different strains of *R. capsulata*. Lines at the top of the figure indicate the *Eco*RI and *Hin*dIII fragments, labelled in kbp, in wild-type *R. capsulata*, that hybridize with pRPA5. The *Eco*RI 7.6-kbp fragment contains the *nifH* gene and most of the *nifD* gene (see Fig. 1 and ref. 6). The 5.0-kbp *Eco*RI fragment contains the *nifK* gene. Location of genes and sites for *Eco*RI(R), *Hin*dIII(H), *Sal*I(S), *Ava*I(A) and *Xho*I(X) are shown for the wild-type strain (SB1003). Strain 1051 carries a Km^r cartridge insertion in the *nifH Sal*I site. DN51 has a 750-bp deletion of the *Xho*I segment in *nifD*. Strain 306 has a 7.0-kbp deletion between *Ava*I sites within the 7.6-kbp *Eco*RI fragment. The *Hin*dIII site in the Km^r cartridge is not shown in this figure.
Methods: Construction of strain 1051 was explained in Fig. 2 legend. To construct strain DN51, the *Sal*I fragment of SB1003 containing sequences from *nifH* to *nifK* was subcloned into pBR322, and then digested with *Xho*I (which does not cut pBR322). To the resulting DNA we ligated the Km^r cartridge, and then transformed *E. coli* HB101, selecting for Tet^r Km^r. The resulting plasmid was mobilized by conjugation into *R. capsulata* Y262 with the helper plasmid pDPT51, selecting for Km^r. GTA was prepared and used to transduce Km^r to SB1003. Substitution of the target sequence by the segment carrying the Km^r cartridge results in the insertion of the cartridge and consequent deletion of the *Xho*I segment. To construct strain 306, we carried out a partial digestion of plasmid pRPA5 with *Ava*I. After inactivation of the enzyme, this DNA was ligated to the Km^r cartridge. Several *Ava*I sites are present in the 11.8-kbp *Hin*dIII fragment (not shown), so several deletions were obtained. Strain 306 is shown as an example. This strain grows normally in minimal medium, indicating that no essential genes were deleted. Its Nif$^-$ phenotype is complemented by pRPA8, which contains only the copy I *nifHDK* operon[7], so there are no other *nif* genes deleted in strain 306.

answer. We would like, however, to discuss two possibilities: first, that the extra copies are pseudogenes and, second, that they are activated in conditions different from those used here.

The first model implies that the extra sequences are permanently silent, which might be expected for evolutionary remnants from a process of lateral diffusion of *nif* genes in nature[12]. The process of activation to produce the pseudorevertants would create a promoter in front of the coding region(s) of one or more copies, or it would consist of mutations which 'repair' the DNA sequence so as to produce active products, or both.

The second possibility is that the extra copies are functional genes which become activated in growth conditions different from those used here. This model would predict that the nitrogenase encoded by the extra copies has properties (such as ammonia switch-off, substrate affinity, cofactor requirements and regulation) different from copy I nitrogenase, and that the cell has the ability selectively to induce different copies according to varying environmental conditions. Pseudorevertants in this case would be mutants in the activation control mechanism. *R. capsulata* can grow and fix nitrogen anaerobically in the dark, provided a strong oxidant (such as dimethyl sulphoxide or trimethylamine oxide) is present in the medium[13]. However, these compounds are not present in natural environments in a concentration high enough to sustain growth. *R. capsulata* is constantly challenged by changing environmental factors, including interactions with other microorganisms, and it might be very difficult or impossible to reproduce these situations in the laboratory. A variation of this model is that the extra copies are actually silent, but a controlled gene rearrangement can bring genes or segments of genes to the copy I region, resulting in the expression of genes whose products have properties different from those of copy I. The phenotypic reversion of strain 1051 to Nif$^+$, Km^s cells could be the consequence of such a process. These possibilities can be distinguished by sequencing and by characterization of the *nif* gene products in the pseudorevertants.

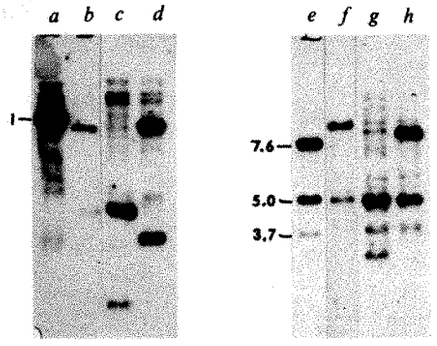


Fig. 4 Characterization of insertion and deletion mutations by Southern hybridization. Total DNA from the following strains was digested with *Hin*dIII (lanes *a–d*) or *Eco*RI (lanes *e–h*): SB1003, lanes *a* and *e*; 1051, lanes *b* and *f*; 306, lanes *c* and *g*; DN51, lanes *d* and *h*. The probe in each case was pRPA5, containing copy I *nifHDK* sequences. Strain 1051 contains the 2.4-kbp Km^r cartridge inserted in *nifH* of copy I (Fig. 3). The cartridge contains a *Hin*dIII site, leading to the two bands seen in lane *b* of 9.9 and 4.3 kbp. Lane *f* shows the 7.6-kbp *Eco*RI fragment increased to 10 kbp by the insert. Strain 306 contains two *Hin*dIII fragments of 4.4 and 2.8 kbp, while the 7.6-kbp *Eco*RI fragment is replaced by one of 3.0 kbp. These changes result from a deletion of 7 kbp and insertion of the 2.4-kbp Km^r cartridge (Fig. 3). Strain DN51 shows two *Hin*dIII fragments of 10.1 and 3.3 kbp, while the 7.6-kbp *Eco*RI fragment is increased to 9.25 kbp. These changes result from a 750-bp deletion and insertion of the Km^r cartridge (Fig. 3).

Methods: Pseudorevertants were isolated as follows: The Km^r Nif$^-$ strains were grown overnight aerobically in RCVB minimal medium at 35 °C[22]. 0.1 ml of these cultures was inoculated into 10 ml of RCVB-NF (nitrogen-free) medium, and grown overnight aerobically at 35 °C to exhaust nitrogen completely. Cells were washed twice with RCVB-NF and approximately 10^9 cells were placed in a 10-ml screwcap tube totally filled with RCVB-NF medium. These cultures were incubated until turbidity was observed (1–2 weeks). Cells were then streaked on RCVB-NF plates and incubated until 2-mm diameter colonies formed (3–4 days). The Km resistance phenotype was tested by replica plating onto RCVB and RCVB-Km. Strain 1051 gave two kinds of pseudorevertants: Km^r (R1051r) and Km^s (R1051s). Only Km^r Nif$^+$ revertants were isolated from the deletion strains. To test nitrogenase activity, cells were grown overnight photosynthetically in RCVB, washed three times with RCVB-NF, resuspended in RCVB-NF and incubated photosynthetically for 8 h before determining acetylene reduction activity[23].

This work adds one more example of bacterial gene families. *Rhizobium phaseoli* has reiterated *nif* sequences[14] and the cyanobacteria *Anabaena*[15] and *Calothrix*[16] have more than one copy of the *nifH* gene. Extra copies of structural genes other than *nif*, of which only one is normally expressed, have been described in other bacterial species. There are two non-identical copies of the spore coat protein gene of *Myxococcus xanthus*[17], separated by a 1.2-kbp spacer. Only the downstream gene that codes precisely for the sequenced coat protein is expressed. A similar situation was found in *Caulobacter crescentus*: of at least three genes that could code for flagellin, the major flagellar protein, only one is normally expressed (N. Agabian, personal communication).

We thank Peter Lammers, Helen Revel, Steven J. Robinson and Nilgun Erekun Tumer for helpful comments. This research was supported by PHS grant GM 21823 and, in part, by a postdoctoral fellowship to P.A.S. from the National Institute of General Medical Sciences.

Received 19 August; accepted 25 October 1983.

1. Mortenson, L. E. & Thorneley, N. F. *A. Rev. Biochem.* **48,** 387–418 (1979).
2. Ausubel, F. M. & Cannon, F. C. *Cold Spring Harbr. Symp. quant. Biol.* **45,** 487–497 (1980).
3. Brill, W. *Microbiol. Rev.* **44,** 49–467 (1980).
4. Cannon, F. C., Riedel, G. E. & Ausubel, F. M. *Molec. gen. Genet.* **174,** 59–66 (1979).
5. Ruvkun, G. B. & Ausubel, F. M. *Proc. natn. Acad. Sci. U.S.A.* **77,** 191–195 (1980).
6. Avtges, P., Scolnik, P. A. & Haselkorn, R. *J. Bact.* **156,** 251–256 (1983).
7. Ditta, G., Stanfield, S., Corbin, D. & Helinski, D. *Proc. natn. Acad. Sci. U.S.A.* **77,** 7347–7354 (1980).
8. Marrs, B. L. *Proc. natn. Acad. Sci. U.S.A.* **71,** 971–973 (1974).
9. Yen, H. C., Hu, N. T. & Marrs, B. L. *J. molec. Biol.* **131,** 157–168 (1979).
10. Jorgensen, R. A., Rothstein, S. J. & Reznikoff, W. S. *Molec. gen. Genet.* **177,** 65–72 (1979).
11. Ruvkun, G. B. & Ausubel, F. M. *Nature* **289,** 85–89 (1981).
12. Postgate, J. R. *Symp. Soc. gen. Microbiol.* **24,** 264–292 (1974).
13. Madigan, M. T. & Gest, H. *Archs Microbiol.* **117,** 119–122 (1978).
14. Quinto, C. *et al. Nature* **299,** 724–726 (1982).
15. Rice, D., Mazur, B. J. & Haselkorn, R. *J. biol. Chem.* **257,** 13157–13163 (1982).
16. Kallas, T., Rebiere, M. C., Rippka, R. & Tandeau de Marsac, N. *J. Bact.* **155,** 427–431 (1983).
17. Inouye, S., Ike, Y., & Inouye, M. *J. biol. Chem.* **258,** 38–40 (1983).
18. Bendich, A. J., Anderson, R. S. & Ward, B. L. in *Genome Organization and Expression in Plants* (ed. Leaver, C. J.) 31–35 (Plenum, New York, 1980).
19. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
20. Taylor, D. *Plasmid* **8,** 100 (1982).
21. Parker, R. C., Watson, R. M. & Vinograd, J. *Proc. natn. Acad. Sci. U.S.A.* **74,** 851–855 (1977).
22. Johansson, B. C. & Gest, H. *Eur. J. Biochem.* **81,** 365–371 (1977).
23. Dilworth, M. *Biochim. biophys. Acta* **127,** 285–294 (1966).

Transposition of Tn*554* does not generate a target duplication

Ellen Murphy & Sven Löfdahl*

Department of Plasmid Biology, Public Health Research Institute of the City of New York Inc., New York, New York 10016, USA

Transposable elements from prokaryotic and eukaryotic organisms are discrete DNA segments bounded by inverted or directly repeated sequences that insert into non-homologous DNA in a reaction that is independent of the general recombination functions of the host. The mechanisms proposed generally involve a staggered double-stranded scission of the target DNA, ligation to the nicked ends of the transposable element, and replication of the element, resulting in the generation of a directly repeated oligonucleotide target sequence flanking the new copy of the element[1–3]. Most transposons have a relatively low degree of target site specificity coupled with a low insertion frequency. Tn*554*, a *Staphylococcus aureus* transposon which specifies resistances to erythromycin and spectinomycin, displays an unusually high degree of insertion specificity. Tn*554* transposes with high efficiency to a unique ('primary') site in the *S. aureus* chromosome[4,5] and only rarely ($<10^{-6}$ per transductant) to other, secondary sites[6]. We report here the nucleotide sequences surrounding the junctions of Tn*554* in three independent 'primary' insertions and two 'secondary' insertions of the transposon. Two unusual features are revealed: first, the termini of Tn*554* contain neither inverted nor directly repeated sequences. Second, transposition of Tn*554* does not generate the short direct repeats of the target DNA that are characteristic of other transposable elements. These results suggest that the mechanism of Tn*554* insertion may be significantly different from that of other transposons.

Previous blot hybridization experiments (ref. 5 and unpublished data) indicated that insertion of Tn*554*, in each of 25 cases examined, occurred in the same location in the *S. aureus* chromosome, within the limits of resolution of this method. To determine the insertion specificity at the nucleotide level, the sequences of several independent transpositions of Tn*554* into the chromosomes were determined. The following strategy was used to obtain multiple, independent insertions of Tn*554* in the primary target. The transposon was first cloned from the *S. aureus* chromosome into *Escherichia coli* (Fig. 1). The flanking chromosomal sequences contained on this plasmid were then used as a probe to screen for a recombinant plasmid containing the target region from a Tn*554*-negative strain, RN450. The chromosomal insert was then recloned into a *S. aureus* vector (pRN8057) and this construct, pEM9605, was used as a target for transposition of Tn*554*. This method was used effectively

* Present address: Department of Bacteriology, National Bacteriological Laboratory, Stockholm, Sweden.

0028–0836/84/030292—03$01.00

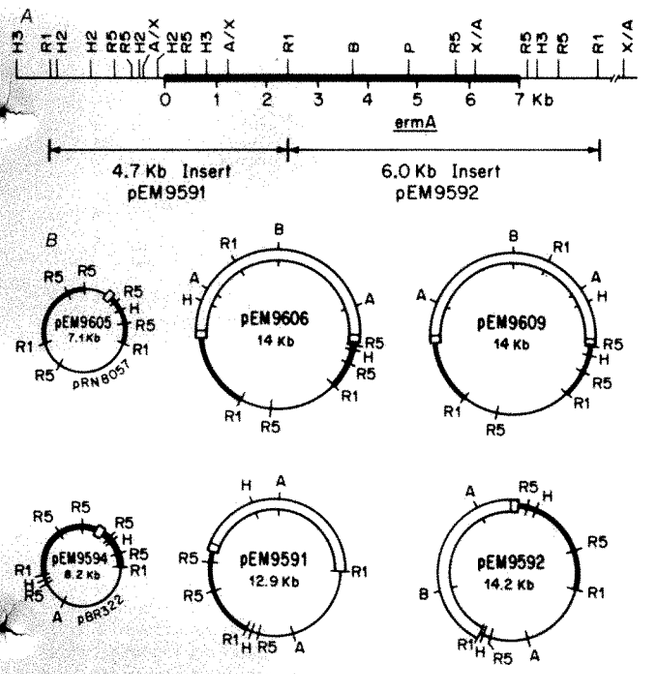


Fig. 1 *A*, Restriction map of Tn*554* and flanking chromosomal DNA. Thick line, Tn*554*; thin lines, flanking DNA. Enzymes are: A, *Aval*; B, *Bgl*II; H2, *Hpa*II; H3, *Hin*dIII; R1, *Eco*RI; R5, *Eco*R5; P, *Pst*I; X, *Xho*I. *B*, Restriction maps of Tn*554*-containing clones. Clones containing the primary (chromosomal) site sequences were obtained as follows: high molecular weight DNA from *S. aureus* strain RN2864 (Tn*554*$^{+}$) was digested with *Eco*RI and the cleaved DNA fractionated on a 10–30% neutral sucrose gradient. Fractions were collected and aliquots loaded onto a 0.7% agarose gel; those containing DNA in the range of 3–7 kb were pooled, the DNA was ethanol-precipitated, and ligated to *Eco*RI-cleaved, alkaline phosphatase-treated pBR322. This preparation was used to transform *E. coli* HB101 with selection for carbenicillin resistance. Transformants were transferred to nitrocellulose filters for colony hybridization[20], using as a probe a 4.9-kb *Aval* fragment from pRN4177, a secondary-site insertion of Tn*554*[6]. As Tn*554* contains a single *Eco*RI site, clones were isolated that contained either the left (pEM9591) or right (pEM9592) half of Tn*554*, as indicated above. The latter carries the Em^{r} locus, which is expressed in *E. coli*. A clone containing the primary chromosomal insertion site (but lacking Tn*554*) was obtained similarly, using *Eco*RI-cleaved DNA from RN450, a standard laboratory host which does not contain Tn*554*. Sucrose gradient fractions containing DNA of the predicted size of the *Eco*RI fragment (3.8 kb) were used for the ligation. The probe for the colony hybridization was the *Eco*RI insert from pEM9592, containing the right half of Tn*554* and 1.5 kb of flanking chromosomal DNA. Positive colonies contained a plasmid with a 3.8-kb *Eco*RI insert which matched the predicted restriction map of the target region. The 3.8-kb insert was subsequently recloned onto pRN8057, a temperature-sensitive derivative of the tetracycline resistance plasmid pT181, and transferred back to *S. aureus* (namely pRN9605).

for transposon Tn*7*, which exhibits an insertion preference for the *E. coli* chromosome that is similar to that of Tn*554*[7,8].

Insertions into pEM9605 were obtained by transduction, using as donors a strain having any one of five penicillinase plasmids containing Tn*554* in a secondary site. We have previously observed that Tn*554* transpositions obtained in this manner are usually associated with loss of the donor plasmid[6]. Erythromycin-resistant (Em^{r}) transductants were therefore screened for the absence of the donor plasmid markers (cadmium and penicillin resistances) and their plasmid contents analysed on agarose gels. Transductants fell into three classes: those containing only the 7.1-kilobase (kb) target plasmid pEM9605 and a chromosomally inserted Tn*554*, those containing only a 14-kb plasmid resulting from the transposition of Tn*554* into pEM9605, and those containing plasmids of various

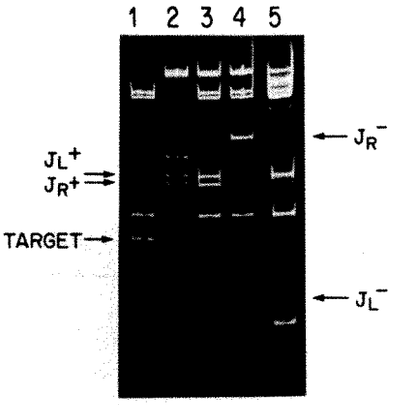


Fig. 2 Restriction analysis of transpositions into pEM9605. Plasmid DNA was prepared as described previously[21], cleaved with *Eco*RV and fractionated on a 5% polyacrylamide gel. Lane 1, pEM9605 (target); lane 2, pEM9595; lane 3, pEM9606 (pRN4176→pEM9605, (+)); lane 4, pEM9609 (pRN4177→pEM9605, (−)); lane 5, pEM9613, a deleted derivative from the cross ϕ11::Tn*554*→pEM9605. *Eco*RV junction fragments J_L^+ and J_R^+, for the (+) insertion, are approximately 1,200 and 980 bp, respectively; for the (−) insertions they are 402 bp (J_L^-) and approximately 1,800 bp (J_R^-); the *Eco*RV restriction fragment containing the Tn*554* target site is ~550 bp long. (Restriction maps of pEM9605, pEM9606 and pEM9609 are shown in Fig. 1*B*.)

intermediate sizes, representing deleted derivatives of pEM9605::Tn*554* insertions. As reported for Tn*7* (ref. 7), transposition into the cloned site does not seem to be as efficient as insertion into the chromosome itself.

Restriction analysis of plasmid DNA from these transductants indicated that Tn*554* inserted into the target fragment present in pEM9605 in the same location into which it inserts in the chromosome (Fig. 2). However, in contrast to the chromosomal insertions, which are orientation and site-specific, insertions into pEM9605 were recovered in both orientations. Recombination between flanking homologous sequences cannot account for the observed orientation specificity of the chromosomal insertions, as this specificity is maintained in the absence of flanking homology (that is, from non-homologous donors) as well as in the *recA* host[5].

Most of the plasmids containing Tn*554* insertions in the same orientation as in the chromosome (designated '+') were structurally unstable and could be detected only in the primary transductant colonies; these gave rise to deleted derivatives that had preferentially lost most of Tn*554*. Insertions into pEM9605 in the opposite orientation (designated '−') and some of the (+) orientation insertions, were stable. It is likely that insertions occurred with equal frequency in both orientations, but that only one class is recovered. Differences between the sequences present on the chromosome beyond the cloned region, and on the vector pRN8057, might account for the instability of the (+) transpositions in pEM9605. Note that the 3.8-kb insert of pEM9605 could itself be cloned in only one orientation into pRN8057; repeated efforts to reverse the orientation of the target fragment failed. In addition, experiments using a host strain carrying a chromosomal deletion of the primary insertion site suggest that the instability may be a consequence of recombinational events between the chromosome and the cloned chromosomal sequences (M. Bastos and E.M., unpublished data).

The nucleotide sequences of the junctions of several independent Tn*554* insertions are shown in Fig. 3. Two unusual features of the Tn*554* junctions are apparent. First, the ends of Tn*554* lack any direct or inverted repeat sequences. Second, insertion of Tn*554* does not generate a repeat of the target DNA. These results contrast with all other known transposable elements except for bacteriophage Mu, which also has asymmetric ends, but which generates a 5-base pair (bp) duplication[9]. Tn*7*, although similar to Tn*554* in its insertion specificity with respect

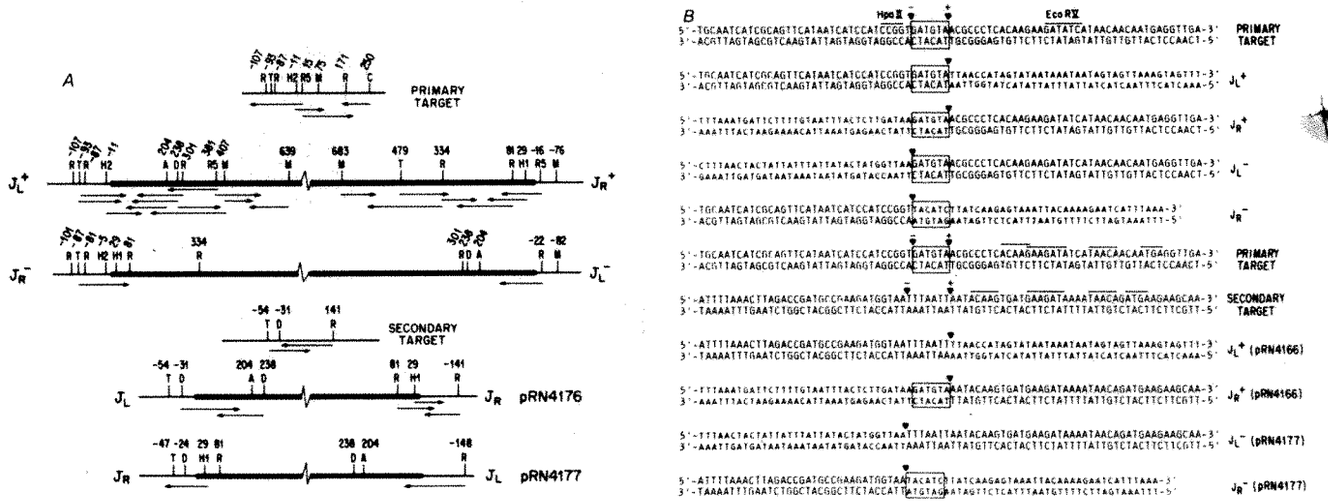


Fig. 3 ***A***, Strategy for sequencing the Tn*554* junctions. Thick lines represent Tn*554* DNA; thin lines, flanking sequences. Fragments were sequenced in the direction of the arrows. DNA fragments isolated from acrylamide gels were 5′-end labelled with polynucleotide kinase (P-L Biochemicals) and cleaved with a second restriction enzyme or strand-separated. Sequencing was done by the method of Maxam and Gilbert[22]. The chromosomal target sequence was determined twice using pEM9594 and pEM9605, which carry the 3.8-kb *Eco*RI target fragment cloned into pBR322 and pRN8057, respectively. pEM9591 and pEM9592 contain the chromosomal junctions J_L^+ and J_R^+, respectively, cloned directly from the Tn*554*-containing *S. aureus* chromosome (RN2864); pEM9606 and pEM9607 carry insertions of Tn*554* in the same orientation as the chromosome (+), into the 3.8-kb target fragment cloned in pEM9605. The junctions of two (−) orientation insertions were obtained from pEM9609 and pEM9612. The sequences of the secondary-site target and insertion junctions were derived from pRN4176, pRN4177 and pEM6271; the latter contains a 2.5-kb *Bgl*II fragment containing the targets for the pRN4176 and pRN4177 insertions. Enzymes are: A, *Alu*I; C, *Cfo*I; D, *Dde*I; H1, *Hin*fI; H2, *Hpa*II; M, *Mbo*I; R5, *Eco*RV; R, *Rsa*I; T, *Taq*I. ***B***, sequences of the Tn*554* insertion junctions. The terminal 40 bp of Tn*554* at each end and 80 bp of the target DNA are shown. Homology between the primary and secondary target sites is indicated by the lines over the sequences. The GATGTA sequence derived from either Tn*554* (smaller typeface) or the target DNA (larger typeface) is boxed.

to the chromosome, also provokes a 5-bp target repeat[10]. In these respects Tn*554* is more similar to temperate bacteriophages such as λ.

Sequences were obtained for the chromosomal insertion that was cloned directly from the *S. aureus* chromosome, for two independent (+) insertions of Tn*554* into pEM9605 (pEM9606 and pEM9607), and for two (−) insertions into pEM9605 (pEM9609 and pEM9612). The junctions of two secondary site penicillinase plasmid insertions (pRN4176 and pRN4177) were also sequenced. All three of the (+) insertions sequenced were found to have inserted between the same nucleotide pair, 3′ to the sequence 5′-GATGTA-3′. This sequence is also present at the right terminus of Tn*554*. The two (−) insertions sequenced are just 5′ of this same sequence, six nucleotides upstream from the site of the (+) insertions. The two secondary-site insertions are 7 bp apart, and in opposite orientations from one another; while they are separated by the sequence TTTAATT, there is a near-match (GATGGTA) to the GATGTA, immediately 5′ to the pRN4177 site (Fig. 3*B*). However, this sequence is unlikely to be sufficient to account for the insertional specificity, as it would be expected to occur approximately 1,000 times in the *S. aureus* chromosome, assuming that these sequences are not restricted. Comparison of the primary and secondary targets revealed some homology 3′ to the insertion sites (Fig. 3*B*); however, these sequences differ greatly in their A+T content: ~47% and 76% for the 30 bp surrounding the primary and secondary sites, compared with 65% for *S. aureus* DNA[11]. The Tn*554* termini are themselves highly A+T-rich (≥80%), a fact which may contribute to the efficiency of transposition, as A+T-rich DNA is highly recombinogenic.

Earlier data concerning excision of Tn*554* (ref. 4) had led to the suggestion that Tn*554* inserts and excises like bacteriophage λ[12], using a free circular intermediate. The presence of the common GATGTA sequence at both the right end of Tn*554* and at the site of insertion, and the absence of a target duplication, are consistent with this hypothesis. However, if Tn*554* inserted via recombination between the 'common core' sequence GATGTA, integration would of necessity be orientation-specific and would not account for the insertions in the (−) orientation into pEM9605 or for the opposite orientations of the two secondary-site insertions. Our sequence data do not rule out the possibility of a circular intermediate assuming that the strands can be cut and rejoined on either side of the GATGTA sequence. Recent experiments suggest that the donor replicon may not be recovered following transposition (I. Edelman and R. Novick, personal communication); the simplest, but not the only, mechanism that could account for these results is a conservative, double-stranded transfer of Tn*554* to a flush, double-stranded break in the recipient DNA molecule. Conservative mechanisms have been suggested for the transposition of Tn*5* (ref. 13) and for the initial insertion of bacteriophage Mu[9,14,15]. The Mu prophage is flanked by 5-bp repeats[16,17] but the mature bacteriophage apparently is not: it is probable that prophage insertion and replicative transposition use two different pathways[9,18,19].

We thank Dr Carol J. Lusty for help with the DNA sequencing and Leslie Huwyler for technical assistance. This work was supported by NIH grant GM27253 to E.M.

Received 11 July; accepted 25 October 1983.

1. Shapiro, J. A. *Proc. natn. Acad. Sci. U.S.A.* **76,** 1933–1977 (1979).
2. Grindley, N. & Sherratt, D. *Cold Spring Harb. Symp. quant. Biol.* **45,** 125–134 (1980).
3. Kleckner, N. *A. Rev. Genet.* **15,** 341–404 (1981).
4. Phillips, S. & Novick, R. P. *Nature* **278,** 476–478 (1979).
5. Krolewski, J. J., Murphy, E., Novick, R. P. & Rush, M. G. *J. molec. Biol.* **152,** 19–33 (1981).
6. Murphy, E., Phillips, S., Edelman, I. & Novick, R. P. *Plasmid* **5,** 292–305 (1981).
7. Lichtenstein, C. & Brenner, S. *Molec. gen. Genet.* **183,** 380–387 (1981).
8. Barth, P. T., Datta, N., Hedges, R. W. & Grinter, N. J. *J. Bact.* **125,** 800–810 (1976).
9. Kamp, D. & Kahmann, R. *Cold Spring Harb. Symp. quant. Biol.* **45,** 329–336 (1980).
10. Lichtenstein, C. & Brenner, S. *Nature* **297,** 601–603 (1982).
11. Marmur, J., Falkow, S. & Mandel, M. *A. Rev. Microbiol.* **17,** 329–372 (1963).
12. Nash, H. *A. Rev. Genet.* **15,** 143–167 (1981).
13. Berg, D. *Proc. natn. Acad. Sci. U.S.A.* **80,** 792–796 (1983).
14. Liebhart, J. C., Ghelardini, P. & Paolozzi, L. *Proc. natn. Acad. Sci. U.S.A.* **79,** 4362–4366 (1983).
15. Akroyd, J. E. & Symonds, N. *Nature* **303,** 84–86 (1983).
16. Allet, B. *Cell* **16,** 123–130 (1979).
17. Kahmann, R. & Kamp, D. *Nature* **280,** 247 (1979).
18. Chaconas, G., Harshey, R. M., Sarvetnick, N. & Bukhari, A. I. *J. molec. Biol.* **150,** 341–359 (1981).
19. Harshey, R. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2012–2016 (1983).
20. Grunstein, M. & Hogness, D. S. *Proc. natn. Acad. Sci. U.S.A.* **72,** 3961–3965 (1975).
21. Novick, R. P., Murphy, E., Gryczan, T. J., Baron, E. & Edelman, I. *Plasmid* **2,** 109–129 (1979).
22. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499–560 (1980).

Sedimentation in Loch Earn and Loch Lubnaig, Scotland

IN their paper about sedimentary features associated with slumping in Loch Earn and Loch Lubnaig, McManus and Duck[1] indicate that there is no prior record of the use of sonar devices in lakes for the investigation of "subaqueous landforms". This is a curious statement given our[2] earlier reported work in their own field area and the extensive surveys of the Canadian Centre for Inland Waters in for example, the Great Lakes (see ref. 3), where acoustic techniques have become standard for mapping both lakebed and sub-bottom landforms, whose recognition is regarded as a normal prerequisite for any engineering undertaking.

While McManus and Duck[1] recognize that their observed slumps occur at depths below the influence of surface water waves, they do not consider the possibility that longer period waves, such as seiches or internal waves, may influence bed forms. It is instructive to investigate the theoretical seiche[4] for any lake under sedimentological investigation to see if water velocities expected at the lakebed may be great enough to cause sediment movement. I have done this for a water body of rectangular cross-section (length 2,500 m, depth 30 m) as a crude model of the Stank Basin[5] of Loch Lubnaig and conclude that a water particle velocity of 5 cm s^{-1} is possible for a seiche of only 0.1-m amplitude, the period of oscillation being ~5 min. Seiche observations by Gill[6] on Llyn Gwellyn, a lake of comparable size to the Stank Basin, suggest that a real seiche would be of longer period than the approximate theory predicts, but that particle velocities at the base of the water column would still be sufficient to transport silts and finer grains (Graf and Acaroglu[7]) even if the seiche amplitude were only a few centimetres. We[2] have observed three sediment waves or mounds in the bed of the Stank Basin. They rise, with gradients of 1:5, to a height of 5 m above the general level of the fine lakebed sediment, which is itself no more than 5 m thick. The sediment waves are symmetrical and show layering which parallels the sediment–water interface. Such layering and symmetry would not arise from slumping, but could be produced by a seiche-driven oscillatory water current. McManus and Duck[1] have drawn attention to the effect that sediment disturbance from slumping will have on the interpretation of lakebed sediment cores retrieved for palaeomagnetic or palaeoecological purposes. Disturbance in the manner I propose will be a more subtle effect because of the presence of seemingly undisturbed layers.

In Scottish lochs, the use of modern acoustic equipment (with the exception of its largely ludicrous use in Loch Ness) has been neglected. This is not the tribute that Murray and Pullar[7] would have wished. As well as conventional sidescan and profiling records, sediment acoustic velocity determinations are required[8,9] to help assign proper depth scales and to assess sediment gas content, cited by Monroe[10] as an important factor in causing slumping. I hope that McManus and Duck[1] and others will receive support to extend their work to other lochs and perhaps to map more completely the mounds in the Stank Basin and to verify if they are indeed associated with seiche activity induced by the winds howling down the glen at Ardchullarie Mor.

ALASDAIR G. MCKAY
Atlantic Geoscience Society,
35 Edward Street, Dartmouth,
Nova Scotia, Canada B2Y 2PC

1. McManus, J. & Duck, R. W. *Nature* **303**, 161–162 (1983).
2. McKay, A. G. & McEwen, I. J. *Mar. Sedim. Atlantic Geol.* **18**, 58 (1982).
3. Thomas, R. L., Demp, A. L. & Lewis, C. F. M. *Can. J. Earth. Sci.* **10**, 226–271 (1973).
4. Neumann, G. & Pierson, W. J. *Principles of Physical Oceanography*, 291 (Prentice-Hall, Englewood Cliffs, 1966).
5. Gill, W. W. S. thesis, Univ. Coll. North Wales (1967).
6. Graf, W. H. & Acaroglu, E. R. *Bull. Int. Ass. Sci. Hydrol.* **11**, 27–43 (1966).
7. Murray, J. & Pullar, L. *Bathymetric Survey of the Scottish Freshwater Lochs* (Challenger Office, Edinburgh, 1910).
8. Bell, D. L. & Porter, W. J. in *Physics of Sound in Marine Sediments* (ed. Hampton, L.) (Plenum, New York, 1974).
9. McKay, A. G. & McKay, P. M. *J. acoust. Soc. Am.* **71**, 871–878 (1982).
10. Monroe, J. N. *Science* **161**, 1394–1395 (1969).

DUCK AND MCMANUS REPLY—We welcome the comments of McKay concerning our recent contribution[1]. He is correct to draw attention to sonar work in the North American Great Lakes. In view of their enormity these water bodies behave as inland seas and are not directly comparable with even the largest of the Scottish lochs.

Our contribution[1] specifically referred to sidescan sonar surveys and not to the use of sonar devices in general. The principal work reported was undertaken on Loch Earn and mention was made of Loch Lubnaig to illustrate that the subaqueous landforms recognized are not mere curiosities confined to one water body. We have since detected them in other lochs.

We were unaware of the boomer seismic work of McKay and McEwen[2] in Loch Lubnaig as their brief abstract was published in a Canadian journal not known for its contributions to Scottish environmental studies. The report of sediment waves or mounds in the Stank Basin[2] is interesting but, despite re-examination of sonographs and echograms[3], we have been unable to confirm their presence due to lack of information regarding their position or orientation. However, marginal spurs are recognized extending into the basin floor from the bounding slopes.

Seiches or internal waves may indeed influence loch bedforms. However, as yet, we have insufficient hydrodynamic data to corroborate this. Furthermore we have reservations about the seiche-associated current velocities calculated by McKay for Loch Lubnaig. Although we have not undertaken seiche observations in the loch there is good reason to believe that the 0.1-m surface seiche amplitude used in McKay's model, and to which the horizontal current velocity is proportional[4], is excessively large. Chrystal[5] demonstrated that seiche activity is poorly developed in Loch Lubnaig due to its shallow nature (mean depth, 13 m), its very irregular bottom and its orientation across the path of atmospheric disturbances. During a 6-week period only four instances of definite, but short-lived, seiche activity, with a period of about 24 min, were recognized[5]. For most of the period "... nothing was found but wind embroidery and sub-permanent wind denivellation, such as would be naturally expected in a shallow lake"[5]. Moreover, the maximum seiche amplitude observed by Chrystal[5] (Fig. 22) was only about 0.5 cm. On the basis of McKay's model for the Stank Basin the maximum current velocity, at a seiche node, associated with such activity would be less than 0.3 cm s^{-1}. Average current velocities would be about half this value and tend to die away rapidly with time[6]. Thus currents resulting from surface seiches are unlikely to be responsible for the bedforms described.

To our knowledge the thermal behaviour of Loch Lubnaig has not been investigated. However, it is likely that internal wave (internal seiche[7]) activity will occur in association with summer stratification. Horizontal current velocity components generated by such water movements are known to be up to five times greater than those associated with corresponding surface seiches[8,9]. Moreover, these currents can persist for several days[8]. Hence it is possible that internal waves may have a role in the formation of the structures recognized by McKay and McEwen[2], perhaps in the manner advocated by Mortimer[10].

ROBERT W. DUCK
JOHN MCMANUS
Department of Geology,
The University,
Dundee DD1 4HN, UK

1. McManus, J. & Duck, R. W. *Nature* **303**, 161–162 (1983).
2. McKay, A. G. & McEwen, I. J. *Mar. Sedim. Atlantic Geol.* **18**, 58 (1982).
3. Asaad, N. M. thesis, Univ. Dundee (1982).
4. Proudman, J. *Dynamical Oceanography* (Wiley, London, 1953).

© 1984 Macmillan Journals Ltd

5. Chrystal, G. in *Bathymetrical Survey of the Scottish Freshwater Lochs* Vol. 1, 29–90 (Challenger Office, Edinburgh, 1910).
6. Smith, I. R. *Freshwater Biol.* **9**, 119–145 (1979).
7. Wilson, B. W. *Adv. Hydrosci.* **8**, 1–94 (1972).
8. George, D. G. *Freshwat. Biol.* **11**, 37–60 (1981).
9. Duck, R. W. thesis, Univ. Dundee (1982).
10. Mortimer, C. H. *Phil. Trans. R. Soc.* B**236**, 355–404 (1952).

Control of fertility in red deer

LOUDON *ET AL.*[1] have recently invoked a suckling-mediated mechanism for the control of fertility in red deer hinds. We suggest that their emphasis on the role of prolactin is not justified by their data and that other explanations are equally likely.

Although plasma prolactin concentrations are positively associated with the frequency of suckling[2], the authors fail to show a significantly higher plasma prolactin level after 80 days, between groups of hinds on low versus high planes of nutrition, despite the persistence of high suckling frequencies in the former. There is a minimum lag of some 30 days separating this latest detection of a difference in plasma prolactin levels between the groups and the earliest possible conception on day 110—when the stag was introduced. It is questionable as to whether there would be an ovulation-inhibiting effect of increased plasma prolactin levels this far in advance of ovulation, even if, as has been suggested[3], such an effect were to operate on the follicular stage of the 18.3 day oestrous cycle[4]. This is assuming that prolactin has a causal influence on oestrus cycle activity which itself remains in doubt[5].

Loudon *et al.* do not attempt to separate from the suckling data the confounding effect of differences in mean body weight between the two groups. It is well established that body weight not only influences fertility rates but may also affect the onset of oestrus in mammals[6]. In farmed red deer small hinds calve later than heavy hinds: a 1 kg increase advancing calving by between 0.3 and 1.0 days (ref. 7 and A.S.I. Loudon, personal communication). Furthermore, in the wild the average non-lactating hind calves approximately 2 days earlier for every 1 kg increase in mean autumn body weight[8]. Therefore a 4.1 kg difference in mean body weights between the two groups could explain the 6.5 day difference in return to oestrus and could be tested with their data. However, even if this is plausible and weight for weight hinds on a low nutritional plane return to oestrus later, this may be for reasons other than lactational control. For example, it is well known that in sheep, which cease lactation many weeks before the rut, body condition interacts with the plane of nutrition immediately before breeding[9]. At a given weight individuals that are rapidly improving in condition, as might be expected in the group of hinds on permanent grass pasture, show earlier ovulation and higher ovulation rates.

In summary, Loudon *et al.* concur with at least one other study in failing to show any influence of the plane of nutrition on plasma prolactin levels during or immediately before the onset of the reproductive period[10]. As a consequence, they have little reason to invoke elevated levels of prolactin as a mechanism delaying conception in poorly nourished hinds. Finally, by not controlling in any way for maternal body weight and/or condition, the major confounding variable is not eliminated nor its contribution to the observed effect even brought into question.

S. D. ALBON
G. R. IASON
Large Animal Research Group,
Department of Zoology,
University of Cambridge,
Downing Street,
Cambridge CB2 3EJ, UK

1. Loudon, A. S. I., McNeilly, A. S. & Milne, J. A. *Nature* **302**, 145–147 (1983).
2. McNeilly, A. S. *Br. med. Bull.* **35**, 151–154 (1979).
3. McNeilly, A. S., Glasier, A., Jonassen, J. & Howie, P. W. *J. Reprod. Fert.* **65**, 559–569 (1982).
4. Guinness, F. E., Lincoln, G. A. & Short, R. V. *J. Reprod. Fert.* **27**, 427–438 (1971).
5. Webster, G. M. & Haresign, W. *J. Reprod. Fert.* **67**, 465–471 (1983).
6. Sadleir, R. M. F. S. *The Ecology of Reproduction in Wild and Domestic Mammals* (Methuen, London, 1969).
7. Hamilton, W. J. & Blaxter, K. L. *J. agric. Sci., Camb.* **95**, 261–273 (1980).
8. Mitchell, B. & Lincoln, G. A. *J. Zool.* **171**, 141–152 (1973).
9. Gunn, R. G. & Doney, J. N. *J. agric. Sci., Camb.* **85**, 465–470 (1975).
10. Rhind, S. M., Robinson, J. J., Chesworth, J. M. & Crofts, R. M. J. *J. Reprod. Fert.* **58**, 145–152 (1980).

LOUDON *ET AL.* REPLY—In our paper we drew attention to the influence of pasture type on the milk yield, suckling patterns and fertility of red deer hinds. We were alerted to the physiological implications of the differences in suckling pattern when we considered our data on prolactin. These data, in common with a number of other studies, showed that prolactin levels were correlated with suckling frequency. At no point in our paper did we suggest that prolactin *per se* was ultimately involved in the regulation of fertility in red deer. Indeed we would suggest that in seasonally breeding mammals such as deer and sheep prolactin may have little influence on fertility. Thus, sheep treated with bromocriptine to block the normal high levels of prolactin in mid-summer resumed fertility at an identical time to untreated control sheep[1,2]. Thus although prolactin may have an important function in regulating fertility in some mammals including humans, we agree with Albon and Iason that there is little evidence that it may be directly important in the case of red deer.

We know of only one study that attempted to control female weight, food intake, milk yield and offspring growth rate while manipulating suckling frequency. In this study, ewes compelled to suckle their offspring twice a day resumed oestrous activity at an earlier date than those permitted to suckle five times a day[3]. We acknowledge that there is clear evidence that the body condition of non-lactating sheep prior to mating has an effect on their fertility but suggest that in the cases of wild mammals, including red deer, which, unlike sheep, frequently suckle their offspring through the mating period, the pattern of suckling activity may have a profound effect on their subsequent fertility. Body weight *per se*, like prolactin, simply correlates with differences in return to oestrus in red deer. We are unable to attribute differences in body weight between the two groups of hinds to differences in body condition or differences in gut fill associated with the complex effects of grazing different grass pastures.

A. S. I. LOUDON
Institute of Zoology,
Regents Park,
London NW1 4RY, UK
A. S. MCNEILLY
MRC Reproductive Biology Unit,
Centre for Reproductive Biology,
37 Chalmers Street,
Edinburgh EH3 9EW, UK
J. A. MILNE
Hill Farming Research Organisation,
Bush Estate, Penicuik,
Midlothian EH26 0PY, UK

1. Fitzgerald, B. P. & Cunningham, F. J. *J. Reprod. Fert.* **61**, 141–148 (1981).
2. Land, R. B., Carr, W. R., McNeilly, A. S. & Preece, R. D. *J. Reprod. Fert.* **59**, 73–78 (1980).
3. Dingwall, W. S., Robinson, J. S. & Atkinson, T. *Anim. Prod.* **34**, 370 (1982).

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

0028–0836/84/030296—01$01.00

Current Topics in Microbiology and Immunology

Editors: M. Cooper, W. Goebel, P. H. Hofschneider, H. Koprowski, F. Melchers, M. Oldstone, R. Rott, H. G. Schweiger, P. K. Vogt, R. Zinkernagel

Volume 96

Gene Cloning in Organisms Other Than E. coli

Editors: P. H. Hofschneider, W. Goebel
1982. 63 figures. VIII, 259 pages. Cloth DM 92,–; approx. US $ 35.70
ISBN 3-540-11117-4

Volume 98

Retrovirus Genes in Lymphocyte Function and Growth

Editors: E. Wecker, I. Horak
1982. 8 figures. VIII, 142 pages. Cloth DM 66,–; approx. US $ 25.70
ISBN 3-540-11225-1

"This timely little volume ...(is a) coherent and crisp publication ...summarizing much of the important work and thinking in the field (of RNA tumor virus) ...welcomed by many who want a competent overview or an update."
Immunology Today

Volume 101

Tumorviruses, Neoplastic Transformation and Differentiation

Editors: T. Graf, R. Jaenisch
1982. 27 figures. VIII, 198 pages. Cloth DM 96,–; approx. US $ 37.30
ISBN 3-540-11665-6

Tumorviruses are widely used as tools to analyze the cellular processes which lead to malignant transformation. One of the most intriguing and important aspects of tumorvirus-cell interactions is the relationship between the differentiated state of the host cell and its susceptibility to infecting viruses. This book presents a series of reviews dealing with experimental systems in which only cells at a specific stage of differentiation are permissive for viral transformation and/or replication.

Volume 103

Retroviruses 1

Editors: P. K. Vogt, H. Koprowski
1983. 16 figures. V, 146 pages.
ISBN 3-540-12167-6

Contents: *U. G.* Rovigatti, *S. M. Astrin:* Avian Endogenous Viral Genes. – *S. H. Hughes:* Synthesis, Integration, and Transcription of the Retroviral Provirus. – *J. C. Neil:* Defective Avian Sarcoma Viruses. – *N. G. Famulari:* Murine Leukemia Viruses with Recombinat *env* Genes: A Discussion of Their Role in Leukemogenesis. – *M. J. Hayman:* Avian Acute Leukemia Viruses. – *O. N. Witte:* Molecular and Cellular Biology of Abelson Virus Transformation.

Volume 106

Mouse Mammary Tumor Virus

Editors: P. K. Vogt, H. Koprowski
1983. 12 figures. VII, 103 pages. Cloth DM 58,–; approx. US $ 22.50
ISBN 3-540-12828-X

Contents: C. Dickson, G. Peters: Proteins Encoded by Mouse Mammary Tumor Virus. – V. Traina-Dorge, J. C. Cohen: Molecular Genetics of Mouse Mammary Tumor Virus. – R. Michalides, A. van Ooyen, R. Nusse: Mouse Mammary Tumor Virus Expression and Mammary Tumor Development. – G. M. Ringold: Regulation of Mouse Mammary Tumor Virus Gene Expression by Glucocorticoid Hormones.

Volume 107

Retroviruses 2

Editors: P. K. Vogt, H. Koprowski
1984. 26 figures. Approx. 190 pages. Cloth DM 96,–; approx. US $ 37.30
ISBN 3-540-12384-9

Contents: P. Besmer: Acute Transforming Feline Retroviruses. – A. Tereba: Asymmetric Chromosomal Distribution of Endogenous Retrovirus Loci in Chickens and Mice. – J. G. Krueger, E. A. Garber, A. R. Goldberg: Subcellular Localization of pp 60^{src} in RSV-Transformed Cells. – J. A. Cooper, T. Hunter: Regulation of Cell Growth and Transformation by Tyrosine-Specific Protein Kinases: The Search for Important Cellular Substrate Proteins. – J. G. Foulkes: Phosphotyrosyl-Protein Phosphatases.

Volume 109

The Molecular Biology of Adenoviruses 1

30 Years of Adenovirus Research 1953–1983

Editor: W. Doerffler, University of Cologne, Germany
1984. 69 figures. Approx. 240 pages. Cloth DM 118,–; approx. US $ 45.80
ISBN 3-540-13034-9

2484/4/1a

Springer-Verlag
Berlin
Heidelberg
New York
Tokyo

Tiergartenstr. 17, D-6900 Heidelberg 1, 175 Fifth Ave., New York, NY 10010, USA,
37-3, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan

Circle No.04 on Reader Service Card.

Wiley

NEW YORK · CHICHESTER · BRISBANE · TORONTO · SINGAPORE

AQUATIC TOXICOLOGY

edited by **J.O. Nriagu,** *National Water Research Institute, Burlington, Canada*

This emphasizes the effects of aquatic toxicology (pollutants) on aquatic biota and discusses the physiological and biochemical effects of aquatic pollutants on aquatic animals. It includes coverage of the most current methodologies for detecting/analyzing toxicant-induced effects. It describes and analyzes toxic effects at the subcellular/cellular, tissue, organism, and community levels.

Advances in Environmental Science and Technology, Vol. 13.

December 1983 — 542pages
0471 88901 6 — $103.45/£73.75

WASTES IN THE OCEAN, Volume III

Radioactive Wastes in the Ocean

edited by **P.K. Park,** *National Oceanic and Atmospheric Administration;* **D.R. Kester; I.W. Duedall,** *S.U.N.Y., Stony Brook;* and **B.H. Ketchum,** *University of Rhode Island*

This is a comprehensive guide to evaluating methods of disposing radioactive wastes into the ocean, reviewing current research and selected case studies. It covers the interrelationship of oceanographic properties and processes to waste disposal practices and examines the behavior and fates of radioactive wastes in the marine environment, their effects on ocean life, and implications for human health. Strategies for determining optimal site selection, packaging, and engineering design of waste disposal are presented.

Environmental Science and Technology: A Wiley-Interscience Series of Texts and Monographs.

January 1984 — 544 pages
0471 09770 5 — $97.95/£69.95

TREES AND NETWORKS IN BIOLOGICAL MODELS

by **N. MacDonald,** *Department of Natural Philosophy, Glasgow University*

The first half of this book deals with the description of abstract networks, using the terminology of graph theory. Part I is concerned with the characterisation of overall properties of networks in terms of appropriate numerical indices, and Part II with stability of steady states and oscillations in networks. The second half (Part III) gives the first available general review of the description, biophysics and simulation of real branching structures, with illustrations from botany, zoology and the physiology of arteries, lung airways and nerve cells.

December 1983 — 226 pages
0471 10508 2 — $33.75/£18.00

BIOCHEMICAL PLANT PATHOLOGY

edited by **J.A. Callow,** *Department of Plant Biology, University of Birmingham*

International authorities present critical, stimulating accounts that reflect the currently exciting prospects in the rapidly expanding field of biochemical pathology.

December 1983 — 496 pages
0471 90092 3 — $65.00/£34.50

MARINE ECOLOGY

Volume V: Ocean Management, Part 2
Ecosystems and Organic Resources

edited by **O. Kinne,** *Biologische Anstalt Helgoland Zentrale, Hamburg, West Germany*

MARINE ECOLOGY provides an exhaustive, systematic exposition summarizing and evaluating the information obtained thus far on living systems in the sea and in littoral areas. It has been conceived with the increasing number of individuals in mind who are professionally concerned with life in the marine environment — especially investigators, teachers and students.

Series: Marine Ecology: A Comprehensive, Integrated Treatise on Life in Oceans and Coastal Waters, Volume V Part 2.

November 1983 — 466 pages
0471 90159 8 — $85.00/£45.00

EQUILIBRIA, NONEQUILIBRIA, AND NATURAL WATERS, Volume II

by **R.M. Pytkowicz,** *Oregon State University*

This book develops aquatic chemistry, geology, and hydrology from first principles, and applies them to the aquatic environment and complex solutions found in oceans, rivers, lakes, and laboratories. It examines processes in natural waters within the framework of thermodynamics and heterogeneous kinetics and presents serious research applications. It covers acids, new solid-solution equilibrium work, complexes, redox reactions, the geochemistry of trace metals, and gas-water interactions.

November 1983 — 372 pages
0471 89111 8 — $57.45/£40.95

THE GLOBAL BIOGEOCHEMICAL SULPHUR CYCLE, SCOPE 19

edited by **M.V. Ivanov,** *Deputy Director, Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences* and **J.R. Freney,** *CSIRO Division of Plant Industry, Canberra City, Australia*

This book focuses attention on the poorly investigated problems of the global biogeochemical sulphur cycle and opens the way for further study. It examines the possible long term effects on the stratospheric sulphate layer and the consequent effects on climate.

Series: SCOPE Report, Number 19

December 1983 — 496 pages
0471 10492 2 — $79.50/£42.50

CEREAL DISEASES

Their Pathology and Control, Second Edition

by **D.G. Jones,** *Department of Agricultural Botany, The University College of Wales, Aberystwyth* and **B.C. Clifford,** *Welsh Plant Breeding Station, Aberystwyth.*

This new edition aims to help towards increased crop yields by providing the basis for a better understanding of the many problems associated with cereal diseases and their control.

December 1983 — 326 pages
0471 10501 5 — $75.00/£39.50

John Wiley & Sons Limited

Baffins Lane · Chichester · Sussex PO19 1UD · England

Circle No.02 on Reader Service Card.

Super theories

Abdus Salam

Supersymmetry and Supergravity. By Julius Wess and Jonathan Bagger.
Princeton University Press: 1983. Pp.180. Hbk $40, £34.50; pbk $12.50, £10.90.

SUPERSYMMETRY — a possible symmetry between bosons (particles of spins zero or one or two, obeying Bose statistics) and fermions (particles of spins ½ or 3/2 obeying Fermi–Dirac statistics) — is an incredible postulate. It could have been made at any time after 1935, when the canons of quantum theory of fields were established. We had, however, been brought up from the kindergarten to think of spin zero (or spin one or spin two) bosons as intrinsically different from spin ½ (or spin 3/2) fermions. The fermions are individualists. In accordance with Pauli's exclusion principle, no two identical fermions could congregate at the same space point. In contrast the bosons are gregarious. They like to congregate and seek each other's company at the slightest provocation. How could one even try to place such different objects in the same multiplet of a possible symmetry group? In what sense could there be a symmetry between quanta of matter (typically fermions) and quanta of forces (typically bosons)?

In 1971, in the USSR, Golfand and Lichtman first discovered that one could, if one wished, introduce a fundamental symmetry between bosons and fermions — notwithstanding their intrinsic differences. The significance of their work was totally missed, however, and ignored until 1973 when this same symmetry was rediscovered by Julius Wess and Bruno Zumino working in the West. Even then (and despite the self-evident elegance of the symmetry, the relative freedom of supersymmetrized Lagrangians from field-theoretical infinities, as well as the guaranteed positivity of the energy) there was no direct evidence of the symmetry's existence at energies available in laboratories and supersymmetry remained a minority interest. The esoteric nature of the subject was reflected by the complete absence of relevant textbooks; conference proceedings and excellent review reports, yes, but no pedagogical material.

Now, after several years of increasing interest, a new generation of texts has begun to appear. Wess and Bagger's *Supersymmetry and Supergravity*, based on a course of lectures given by the senior author at Princeton, is one of the first. To assess the book, what it contains and what it chooses to leave out, it is necessary to consider the recent history of ideas in this field.

Two of the early developments concerned, first, the recognition of the existence of the so-called extended supersymmetries (N = 2, 3, 4 . . . 8) where internal symmetries are married to simple supersymmetry (N = 1); and second, the superspace and the superfield method of exhibiting N = 1 supersymmetry in field theory. The simple N = 1 supersymmetry admits of two spin zero particles being grouped together with one spin ½ Majorana particle into a single "supermultiplet". All these particles must possess the same mass. The N = 1 superfield is a field-function which describes this entire set of particles; the field is defined in "super-space" which is space-time manifold augmented with an additional fermionic coordinate. The extended N = 2 supersymmetry admits of a supermultiplet with four spin zero and two spin ½ objects. The super-space this supermultiplet would exist on would admit of *two* fermionic coordinates. And so on for N = 3, 4, . . . , this sequence ending finally with the N = 8 supersymmetry — the so-called maximal supersymmetry — which would group together the following into its fundamental supermultiplet: one spin two, eight spin 3/2, twenty-eight spin one, fifty-six spin ½ and seventy spin zero massless objects.

During 1974–1975 renormalizable supersymmetric theories of spin ½ and spin zero objects were elaborated, as were supersymmetric theories of spin one and spin ½ particles, describing gauge quanta and their supersymmetric partners, the gauginos. Then, in 1976, the prize problem of supersymmetrizing gravity was solved. This was the prize problem because a gauging of supersymmetry must inevitably lead to a supersymmetrization of Einstein's gravity. The N = 1 "supergravity" which results is a generally-covariant, local, supersymmetric theory of the spin 2 particle (the hypothetical graviton) and of its supersymmetric partner (the still-more-hypothetical gravitino of spin 3/2). The following years were an exuberant period of writing down of theories of extended supergravities (N = 2, 3, 4, 8). With these — and particularly with N = 8 supergravity — a singular hope was born: that particles of spin two and spin one, mediating *all* the four fundamental forces (gravity, electroweak and strong), together with the appropriate spin-zero Higgs needed to break the electroweak and other gauge symmetries, and interacting with "source" matter of spin ½ quarks and leptons, could all be described by the fundamental supermultiplet of the N = 8 theory interacting with itself. This would not only unite the "marble" of gravity with the "base wood" of matter, as Einstein dreamt; in Hawking's eloquent phrase, it would be the "Theory of Everything".

Unfortunately, the spectrum of particles, even in the maximally extended supergravity theory (N = 8) turned out to be inadequate to accommodate the known quarks, leptons and gauge particles. It was also soon recognized that, after gauging, any such theory must contain two coupling constants — the Newtonian constant determining the strength of the gravitational force and an unacceptably large cosmological constant in lieu of the fine structure constant (phenomenologically wrong by factor 10^{120}!). On the technical side too, there were problems. It came to be clearly appreciated that the geometrization of physics, which started with the Einsteinian revolution of associating gravity with the curvature of space-time, would not be complete until supergravities had been described in terms of geometrical entities (super-curvature and super-torsion) in extended space-times — extended with the addition of the new fermionic coordinates on which the requisite superfields depend.

This was the situation in 1981. We had theories which were tantalizingly elegant but technically incomplete; theories which were relevant, but not quite adequate as far as the known low-energy physics was concerned. Since 1981, however, a fresh look has been taken at supersymmetry theories. The motivation for this has come from a new direction — from the so-called hierarchy problem, the problem of disparate mass scales in physics. In particle physics the relevant scale of masses ranges from the mass of the electron (of around 10^{-3} Gev) to the Planck mass associated with Newton's constant of gravity ($\approx 10^{19}$ Gev). The ratio of these two masses is an enormous number of the order of 10^{22}. And if the electronic neutrino is also massive (with an expected mass of the order of 10^{-8} Gev), the range is even more staggering. Dirac pointed out this problem of large numbers a long time ago; he attempted to solve it within the context of his cosmological theories, with a time-varying gravitational constant. Such theories, however, appear not to be favoured by current experiment.

Around 1981, came the realization that at least one aspect of this problem — the radiative stability of the mass ratios — could be resolved using supersymmetric ideas. With this input, suppose one was not too ambitious and did not ask for a Theory of Everything in one go; suppose one were to go back to 1974 and 1975 and try to invent a humble, realistic supersymmetric model of the electroweak or the electronuclear theories only. One would choose to eschew gravity and with it the spin 3/2 superpartner of the graviton. Such a model theory would contain photons and photinos; Ws and Zs, and their superpartners the winos and zinos; it would contain higgs and higginos. It would also contain quarks and quarkinos, and leptons

and leptinos (quarkinos and leptinos would be spinless; photinos, winos, zinos and higginos would carry spin ½). How near to a successful model could one come in this restricted programme?

To be physically realistic, the most crucial further input needed would be a mechanism for supersymmetry breaking. This would generate a mass-difference between the known particles and their (hitherto undiscovered) superpartners, and would explain our inability in not having detected these superpartners so far. A desirable supersymmetry-breaking mechanism should be spontaneous, if only to conform to the pattern of symmetry breakings already familiar in particle physics.

But such a spontaneous supersymmetry breaking (even if it could be simply accomplished) would pose its own problems. It would lead to the existence of at least one massless spin ½ particle — the goldstino. Could this massless fermion be one of the known neutrinos? Alas, the predicted low-energy behaviour of the goldstino turned out to be quite different from that of neutrinos. The goldstino must be suppressed.

Now the only known mechanism to suppress the appearance of the goldstino is to re-introduce the spin 3/2 gravitino, which would absorb the goldstino (super-Higgs effect) and itself become massive. We are inevitably being led back to supergravity, but this time with supersymmetry-breaking as an important new ingredient. This is not a unification of gravity with other forces (electroweak or electronuclear). But it is a triumph nevertheless, in showing that gravity may already be influencing low-energy particle physics.

Models incorporating these ideas were constructed during 1982. The exciting thing is that this type of supersymmetry-breaking (i.e. through the introduction of $N = 1$ supergravity) can also induce a breaking of the electroweak gauge symmetry. The masses of the Ws and the Zs are then a consequence of supergravity and super-Higgs effect! The spin 3/2 superpartner of the graviton is expected to weigh around 300 Gev. The winos and zinos may be lighter than their supersymmetric partners, the recently discovered Ws and Zs. If experiments at the CERN p–$\bar{p}$ collider show that the Ws and the Zs do indeed decay into winos and zinos, the age of supersymmetry will have come. Further evidence for this may be provided by proton-decay experiments, currently in progress. In the supersymmetric models the favourite decay is likely to be the proton decaying into a muon plus a kaon rather than the non-supersymmetric decay into a positron plus pion (searched for hitherto and apparently not found). The future CERN Large Electron–Positron accelerator (now under construction for Z^0 physics) may, in fact, justify itself much more as a supersymmetry accelerator. My own hunch is that this is what will happen.

But even if $N = 1$ broken supergravity theory, in interaction with $N = 1$ supermatter, is indicated by low energy (a few hundred Gev) experiments, this would still not be a Theory of Everything. We would need to bring back the ideas of extended supergravity and in particular of the $N = 8$ extended theory to achieve this. What then of progress in this area?

Here also there have been developments of great potential significance. Extended supergravities are flourishing with the discovery (in 1979) that the maximal $N = 8$ supergravity theory in four space–time dimensions can be recovered from the simple $N = 1$ supergravity in a space–time of eleven dimensions. This is a super-extension of an original idea of Kaluza and Klein, formulated some 60 years ago, in which the following miraculous happening was noted: an Einsteinian gravitational Lagrangian in *five* dimensions is equivalent to the normal Einsteinian Lagrangian in four dimensions plus Maxwell's electromagnetism, when the fifth dimension is "compactified" to a vanishing size. The new version of this — the super-miracle — occurs in eleven dimensions, where the simple ($N = 1$) supergravity gives rise exactly to the $N = 8$ extended supergravity theory in four dimensions, when the extra seven dimensions are suitably shrunken.

But what if the compactification size is finite, albeit smaller than the Planck size (i.e. $< 10^{-33}$ cm)? What effect is the topology of the seven-dimensional "internal" manifold likely to have on the resulting physics? Do such compactifications yield new (broken) supertheories, more in accord with experiment than the original massless $N = 8$ was? This is the search that is currently on in the context of a fundamental Theory of Everything. Some day, it is hoped, this method of attack will embrace and meet the phenomenological approach mentioned earlier.

And what of Wess and Bagger's new book? It is a beautiful exposition of the original ideas of Wess and Zumino in formulating $N = 1$ supersymmetry and supergravity theories, couched in the language of superfields introduced by Strathdee and the reviewer. The use of differential forms in super-space leads to a succinct exposition of ideas of super-torsion and super-curvature. Not discussed, however, are extended supersymmetries, or extended supergravities. There is also no comparative discussion of other approaches to $N = 1$ supergravity (except that of Wess and Zumino); and, of course, there are no phenomenological applications. But within its compass, the book is masterly. Coming as it does from one of the originators of the subject, all serious students of particle physics would do well to acquire a copy. □

Abdus Salam is Director of the International Centre for Theoretical Physics, Trieste, and a Professor in the Department of Physics at Imperial College, University of London.

Working plants for the ecologist

Peter D. Moore

Physiological Plant Ecology II, III and IV. (Vols 12B, C and D of *Encyclopedia of Plant Physiology*, New Series.)
Edited by O.L. Lange *et al.*
Springer-Verlag: 1983. Vol. 12B, pp.747, DM 288, $111.82; Vol. 12C pp.799, DM 298, $115.70; Vol. 12D pp.644, DM 290, $112.60.

THE publication of Vol. 12D in the new *Encyclopedia of Plant Physiology* marks the completion of the section dealing with the essentially ecological aspects of plant physiology. Since the appearance of Vol. 12A, the first in the set (for review see *Nature* **301**, 181; 1983) which dealt largely with the physical environment, there have been two intervening volumes published.

Volume 12B covers two main topics, water relations and carbon assimilation. In fact, most of the photosynthetic material is actually dealt with in 12A and what remains here is closely related to water balance problems, for example the article on the ecological significance of C_4 metabolism by C.B. Osmond *et al.* Here the competitive advantages are considered mainly in connection with drought, though some mention is made of aquatic plants. On the water relations side, subdivision of the subject consists of the various stages of water passage through the plant and also the water balance of individual cells. Of general interest to ecologists are chapters on drought tolerance, flood tolerance, frost-drought and seed germination.

Volume 12C deals mainly with inorganic aspects of plant nutrition and the ecological orientation of material is here very apparent. Limestone and *p*H effects have their own chapter (H. Kinzel), as do metals (H.W. Woolhouse). Microbial studies also receive some attention, with chapters on halotolerance, lichen symbioses, nitrogen fixation and mycorrhiza. That macabre source of interest the carnivorous plant is also given its own chapter (U. Lüttge) and a timely plea is made for a quantitative, systems study of these well-worked but little understood plants.

Interactions between plants and other organisms forms a second theme in this volume, including such topics as parasitism, the ecology of viruses, pollination (a chapter well supplied with chemical information difficult to find in standard texts) and herbivory, where the phenomenon of compensatory growth is given welcome prominence. It is left to E.I. Newman to cover interactions between plants in just one chapter and he tackles this wide subject by concentrating on mechanisms of interference.

The final volume, 12D, is perhaps the

most remarkable in that it extends the coverage of the series far beyond the scope of earlier encyclopaedias of plant physiology. Its subject is the plant component of ecosystems. Beginning with chapters on nutrient cycling in terrestrial, freshwater and marine ecosystems, it focuses on productivity within the major biomes. The aquatic nutrient cycling chapters concentrate upon specific elements, such as phosphorus and sulphur, and the marine account ties in well with the plant-interactions theme of the previous volume by analysing the influence of nutrient levels on phytoplankton diversity.

The productivity chapters feature the technical problems associated with estimates of production and also provide some detailed accounts of the growth models used to describe production processes on a biome scale. The volume closes with chapters reviewing pollution and eutrophication problems, including topical items such as increasing atmospheric CO_2 levels.

Looking back through the physiological plant ecology section of the *Encyclopedia*, one must be impressed by the amount of information which has been pressed into four volumes. The provision of a subject index at the end of the quartet will enable ecologists of all shades to gain rapid access to a wealth of up-to-date, well-reviewed material with extensive bibliographies. Subject overlap and repetition does exist, but is not excessive; omissions are few, though some topics, such as plant competition, deserved more space. Although somewhat bulky, these four books will render redundant about two shelves of older ecology texts, so it is well worth making room for them. □

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences, King's College, University of London.

Fisher from inside

Cedric A. B. Smith

Natural Selection, Heredity and Eugenics.
Edited and introduced by J.H. Bennett.
Oxford University Press: 1983. Pp.306. £17.50, $47.50.

IN a letter to Hooke, Newton wrote "If I have seen a little further it is by standing on the shoulders of Giants". But the pace of scientific advance has accelerated so that, as Gerald Holton is said to have remarked, "We are now uniquely privileged to sit side by side with the giants on whose shoulders we stand".

As a student, I knew a little about mathematical genetics, and was vaguely aware that the major pioneering work had been done by R.A. Fisher, J.B.S. Haldane and Lancelot Hogben. (I was only later to hear of Sewall Wright.) I had a rosy vision of Fisher, Haldane and Hogben frequently discussing, with great excitement, their latest discoveries and plans for the future.

In fact, after the Second World War, fate decreed that I should become a mathematical geneticist, and get to know Fisher, Haldane and Hogben personally (and even, on occasion, sit side by side with them). What immediately became clear was that relations between them were alas far from ideally cordial and that each in his own way could sometimes be inexplicably prickly, hindering both close intellectual contact and scientific advance.

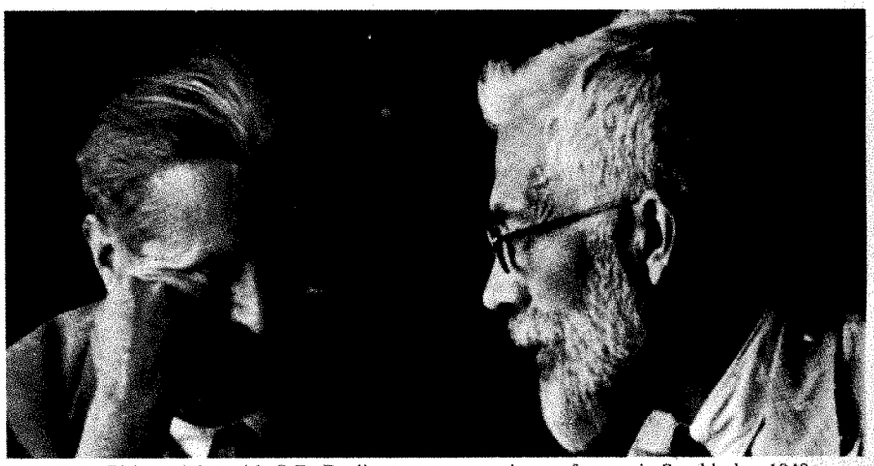
The publication of this book, which mainly consists of correspondence to and from Fisher, does much to throw light on this sensitivity, on the relationship between the three men and especially on the character of Fisher himself. With his closest friends, Fisher was always helpful and generous — perhaps his touchiness with others was a barrier put up in self-defence, or a reaction to the severe disappointment when many of his outstanding papers produced when he was young had been turned down by major scientific journals. In his letters we see the real Fisher, almost always gentle, polite, friendly and idealistic, and most willing to listen to argument. Rather than the almost impenetrable and tortuous prose of much of Fisher's scientific writings we have instead a limpid expression of his thoughts.

Two other points emerge from the correspondence printed here. The idea of evolution through selection acting on rare mutation, which from around 1935 seemed obvious common sense, and the consequent mathematical theory, had achieved this position of seeming to be the most logical and proper approach to evolution only quite recently by reason of the work of Fisher, Haldane and Hogben. And the unnecessarily strained relations between the three protagonists were largely of recent origin — a few years earlier friendship and helpfulness had prevailed.

There are occasions when a new branch of knowledge seems to be born suddenly, rather than by a process of gradual accretion. Fisher's 1918 paper "On the Correlation between Relatives on the Supposition of Mendelian Inheritance" must have been one, and the appearance in 1921 of his book *Statistical Methods for Research Workers* another. The paper on correlations between relatives covered the topic so thoroughly that not a great deal has been added to the conclusions in the 64 years which have followed. It appears that we owe the appearance of this paper to the constant encouragement of Major Leonard Darwin (Charles Darwin's son) who corresponded regularly and frequently with Fisher for 27 years. In their letters important insights emerged, some of which did not find their way into Fisher's published papers. For example, William Hamilton has attributed the inspiration of his theory of the evolution of altruism to remarks by Haldane. But in a letter to Darwin on 27 June 1929, Fisher shows that he had understood the essential point already, namely that an organism's willingness to sacrifice itself may nevertheless improve the chances of survival of the genes it carries because they will also be found in close relatives.

Just over a hundred pages of the book are occupied by the Darwin–Fisher correspondence, and approximately another hundred are taken up by extracts from letters on selection and evolution, including letters to (and sometimes from), W.C. Bond on blood groups, L.C. Dunn on dominance, E.B. Ford on selection in butterflies, J.B.S. Haldane on miscellaneous topics, J.S. Huxley on Family Allowances and S. Wright on topics in the mathematics of selection. The book also contains some other papers by Fisher which are unpublished or not readily accessible. In all, it illuminates in an interesting and informative way the development of the theory of natural selection during the years 1910–1960, both as regards Fisher's own personal contributions and his reactions to those of other pioneers. □

Cedric A. B. Smith is at the Galton Laboratory, University College London.



R.A. Fisher (right) with C.D. Darlington at a genetics conference in Stockholm, 1948.

End of blue-green algae

Sergey V. Shestakov

The Biology of Cyanobacteria.
Edited by N.G. Carr and B.A. Whitton.
Blackwell Scientific/University of California Press: 1982.
Pp.688. £42, $75.

IT IS now unarguable that phototrophic organisms, which have for over a century been called blue-green algae, belong to the prokaryotes and are thus the property of microbiologists rather than botanists. The best proof of this is the appearance of an authoritative book with the word "cyanobacteria" in its title. The term has thus been fully legalized. In the foreword, written shortly before his death, Roger Stanier succinctly defined them as: "Microorganisms that harbour within a typically prokaryotic cell, a photosynthetic apparatus closely similar in structure and function to that located within chloroplasts of phototrophic eukaryotes".

The book is a successor to *The Biology of Blue-Green Algae,* published by Blackwell Scientific and University of California Press in 1973, and clearly reflects the progress in cyanobacteriology made in the intervening decade. The contributors are all leading experts and between them provide comprehensive reviews of the morphology, physiology, biochemistry and ecology of the cyanobacteria, and (in the final chapter) of palaeomicrobiological aspects of their origin and evolution.

The role of cyanobacteria in freshwater and marine ecosystems is described in great detail, and the peculiarities of structure and function of membranes and cell wall, heterocysts and akinetes, phycobilisomes and gas vesicles are discussed in a new light. A lot of attention is also paid to photosynthesis, carbon, nitrogen and phosphate metabolism, and to motility and taxis. Particularly stimulating are the chapters on genetics and molecular evolution, in which the genome of cyanobacteria and their phylogenetic position within the prokaryotes and in relation to eukaryotes are for the first time exhaustively analysed. The genetic relatedness of cyanobacteria and plastids of eukaryotic photosynthesizers is well argued.

The book is not without its disappointments, however. Cyanoviruses, symbiotic systems and soil ecology of cyanobacteria are not included, while the omission of certain contemporary issues in applied biology — the use of cyanobacteria in agriculture and biotechnology, for example — is especially regrettable. (Cyanobacteria are promising subjects for the genetic engineer, and may in time provide the clues by which we unravel the intricacies of such fundamental processes as nitrogen fixation, membrane biogenesis and cell differentiation.) The editors have in general done an excellent job; but some of the authors, although giving an impressive show of their knowledge, have sadly failed to give pointers to the immediate research problems to be tackled in their specific areas.

But the plus points easily outweigh the minus. The book is extensively illustrated and thoroughly referenced, even including very recent data, which will make it a good source for advanced students and new research workers. And since cyanobacteria are now attracting an increasing number of scientists from a variety of backgrounds, this highly sophisticated book will certainly be appreciated not only by microbiologists, but also by biochemists, cytologists, ecologists, molecular biologists and other specialists. □

Sergey V. Shestakov is Head of and a Professor in the Department of Genetics at Moscow State University.

In colour

Colour Vision: Physiology and Psychophysics, a book stemming from the conference on the subject held in Cambridge, UK, in 1982, has recently been published by Academic Press. Editors are J.D. Mollon and L.T. Sharpe; price is £28, $49. Issues raised at the conference were discussed in a News and Views article in *Nature* **300**, 320; 1982.

Life dictionary

A new edition of E.A. Martin's *Dictionary of Life Sciences,* which first appeared in 1976, has been published by Macmillan Reference Books, London. According to the editor, revision of the *Dictionary* has taken account of advances in genetics, molecular biology, microbiology and immunology, while coverage of the more traditional aspects of biology has been reduced slightly. Price is £22.50.

Algebraic astronomy

P.K. Seidelmann

Vectorial Astrometry.
By C.A. Murray.
Adam Hilger, Bristol/Heyden, Philadelphia: 1983. Pp.353. £25, $49.

THE lack of a comprehensive yet readable text on spherical, or practical, astronomy is generally recognized. Instead, a number of good, specific-purpose texts*, and several, much older textbooks† which are still valuable reference works when wisely used, have had to suffice.

While astrophysics has changed drastically in the last century, astrometry has changed by degree. Thus, an astrophysics textbook from the last century would be wrong, but a spherical astronomy volume from the nineteenth century would only omit recent developments and lack the accuracy of current standards. This evolutionary progress in astrometry may explain the previous absence of a textbook based on vector and matrix algebra.

C.A. Murray's *Vectorial Astrometry* is not the much-needed comprehensive, readable text. Rather it attempts to apply vector and matrix algebra systematically to spherical astronomy. At the same time the International Astronomical Union reference system, time scales and constants, introduced at the beginning of 1984, are explained and used throughout the volume.

The personality and interests of Andrew Murray are accurately reflected by the strengths of the book. Photographic astrometry, relativistic astrometry, the theory of refraction, precession and nutation are well presented. For many subjects the latest developments are included and the vectorial and matrix equations are derived.

The volume would be significantly improved by good diagrams, however. The present two-dimensional, captionless line drawings add little to the clarity of the treatment. On subjects that are not associated with previous interests of the author, there is little in the way of descriptive explanation; the purpose appears to be the derivation of the necessary equations rather than communication of their reason and purpose. Several chapters contain individual sections without any apparent connection between the subjects. Also, I suggest reading the appendices first, so the notation and basis of some equations are understood.

While many new textbooks provide the cookbook formulae and methods for performing specific computations, this volume emphasizes the theory and derivation of equations and avoids the practical applications. Thus, under the title of astrometry through the atmosphere, the theory of refraction is discussed in great detail, but there is no mention of practical methods of calculating refraction.

For those who believe that astrometry has never entered the twentieth century, this is the book to change their minds. If you wish to apply vector or matrix algebra to spherical astronomy, if you need the relativistic formulation of astrometric calculations, and if you are interested in the latest developments and most accurate methods of astrometry, you will find this book to be interesting and useful. For all astrometrists, and for astronomers who must make accurate spherical astronomical computations, it will be an important reference volume. □

P.K. Seidelmann is at the US Naval Observatory, Washington DC.

*e.g. I.I. Mueller's *Spherical and Practical Astronomy as Applied to Geodesy* (Frederick Ungar, 1969); E.W. Woolard and G.M. Clemence's *Spherical Astronomy* (Academic, 1966); L.G. Taff's *Computational Spherical Astronomy* (Wiley, 1981); P. Duffett-Smith's *Practical Astronomy with your Calculator* (Cambridge University Press, 1979).

†e.g. S. Newcomb's *A Compendium of Spherical Astronomy* (Macmillan, 1906/Dover, 1960); W. Chauvenet's *A Manual of Spherical and Practical Astronomy* (Lippincott, 1863/Dover, 1960); W.M. Smart's *Textbook on Spherical Astronomy* (Cambridge University Press, 1931).

Biological Sciences

ADIYODI, K.G. and R.G. (eds). Reproductive Biology of Invertebrates, Vol.1: Oogenesis, Oviposition, and Oosorption. Pp.770. ISBN 0-471-10128-1. (Wiley: 1983.) £48.50, $95.

ALCAMO, I.E. Fundamentals of Microbiology. Pp.834. ISBN 0-201-10068-1. (Addison-Wesley: 1983.) £23.95.

ANGELI, A., BRADLOW, H.L. and DOGLIOTTI, L. (eds). Endocrinology of Cystic Breast Disease. Pp.343. ISBN 0-89004-862-2. (Raven: 1983.) $59.52.

BARKER, J.S.F. and STARMER, W.T. (eds). Ecological Genetics and Evolution: The Cactus–Yeast–*Drosophila* Model System. Pp.362. ISBN 0-12-078820-9. (Academic: 1982.) $38.

BARR, C.A. Jewels of the Plants: Wild Flowers of the Great Plains Grasslands and Hills. Pp.236. ISBN 0-8166-1127-0. (University of Minnesota Press: 1983.) $19.95.

BECK, B.B. and WEMMER, C. (eds). The Biology and Management of An Extinct Species: Pere David's Deer. Pp.193. ISBN 0-8155-0938-3. (Noyes: 1983.) $26.

BERNÁT, I. Iron Metabolism. Pp.415. ISBN 0-306-30829-0. (Plenum: 1983.) $39.50.

BOYLE, R.H. and BOYLE, R.A. Acid Rain. Pp.146. Hbk ISBN 0-8052-3854-9; pbk ISBN 0-8052-0746-5. (Nick Lyons: 1983.) Hbk $14.95; pbk $8.95.

BRANDHAM, P.E. and BENNETT, M.D. (eds). Kew Chromosome Conference II. Proceedings of the 2nd Conference held September 1982, England. Pp.382. ISBN 0-04-575022-X. (George Allen & Unwin: 1983.) £20.

BRAY, C.M. Nitrogen Metabolism in Plants. Pp.214. Pbk ISBN 0-582-44640-6. (Longman: 1983.) £7.95.

BREGMAN, A.A. Laboratory Investigations in Cell Biology. Pp.253. ISBN 0-471-86241-X. (Wiley: 1983.) £12.30, $18.35.

BROWN, V.K. Grasshoppers. Naturalists' Handbooks 2. Pp.64. Hbk ISBN 0-521-23903-6; pbk ISBN 0-521-28299-3. (Cambridge University Press: 1983.) Hbk £8; pbk £2.95.

CALABRESE, E.J. Principles of Animal Extrapolation. Pp.603. ISBN 0-471-08762-9. (Wiley: 1983.) £57.75, $86.50.

CAPPUCCINO, J.G. and SHERMAN, N. Microbiology: A Laboratory Manual. Pp.466. Pbk ISBN 0-201-11160-8. (Addison-Wesley: 1983.) £12.70.

DALEY, M. and WILSON, M. Sex, Evolution, and Behaviour, 2nd Edn. Pp.402. Pbk ISBN 0-87150-767-6. (Willard Grant Press: 1983.) Np.

DANIELLI, J.F. (ed.). Aspects of Cell Regulation. International Review of Cytology Supplement 15. Pp.283. ISBN 0-12-364376-1. (Academic: 1983.) $37.50.

DAVIDOFF, R.A. (ed.). Handbook of the Spinal Cord, Vol.1: Pharmacology. Pp.560. ISBN 0-8247-1708-2. (Dekker: 1983.) SwFr. 185.

DAVIS, B.N.K. Insects on Nettles. Naturalists' Handbooks 1. Pp.64. Hbk ISBN 0-521-23904-4; pbk ISBN 0-521-28300-0. (Cambridge University Press: 1983.) Hbk £8; pbk £2.95.

DELMONT, J. (ed.). Milk Intolerances and Rejection. Pp.172. ISBN 3-8055-3546-5. (Karger: 1983.) SwFr. 140, DM 168, $84.

DOWBEN, R.M. and SHAY, J.W. (eds). Cell and Muscle Motility, Vol.4. Pp.333. ISBN 0-306-41227-6. (Plenum: 1983.) $39.50.

EAZIN, S. and TULLY, J.G. (eds). Methods in Mycoplasmology, Vol.1: Mycoplasma Characterization. Pp.504. ISBN 0-12-583801-8. (Academic: 1983.) $57.

ECKERT, R. and RANDALL, D. Animal Physiology: Mechanisms and Adaptations, 2nd Edn. Pp.830. Hbk ISBN 0-7167-1423-X; pbk ISBN 0-7167-1422-1. (W.H. Freeman: 1983.) Hbk £29.95; pbk £17.50.

ELIOT, J.N. and KAWAZOÉ, A. Blue Butterflies of the Lycaenopsis Group. Pp.309. ISBN 0-565-00860-9. (British Museum (Natural History): 1983.) £28.

ELSON, E., FRAZIER, W. and GLASER, L. (eds). Cell Membranes: Methods and Reviews, Vol.1. Pp.197. ISBN 0-306-41298-5. (Plenum: 1983.) $29.50.

ELWOOD, R.W. (ed.). Parental Behaviour of Rodents. Pp.296. ISBN 0-471-10252-0. (Wiley: 1983.) £26, $48.50.

ENGLAND, M.A. A Colour Atlas of Life Before Birth: Normal Fetal Development. Pp.216. ISBN 0-7234-0775-4. (Wolfe Medical Publications: 1983.) £25.

EWERT, J.-P., CAPRANICA, R.R. and INGLE, D.J. (eds). Advances in Vertebrate Neuroethology. NATO Advanced Science Institutes Series, Vol.56. Pp.1238. ISBN 0-306-41197-0. (Plenum: 1983.) $150.

FISHER, M.M. and ROY, C.C. (eds). Pediatric Liver Disease. Hepatology, Vol.5. Pp.296. ISBN 0-306-41164-4. (Plenum: 1983.) $42.50.

FRAZER, D. Reptiles and Amphibians in Britain. Pp.256. ISBN 0-00-219706-5. (Collins: 1983.) £11.

GALLIN, J.I. and FAUCI, A.S. (eds). Lymphoid Cells. Advances in Host Defence Mechanisms, Vol.2. Pp.383. ISBN 0-89004-729-4. (Raven: 1983.) $66.96.

GERSHENSON, S.M. Principles of Modern Genetics, 2nd Edn. Pp.557. No ISBN. (Naukova Dumka, Kiev: 1983.) Np.

GRAY, C.H. and JAMES, V.H.T. (eds). Hormones in Blood, 3rd Edn, Vols 4 and 5. Vol.4 pp.510; Vol.5 pp.316. Vol.4 ISBN 0-12-296204-4; Vol.5 ISBN 0-12-296205-2. (Academic: 1983.) Vol.4 £45, $72; Vol.5 £30.80, $50.

GREENWALD, G.S. and TERRANOVA, P.F. (eds). Factors Regulating Ovarian Function. Pp.507. ISBN 0-89004-945-9. (Raven: 1983.) $55.80.

HALL, J.L. and MOORE, A.L. (eds). Isolation of Membranes and Organelles from Plant Cells. Pp.328. ISBN 0-12-318820-2. (Academic: 1983.) £27, $45.

HELÈNE, C. (ed.). Structure, Dynamics, Interactions and Evolution of Biological Macromolecules. Proceedings of a Colloquium held July 1982, France, to Celebrate the 80th Birthday of Professor Charles Sadron. Pp.415. ISBN 90-277-1531-9. (Reidel: 1983.) Np.

HERBERMAN, R.B. (ed.). NK Cells and Other Natural Effector Cells. Pp.1566. ISBN 0-12-341360-5. (Academic: 1982.) $74.50.

HILL, D.S., HORE, P.M. and THORNTON, I.W.B. Insects of Hong Kong. Pp.503. Pbk ISBN 962-209-008-7. (Hong Kong University Press: 1982.) $126.50 (Hong Kong).

HIRS, C.H.W. and TIMASHEFF, S.N. (eds). Enzyme Structure Part 1. Methods in Enzymology, Vol.91. Pp.693. ISBN 0-12-181991-4. (Academic: 1983.) $45.60.

HOAR, W.S. General and Comparative Physiology, 3rd Edn. Pp.851. ISBN 0-13-349308-3. (Prentice-Hall: 1983.) £27.85.

INMAN, F.P. and KINDT, T.J. (eds). Contemporary Topics in Molecular Immunology, Vol.9. Pp.245. ISBN 0-306-41304-3. (Plenum: 1983.) Np.

KHAN, A. and HILL, N.O. (eds). Human Lymphokines: The Biological Immune Response Modifiers. Pp.733. ISBN 0-12-406080-3. (Academic: 1982.) $54.

KILIMANN, S.V. -A.A., CRONKITE, E.P. and MULLER-BERAT, C.N. (eds). Haemopoietic Stem Cells: Characterization, Proliferation, Regulation. Proceedings of the Alfred Benzon Symposium held June 1982, Copenhagen. Pp.427. ISBN 87-16-09396-8. (Munksgaard: 1983.) D.Kr.325.

KIMBALL, J.W. Biology, 5th Edn. Pp.974. Pbk ISBN 0-201-11688-X. (Addison-Wesley: 1983.) £14.95.

KROMBEIN, K.V. Biosystematic Studies of Ceylonese Wasps, XI: A Monograph of the Amiseginae and Loboscelidiinae (Hymenoptera: Chrysididae). Smithsonian Contribution to Zoology No.375. Pp.79. No ISBN. (Smithsonian Institution Press: 1983.) Np.

KUNIN, A.S. and SIMMONS, D.J. (eds). Skeletal Research: An Experimental Approach, Vol.2. ISBN 0-12-429002-7. (Academic: 1983.) $37.

LAJTHA, A. (ed.). Handbook of Neurochemistry, 2nd Edn., Vol.3: Metabolism in the Nervous System. Pp.698. ISBN 0-306-41153-9. (Plenum: 1983.) $89.50.

LAJTHA, A. (ed.). Handbook of Neurochemistry, 2nd Edn., Vol.4: Enzymes in the Nervous System. Pp.549. ISBN 0-306-41210-1. (Plenum: 1983.) $72.50.

LANGE, O.L. *et al.* (eds). Physiological Plant Ecology III: Responses to the Chemical and Biological Environment. Encyclopedia of Plant Physiology, New Series Vol.12, Part C. Pp.799. ISBN 3-540-10907-2/0-387-10907-2. (Springer-Verlag: 1983.) DM 298, $128.50.

LARGE, P.J. Methylotrophy and Methanogenesis. Aspects of Microbiology, Vol.8. Pp.86. Pbk ISBN 0-442-30528-1. (Van Nostrand Reinhold: 1983.) £4.75.

LEIGH, E.G. Jr., RAND, A.S. and WINDSOR, D.M. (eds). The Ecology of a Tropical Forest: Seasonal Rhythms and Long-Term Changes. Pp.468. Pbk ISBN 0-19-858603-5. (Oxford University Press: 1983.) £19.

LENHOFF, H.M. (ed.). Hydra: Research Methods. Pp.463. ISBN 0-306-41086-9. (Plenum: 1983.) $59.50.

MAYES, A. (ed.). Memory in Animals and Humans: Some Comparisons and their Theoretical Implications. Pp.338. ISBN 0-442-30524-9. (Van Nostrand Reinhold: 1983.) £23.50.

MESSMER, K. and HAMMERSEN, F. (eds). Structure and Function of Endothelial Cells. Proceedings of the 1st Bodensee Symposium held October 1982, Bodensee. Mikrozirkulation in Forschung und Klinik. Progress in Applied Microcirculation, Vol.1. Pp.138. Pbk ISBN 3-8055-3635-6. (Karger: 1983.) SwFr.46, DM 55, $27.75.

MIKAMI, S-I., HOMMA, K. and WADA, M. (eds). Avian Endocrinology: Environmental and Ecological Perspectives. Pp.334. ISBN 4-7622-6344-3/3-540-11871-3/0-387-11871-3. (Japan Scientific Societies Press/Springer-Verlag: 1983.) DM 118, $48.80.

MILAZZO, G. (ed.). Topics in Bioelectrochemistry and Bioenergetics, Vol.5. Pp.305. ISBN 0-471-10531-7. (Wiley: 1983.) £48.

MILAZZO, G. and BLANK, M. (eds). Bioelectrochemistry I: Biological Redox Reactions. Ettore Majorana International Science Series, Vol.11. Pp.348. ISBN 0-306-41340-X. (Plenum: 1983.) $52.50.

MULLER-SCHWARZE, D. and SILVERSTEIN, R.M. (eds). Chemical Signals in Vertebrates, Vol.3. Pp.368. ISBN 0-306-41254-3. (Plenum: 1983.) $52.50.

Environmental Sciences

HESKETH, H.E. and CROSS, F.L. Jr. Fugitive Emissions and Controls. The Environment and Energy Handbook Series. Pp.145. ISBN 0-250-40448-6. (Ann Arbor: 1983.) £21.

MACKAY, D. *et al.* (eds). Physical Behaviour of PCBs in The Great Lakes. Pp.442. ISBN 0-250-40584-9. (Ann Arbor: 1983.) £37.

MIYAMOTO, J. and KEARNEY, P.C. (eds). Pesticide Chemistry: Human Welfare and the Environment. Proceedings of the 5th International Congress held August–September 1982, Japan. 4 Vols Set ISBN 0-08-029219-4. (Pergamon: 1983.) £195, $390.

ULRICH, B. and PANKRATH, J. (eds). Effects of Accumulation of Air Pollutants in Forest Ecosystems. Proceedings of a Workshop held May 1982, West Germany. Pp.389. ISBN 90-277-1476-2. (Reidel: 1983.) Dfl. 135, $58.50.

Applied Biological Sciences

BJERRUM, O.J. (ed.). Electroimmunochemical Analysis of Membrane Proteins. Pp.476. ISBN 0-444-80461-7. (Elsevier Biomedical/North Holland: 1983.) $106.50, Dfl.250.

BOYNTON, A.L., McKEEHAN, W.L. and WHITFIELD, J.F. (eds). Ions, Cell Proliferation, and Cancer. Pp.551. ISBN 0-12-123050-3. (Academic: 1982.) $39.50.

BRIDGES, J.W. and CHASSEAUD, L.F. (eds). Progress in Drug Metabolism, Vol.7. Pp.446. ISBN 0-471-10487-6. (Wiley: 1983.) £45, $87.50.

CASTEGNARO, M. *et al.* (eds). Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Polycyclic Aromatic Hydrocarbons. IARC Scientific Publications No.49. Pp.81. Pbk ISBN 92-8-321149-9. (WHO: 1983.) SwFr.20, $10.

CHANDRA, P. (ed.). Biochemical and Biological Markers of Neoplastic Transformation. NATO Advanced Science Institute Series, Vol.57. Pp.639. ISBN 0-306-41240-3. (Plenum: 1983.) $89.50.

CHARLES-EDWARDS, D.A. Physiological Determinants of Crop Growth. Pp.161. ISBN 0-12-169360-0. (Academic: 1983.) $26.

COYLE, J.T. and ENNA, S.J. (eds). Neuroleptics: Neurochemical, Behavioral, and Clinical Perspectives. Central Nervous System Pharmacology. Pp.341. ISBN 0-89004-735-9. (Raven: 1983.) $68.20.

CURRY, S.H. and WHELPTON, R. Manual of Laboratory Pharmacokinetics. Pp.189. ISBN 0-471-10247-4. (Wiley: 1983.) £12.50, $25.

DAS, K.G. (ed.). Controlled-Release Technology: Bioengineering Aspects. Pp.225. ISBN 0-471-08680-0. (Wiley: 1983.) £44.50, $66.50.

DOBBING, J. (ed.). Prevention of Spina Bifida and Other Neural Tube Defects. Pp.272. ISBN 0-12-218860-8. (Academic: 1983.) £17.60, $29.

EDWARDS, D.I. and HISCOCK, D.R. (eds). Chemotherapeutic Strategy. Proceedings of the Symposium held June 1982, London. Pp.232. ISBN 0-333-34318-2. (Macmillan Press, London: 1983.) £25.

EGAN, H. (ed.). Environmental Carcinogens Selected Methods of Analysis, Vol.5: Some Mycotoxins. IARC Publications No.44. Pp.455. ISBN 92-8-321144-8. (WHO: 1982.) SwFr.60, $30.

FANBURG, B.L. (ed.). Sarcoidosis and Other Granulomatous Diseases of The Lung. Lung Biology in Health and Disease Series, Vol.20. Pp.544. ISBN 0-8247-1866-6. (Dekker: 1983.) SwFr.159.

GOTTSCHALK, W. and MÜLLER, H.P. (eds). Seed Proteins: Biochemistry, Genetics, Nutritive Value. Advances in Agricultural Biotechnology. Pp.542. ISBN 90-247-2789-8. (Martinus Nijhoff: 1983.) Dfl.250, $109.

HINDLEY, J. DNA Sequencing. Laboratory Techniques in Biochemistry and Molecular Biology, Vol.10. Pp.384. Hbk ISBN 0-444-80497-8; pbk ISBN 0-444-80385-8. (Elsevier Biomedical/North Holland: 1983.) Hbk np; pbk $27.75, Dfl.65.

HOLZL, R. and WHITEHEAD, W.E. (eds). Psychophysiology of the Gastrointestinal Tract: Experimental and Clinical Applications. Pp.363. ISBN 0-306-41089-3. (Plenum: 1983.) $45.

KOSUGE, T., MEREDITH, C.P. and HOLLAENDER, A. (eds). Genetic Engineering of Plants: An Agricultural Perspective. Basic Life Sciences, Vol.26. Pp.499. ISBN 0-306-41353-1. (Plenum: 1983.) $69.50.

KOZLOWSKI, T.T. (ed.). Water Deficits and Plant Growth, Vol.7: Additional Woody Crop Plants. Pp.251. ISBN 0-12-424157-3. (Academic: 1983.) $37.

LAMBLE, J.W. (ed.). Drug Metabolism and Distribution: Current Reviews in Biomedicine 3. Pp.185. Pbk ISBN 0-444-80510-9. (Elsevier Biomedical Press: 1983.) $17.50, £8.50.

LANGONE, J.J. and VAN VUNAKIS, H. (eds). Immunochemical Techniques, Part E: Monoclonal Antibodies and General Immunoassay Methods. Methods in Enzymology, Vol.92. Pp.647. ISBN 0-12-281992-2. (Academic: 1983.) $65.

LEFER, A.M. and SCHUMER, W. (eds). Molecular and Cellular Aspects of Shock and Trauma. Proceedings of the U.S.A.–Japan Binational Conference held June 1982, Hawaii. Progress in Clinical and Biological Research, Vol.3. Pp.346. ISBN 0-8451-0111-0. (Alan R. Liss: 1983.) £46.

LEVIN, M.A. *et al.* Applied Genetic Engineering: Future Trends and Problems. Pp.191. ISBN 0-8155-0925-1. (Noyes: 1983.) Np.

LI, P.H. and SAKAI, A. (eds). Plant Cold Hardiness and Freezing Stress: Mechanisms and Crop Implications, Vol.2. Pp.694. ISBN 0-12-447602-3. (Academic: 1982.) $39.50.

LURQUIN, P.F. and KLEINHOFS, A. (eds). Genetic Engineering in Eukaryotes, Vol.61. Pp.282. ISBN 0-306-41275-6. (Plenum: 1983.) $45.

McCRACKEN, A.X. and CAWSON, R.A. Clinical and Oral Microbiology. Pp.629. ISBN 0-07-010296-1. (Hemisphere: 1983.) $32.

MEAD, R. and CURNOW, R.N. Statistical Methods in Agriculture and Experimental Biology. Pp.335. Hbk ISBN 0-412-24230-3; pbk ISBN 0-412-24240-0. (Chapman & Hall: 1983.) Hbk £25; pbk £11.95.

MEERSON, F.Z. The Failing Heart: Adaptation and Deadaptation. Pp.341. ISBN 0-89004-550-X. (Raven: 1983.) $54.56.

MOLINATTI, G.M., MARTINI, L. and JAMES, V.H.T. (eds). Androgenization in Women: Pathophysiology and Clinical Aspects. Pp.216. ISBN 0-89004-854-1. (Raven: 1983.) $52.08.

MONTAGUE, W. Diabetes and the Endocrine Pancreas: A Biochemical Approach. Pp.169. Hbk ISBN 0-85664-887-6; pbk ISBN 0-85664-888-4. (Croom Helm: 1983.) Hbk £14.95; pbk £6.95.

MUNGALL, D.R. (ed.). Applied Clinical Pharmacokinetics. Pp.458. ISBN 0-89004-727-8. (Raven: 1983.) $57.04.

NOWOTNY, A. (ed.). Beneficial Effects of Endotoxins. Pp.581. ISBN 0-306-41147-4. (Plenum: 1983.) $69.50.

REICHARD, S.M., REYNOLDS, D.G. and ADAMS, H.R. (eds). Advances in Shock Research, Vol.10. Proceedings of the 5th Annual Conference held June 1982, Vermont. Part 2. Pp.242. ISBN 0-8451-0609-0. (Alan R. Liss: 1983.) £41.

REICHARD, S.M. and WOLFE, R.R. (eds). Advances in Shock Research, Vol.9. Proceedings of the 5th Annual Conference held June 1982, Vermont. Part 1. Pp.312. ISBN 0-8451-0608-2. (Alan R. Liss: 1983.) £54.

REIVICH, M. and HURTIG, H.I. (eds). Cerebrovascular Diseases. 13th Research (Princeton) Conference. Pp.457. ISBN 0-89004-876-2. (Raven: 1983.) $90.52.

ROSEMAN, T.J. and MANSDORF, S.Z. (eds). Controlled Release Delivery Systems. Pp.424. ISBN 0-8247-1728-7. (Dekker: 1983.) SwFr.153.

ROTHSTEIN, M. (ed.). Review of Biological Research in Aging, Vol.1. Pp.424. ISBN 0-8451-2500-7. (Alan R. Liss: 1983.) £49.

SCHELL, R.F. and MUSHER, D.M. (eds). Pathogenesis and Immunology of Treponemal Infection. Immunology Series, Vol.20. Pp.424. ISBN 0-8247-1384-2. (Dekker: 1983.) SwFr.173.

SPITZER, J.J. (ed.). Myocardial Injury. Advances in Experimental Medicine and Biology, Vol.161. Pp.612. ISBN 0-306-41253-5. (Plenum: 1983.) $85.

SYMONS, L.E.A. *et al.* (eds). Biology and Control of Endoparasites. McMaster Laboratory 50th Anniversary Symposium held November 1981, Sydney. Pp.416. ISBN 0-12-681020-7. (Academic: 1982.) $58.

TOPLISS, J.G. (ed.). Quantitative Structure–Activity Relationships of Drugs. Pp.519. ISBN 0-12-695150-0. (Academic: 1983.) $69.

WESTLEY, J.W. (ed.). Polyether Antibiotics: Naturally Occurring Acid Ionophores, Vol. 2: Chemistry. Pp.432. ISBN 0-8247-1888-7. (Dekker: 1983). SwFr. 185.

WIMER, W.W., RUSSEL, J.A. and KAPLAN, H.L. Alcohols Toxicology. Pp.278. ISBN 0-8155-0948-0. (Noyes: 1983.) $36.

WYBAR, K. and TAYLOR, D. (eds). Pediatric Ophthalmology: Current Aspects. Pp.512. ISBN 0-8247-1841-0. (Dekker: 1983.) SwFr. 198.

Psychology

HAMILTON, V. The Cognitive Structures and Processes of Human Motivation and Personality. Pp.352. ISBN 0-471-10526-0. (Wiley: 1983.) £19.95, $37.50.

KAPLAN, H.S. The Evaluation of Sexual Disorders: Psychological and Medical Aspects. Pp.304. ISBN 0-87630-210-X. (Brunner/Mazel: 1983.) $32.50.

LEWIS, M. and MICHALSON, L. Children's Emotions and Moods: Developmental Theory and Measurement. Pp.472. ISBN 0-306-41209-8. (Plenum: 1983.) $39.50.

MACKINTOSH, N.J. Conditioning and Associative Learning. Oxford Psychology Series, No.3. Pp.316. ISBN 0-19-852101-4. (Oxford University Press: 1983.) £17.50.

MENDLEWICZ, J. and VAN PRAAG, H.M. (eds). Biological Rhythms and Behaviour. Advances in Biological Psychiatry, Vol. 11. Pp.150. Pbk ISBN 3-8055-3672-0. (Karger: 1983.) SwFr. 99, DM 119, $59.50.

MILLER, P.H. Theories of Developmental Psychology. Pp.436. Hbk ISBN 0-7167-1431-0; pbk ISBN 0-7167-1432-9. (W.H. Freeman: 1983.) Hbk £19.50; pbk £10.95.

PETERSON, C. and McCABE, A. Developmental Psycholinguistics: Three ways of Looking at a Child's Narrative. Pp.245. ISBN 0-306-40964-X. (Plenum: 1983.) $27.50.

SALES, B.D. (ed.). The Professional Psychologist's Handbook. Pp.779. ISBN 0-306-40934-8. (Plenum: 1983.) $60.

SPENCE, J.T. (ed.). Achievement and Achievement Motives: Psychological and Sociological Approaches. Pp.381. Hbk ISBN 0-7167-1396-9; pbk ISBN 0-7167-1397-7. (W.H. Freeman: 1983.) Hbk £22.50; pbk £12.50.

VOYAT, G. Cognitive Development Among Sioux Children. Pp.161. ISBN 0-306-41276-4. (Plenum: 1983.) $24.50.

WHITLOCK, F.A. Symptomatic Affective Disorders: A Study of Depression and Mania Associated with Physical Disease and Medication. Pp.218. ISBN 0-12-74580-X. (Academic: 1983.) $24.

Sociology

BAIN, B. (ed.). The Sociogenesis of Language and Human Conduct. Pp.581. ISBN 0-306-41041-9. (Plenum: 1983.) $50.

GERSHUNY, J.I. and MILES, I.D. The New Service Economy: The Transformation of Employment in Industrial Societies. Pp.281. ISBN 0-86187-314-9. (Frances Pinter: 1983.) £16.

GOODY, J. The Development of the Family and Marriage in Europe. Pp.308. Hbk ISBN 0-521-24739-X; pbk ISBN 0-521-28925-4. (Cambridge University Press: 1983.) Hbk £22.50, $39.50; pbk £7.95, $12.95.

MONAHAN, J. and STEADMAN, H.J. (eds). Mentally Disordered Offenders: Perspectives from Law and Social Science, Vol. 6. Pp.302. ISBN 0-306-41151-2. (Plenum: 1983.) $29.50.

SCHULTZ, T.W. Investing in People: The Economics of Population Quality. Pp.173. Pbk ISBN 0-520-04787-7. (University of California Press: 1983.) £5, $7.75.

General

ARMSTEAD, H.C.H. Geothermal Energy: Its Past, Present and Future Contributions to the Energy Needs of Man. Pp.404. ISBN 0-419-1222-6. (E. & F.N. Spon: 1983.) £25.

ATLURI, S.N., GALLAGHER, R.H. and ZIENKIEWICZ, O.C. (eds). Hybrid and Mixed Finite Element Methods. Pp.582. ISBN 0-471-10486-8. (Wiley: 1983.) £45, $85.

BATTY, M. and HUTCHINSON, B. (eds). Systems Analysis in Urban Policy-Making and Planning. NATO Conference Series, Vol. 12. Pp.619. ISBN 0-306-41118-0. (Plenum: 1983.) $75.

BOARDMAN, R. and KEELEY, J.F. (eds). Nuclear Exports and World Politics: Policy and Regime. Pp.256. ISBN 0-333-31514-6. (Macmillan Press, London: 1983.) £20.

BRADY, N.C. (ed.). Advances in Agronomy, Vol. 35. Pp.305. ISBN 0-12-000735-5. (Academic: 1982.) $45.50.

BUTEUX, P. The Politics of Nuclear Consultation in NATO 1965-80. Pp.292. ISBN 0-521-24798-5. (Cambridge University Press: 1983.) £22.50, $39.50.

CAMPBELL-JONES, S. (ed.). Horizon at the Edge of the Universe. Pp.216. Pbk ISBN 0-563-17954-6. (BBC Publications: 1983.) £2.95.

CAMPBELL-JONES, S. (ed.). Horizon at the Frontiers of Medicine. Pp.172. Pbk ISBN 0-563-17955-4. (BBC Publications: 1983.) £2.95.

CHUNG, K.L. (ed.). Pao-Lu Hsu: Collected Papers. Pp.589. ISBN 0-387-90725-4/3-540-90725-4. (Springer-Verlag: 1973.) DM 128, $52.90.

CLINCH, P.E.M. and MOLLAN, R.C. (eds). A Profit and Loss Account of Science in Ireland. Pp.151. ISBN 0-86027-013-0. (The Royal Dublin Society: 1983.) £10.

COHEN, I.B. The Newtonian Revolution. Pp.404. Hbk ISBN 0-521-22964-2; pbk ISBN 0-521-27380-3. (Cambridge University Press: 1983.) Hbk np; pbk £9.95.

DOUGLAS, J.J. A Re-Appraisal of Forestry Development in Developing Countries. Pp.178. ISBN 90-247-2830-4. (Martinus Nijhoff: 1983.) Dfl. 75, $33.

FIEDLER, L.A. Olaf Stapledon: A Man Divided. Pp.236. Hbk ISBN 0-19-503086-9; pbk ISBN 0-19-503087-7. (Oxford University Press: 1983.) Hbk £17.50; pbk £6.95.

FINCH, R. The Primal Place. Pp.243. ISBN 0-393-01623-4. (W.W. Norton: 1983.) $15.

ANNOUNCING

NEW FOR 1984

Pharmaceutical Medicine

The contents will include:

★ **Original papers**
- on methods used to evaluate the efficacy and safety of drugs in man
- with emphasis on the validation of these methods
- covering human pharmacology, clinical research, drug epidemiology, and safety surveillance
- and especially the application of new methods to drug testing

★ **Abstracts of methodological studies published elsewhere**
- either used already in the context of drug investigation
- or methods suitable for drug evaluation

★ **Invited reviews on topics related to pharmaceutical medicine**

★ **Editorials and Commentaries**
- offering opinions
- high-lighting problems

★ **Correspondence, Book Reviews, and Calendar**

Editor
Robert N Smith MD FRCP
Director of the Medical Division, Glaxo Group Research Limited, Greenford; formerly Senior Lecturer and Consultant Physician, Clinical Pharmacology and Therapeutics, University of Sheffield

Editorial Advisory Board
Daniel L Azarnoff MD FACP
President, Research and Development, G D Searle & Company, Chicago; formerly Professor of Medicine and Pharmacology, University of Kansas Medical Center
Michael G Carter MB BPharm
Director of Pharmaceutical Division, Roche Products Limited, Welwyn Garden City
Arthur H Hayes, Jr, MD
Dean and Provost, New York Medical College, New York City; formerly FDA Commissioner, Washington DC
William P Maclay MB DTM&H
Director of Medical Affairs, Sandoz Products Limited London
Sir Abraham Goldberg MD FRCP
Regius Professor of the Practice of Medicine, University of Glasgow; Chairman, Committee on Safety of Medicines
J Desmond Fitzgerald MD FRCP
Medical Director, Imperial Chemical Industries plc, Macclesfield; formerly Professor of Medicine, MacMaster University, Hamilton, Ontario
Cyril Maxwell MB ChB
Medical Director and Chief Executive, Clinical Research Services Limited, London; formerly Medical Director, Richardson-Merrell Limited
Paul Turner MD FRCP
Professor of Clinical Pharmacology, St Bartholomew's Hospital Medical College, London
Derek C Quantock PhD MD
Director of Science and Technology, Fisons Limited, Loughborough; formerly Medical Director, Association of the British Pharmaceutical Industry
Peter S Schönhöfer MD
Research Coordination, Institute für Arzneimittel, Bundesgesundheitsamt, Berlin; formerly Professor of Pharmacology, Medizinsche Hochschule, Hannover
Julian H Shelley BSc MRCP
Head of Medical Research, Boehringer Ingelheim International GmbH, Brackneil; Visiting Lecturer in Pharmacology, King's College, London
Geoffrey R Venning BM FRCP
Director of Research and Development, Janssen Pharmaceutical Limited, Marlow; formerly Senior Medical Officer, Medicines Division, DHSS
William M Wardell DM DPhil
Associate Professor of Pharmacology and Assistant Professor of Medicine; Director, Center for the Study of Drug Development, University of Rochester Medical Center

☐ Please send me a guide to contributors
☐ Please send me further information
☐ Please reserve me a sample copy of issue No. 1

NAME _____

ADDRESS _____

To: David N Guthrie,
Scientific and Medical Division,
The Macmillan Press Ltd.,
Houndmills, Basingstoke, Hampshire
RG21 2XS, U.K.

Subscription Information
Frequency: 4 issues per year
Language: English
Rates per volume of 4 issues:
UK & Eire: £45.00
USA/Canada (surface), US$95.00
Rest of the World (surface), £63.00

SCIENTIFIC & MEDICAL | M | MACMILLAN PRESS LTD

Circle No.67 on Reader Service Card.

NATURE CLASSIFIED

LONDON OFFICE Keith Walesby 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024)
AMERICAN OFFICES NEW YORK Miss Gene Fein 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900
SAN FRANCISCO JJHS (415) 392 6794 ●**PASADENA** JJHS (213) 796 9200
CHICAGO Didier & Broderick (312) 498 4520 ● **BOSTON** CEL Assoc (617) 848 9306
CANADIAN OFFICE TORONTO Peter Drake 17 Pine Crescent, Toronto, Ontario M4E 1L1 (416) 690 2423

RATES UK — Rest of world — £11.00 per centimetre. Minimum 3 cm. £2 is charged for the re-direction of replies to advertisements with a box number.

CONDITIONS. The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.

UNIVERSITY OF READING
PROFESSORSHIP OF PHYSICS

Applications are invited for a Professorship of Physics which will become vacant on the retirement on 30 September 1984 of Professor H H Hopkins, FRS, Professor of Applied Optics.

Candidates should have experience and achievement within the field of applied and modern optics, interpreted in the broadest sense.

The appointment will be made from a date to be arranged with the successful candidate.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Reading RG6 2AH. The closing date for applications is 16 March 1984. (1005)A

ST THOMAS'S HOSPITAL MEDICAL SCHOOL
(United Medical & Dental Schools)
POSTDOCTORAL POSITION IN HUMAN NEUROPHYSIOLOGY

Applications are invited for a post-doctoral position at the Sherrington School of Physiology to study the organization of central synaptic input to motoneurones in man. The position is supported by the MRC and will be available from January 1984. Knowledge of spike train analysis would be an advantage but is not essential.

For more details please contact Dr John Stephens, Sherrington School of Physiology, St Thomas's Hospital Medical School, London SE1 7EH. (1002)A

UNIVERSITY OF CAMBRIDGE
PHYSICIST/ENGINEER RESEARCH ASSISTANT

required to participate in development of new ion-beam equipment for secondary-ion mass spectrometry. Post-graduate experience and familiarity with ultra-high vacuum and electronic measuring techniques an advantage.

3 year appointment with starting salary in the range of £5,880 — £8,080 according to age and experience.

Applications, cv + names of two referees, to Dr J V P Long, Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EQ by 13 February, 1984. (1011)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
TECHNICIAN GRADE 3 OR 5

Required for a 3 year MRC study of embryonic sensory organs. Duties will include dissection of chick embryos, establishment of cell cultures, protein purification and histology. Appointment at T3 (Min £5,151 pa) or T5 (Min £6,279 pa) depending upon experience, plus £700 pa London Allowance.

Application form and further details from Establishment Officer (Quote Ref: TP), St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Closing date 9th February. (1045)A

Rijksuniversiteit Utrecht

In the faculty of Sciences, subfaculty of Geology and Geophysics, is, in the Department Geophysics of the Solid Earth, the post of

PROFESSOR OF GEOPHYSICS (M/F)

vacant

His duties will concern:

- Teaching of subjects in geophysics of the solid earth, in compliance with the program of the department of Geophysics.
- Leading research to be carried out in agreement with the program of the Department.
- Participating in administrative and managerial tasks which are relevant to the Department.

Preference will be given to candidates with proven ability in the field of seismology.

The department of Geophysics of the Solid Earth, at present, comprises groups for Marine Geophysics, Paleomagnetism, Exploration Geophysics and Theoretical Geophysics (including Seismology).

The candidate is expected to give important and meaningful contributions to the further development of geophysics.

The salary scale is according to regulations between Dfl. 7378 — and Dfl. 9283 — per month.

Written applications, accompanied by a curriculum vitae and list of publications should be received before March, 15 1984, addressed to the Secretary of the Advisory Committee, Prof. Dr. N. J. Vlaar, Instituut voor Aardwetenschappen, Rijksuniversiteit Utrecht, Budapestlaan 4, 3584 CD Utrecht, the Netherlands under No 145.031.

Persons who wish to draw the attention of the Committee to potential candidates are invited to express their opinion in writing to the Secretary of the Committee.

(W675)

MACQUARIE UNIVERSITY
Sydney, Australia

SCHOOL OF BIOLOGICAL SCIENCES

LECTURER IN BIOLOGY
(Fixed-Term Appointment)

Applications are invited for the position of lecturer in Biology for a fixed-term of 5 years. Applicants should possess high academic qualifications and have shown capacity for research. Some university level teaching experience would be an advantage. The appointment will be made in one of three fields: biochemistry, molecular biology or immunology, but preference will be given to one or other of the following two specialised areas: (a) cell surface biology, with particular expertise in the production and use of monoclonal antibody reagents, (b) molecular and DNA sequencing. If the successful applicant were chosen from the first area, he or she would be encouraged to become involved with a group working on cell surface aspects of differentiation in simple eukaryotes such as Dictyostelium, while for the second area a person working with higher plants is preferred.

The present Chairs of Biology are held by Professors D W Cooper, G B Sharman and K L Williams.

Salary range $:A23,394 — $A30,734 pa.

Further information about the University and advice regarding the method of application should be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, Macquarie University, North Ryde, New South Wales 2113, Australia.

Applications close on 24 February 1984. (W673)A

ICI

CORPORATE BIOSCIENCE GROUP

RESEARCH SCIENTIST

ICI's Corporate Bioscience Group has an opportunity for an enthusiastic graduate with a good Hons degree in biochemistry, microbiology or related discipline. The successful candidate will be a member of a small team applying microbiological systems to the synthesis of chemicals of interest to ICI. He or she should be able to adapt to a variety of techniques which may include fermentation, cell immobilisation, *in vitro* enzyme studies and analytical methods such as GC and HPLC. Experience in these areas would be desirable.

Please apply enclosing a full CV to Miss E. H. Lauermann, Personnel Officer, PO Box No. 11, The Heath, Runcorn, Cheshire.

Closing date — 2nd February 1984. (1022)A

ARMAUER HANSEN RESEARCH INSTITUTE (AHRI) ADDIS ABABA, ETHIOPIA
Founded and sponsored by the Norwegian and Swedish Save the Children Organizations

DIRECTOR OF AHRI

The position as Director of AHRI is vacant from January 1985. Applicants are invited to apply for a 2-3 years contract.

AHRI was set up as a collaborative project between the Norwegian and Swedish Save the Children Organizations and the University of Bergen in 1969 to undertake basic research in the immunology of leprosy. The Institute has at present 30 positions whereof 5 scientific. AHRI is situated in Addis Ababa, Ethiopia on the compound of an international training and rehabilitation centre for leprosy (ALERT) with 20 in-patient beds and a large out-patient clinic. There are Norwegian, Swedish, English, French and International Community schools in Addis Ababa, and a good furnished family house available.

The research work at AHRI is predominantly dealing with the immunology of leprosy, at present e.g. with detection of *M.leprae* antigens in tissues and fluids, mechanisms of tolerance to *M.leprae*, mechanisms of nerve damage, and the dynamics of cellular responses in natural infection.

The Institute is well equipped for research in these fields and the possibilities for scientific contacts outside the Institute are good. The Director of AHRI is responsible for the research activities and the administrative matters. The Director reports to the AHRI Board.

Qualifications for the position as Director are primarily scientific and may be based on an MD or PhD degree with research experience in a relevant field for the research profile of the Institute in at least the level of associate professor.

Administrative experience will also be taken into account. The Director shall conduct research on his/her own and supervise the other research activities at the Institute.

Further information is available on request from the AHRI Board.

Application in English, curriculum vitae and 3 references should be submitted to:
Professor Morten Harboe,
Chairman of the AHRI Board,
Institute of Immunology and Rheumatology,
Fr. Qvamsgt. 1.
Oslo 1.
Norway.
before 1st March 1984. (W663)A

CSIRO

Chief of Division
Division of Mineralogy
Floreat Park
Perth, WA Australia

Applications for the position of Chief of the Division of Mineralogy are invited from scientists with an established record of personal research achievement and leadership in an appropriate field of science. The position became vacant on October 7, 1983 following the retirement of Mr. A. J. Gaskin FTS.

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology, and Physical Sciences.

Mineralogy is one of the Divisions of the Institute of Energy and Earth Resources. Also in the Institute are the Divisions of Geomechanics, Energy Chemistry, Energy Technology, Groundwater Research, Mineral Chemistry, Mineral Engineering, Fossil Fuels, Mineral Physics and the Physical Technology Unit.

The Division of Mineralogy Headquarters is located at Floreat Park, a suburb in Perth, with a major laboratory at North Ryde in Sydney, NSW, and a small laboratory at Canberra, ACT. The Division has a total staff of 112, which includes 54 professional staff.

The work of the Division, which lies within the broad objectives of the Institute of Energy and Earth Resources, is to develop better techniques for locating new ore bodies and concepts for the delineation of prospective terrain. Basic research on ore genesis and depositional environments forms an important background to this general objective.

The Chief of the Division will be responsible to the Director of the Institute of Energy and Earth Resources for the leadership, scientific direction and integration of the research programs of the Division and will encourage promotion of active collaboration with other CSIRO Divisions, Universities, appropriate State Departments, industry representatives and other institutions conducting research in relevant areas.

The salary will not be less than $A52,780 per annum plus an expenses of office allowance of $A1,250.

Appointment to CSIRO is for an indefinite period and carries Australian Government superannuation privileges, subject to normal conditions. The position of Chief of Division is offered for a negotiable term of the order of five to seven years, with subsequent options for a further term, if mutually desired, or for a senior position in the Organization.

The Division has recently been reviewed by a committee appointed by the Executive of CSIRO and a copy of the Review Committee's Report will be made available on request. The Director of the Institute of Energy and Earth Resources would be pleased to provide further information about the position and to discuss the position with potential applicants. He would also be pleased to receive advice concerning the appointment from people with a particular interest in the position. The Director, Mr. I. E. Newnham, May be contacted by telephone in Melbourne (03) 2687202.

Applications stating full personal and professional details, and names of at least three professional referees and quoting reference number A3804 should be directed to: The Director, Institute of Energy and Earth Resources, CSIRO, 9 Queens Road, MELBOURNE VIC 3004 AUSTRALIA. Applications Close: February 24, 1984. (W666)A

THE UNIVERSITY OF MANITOBA FACULTY OF MEDICINE
has positions available as

ASSISTANT PROFESSOR

These positions are available in response to the development of a multi-disciplinary program in the area of recombinant DNA technology as applied to neurosciences or endocrinology. The successful applicant(s) will be expected to develop an independent research program utilizing recombinant DNA techniques.

Send curriculum vitae including a brief outline of future research interest to Dr. Henry G. Friesen, Professor and Head, Department of Physiology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba R3E 0W3 Canada. (NW225)A

Faculty Position Available Immediately

University of Massachusetts Medical School

The Department of Surgery seeks a full-time research scientist to direct its Division of Surgical Research. The candidate must have the Ph.D., M.D., or equivalent degree, as well as the demonstrated ability to conduct independent research. The appointee will be expected to conduct original research, as well as to coordinate, assist and direct the research of others. Research facilities and support will be provided.

The research interests of the appointee should lie in the disciplines of Physiology, Biochemistry or other basic sciences pertinent to the field of Surgery. A joint appointment in the appropriate basic science department is anticipated.

The level of appointment and salary will be commensurate with qualifications. Applications should include a curriculum vitae and the names and addresses of three references. Applications should be forwarded to:

Dr. H. Brownell Wheeler
Professor and Chairman
Department of Surgery
Univerisity of Massachusetts Medical School
Worcester, Mass. 01605

Further information may be obtained from Dr. H. Brownell Wheeler at (617) 856-2201. The University of Massachusetts Medical School is an equal opportunity employer. (NW221)A

CHAIRPERSON

Department of Physiology

The Mount Sinai School of Medicine of the City University of New York

The Mount Sinai School of Medicine of the City University of New York invites applications and nominations for the position of Chairperson of the Department of Physiology. The medical school seeks a biomedical scientist with an outstanding research program who is committed to the educations of M.D. students, PhD students and MD-PhD students.

Please send cv and list of publications to: Dr. J. P. Green, Chairman, Search Committee, Department of Pharmacology, One Gustave L. Levy Place, New York, NY 10029. An Equal Opportunity Employer.

The Mount Sinai School of Medicine

(NW220)A

UNIVERSITY OF FORT HARE
SOUTH AFRICA
CHIEF TECHNICIAN

Applications are invited from suitably qualified persons, regardless of sex, religion, race, colour or national origin, for the post of Chief Technician in the Electron Microscope Unit.

The Unit contains an H500 TEM and S450 SEM, both equipped with fully quantitative x-ray analysers, plus the usual peripheral equipment. Prospective candidates should have suitable experience in handling biological material for TEM and SEM and material for x-ray analysis, although the latter is not a prerequisite for appointment.

The University is rurally situated some 110 km from Rhodes University in Grahamstown.

The Salary will be in the range R12,030 — R13,740 per annum. A pensionable allowance of 12% is also payable. The initial salary will be determined according to qualifications and experience. (R1.68 = £1.00 approx).

Service benefits available to staff include, under certain conditions, financial assistance with removal of personal effects and travelling expenses, service bonus, foreign allowance, group life assurance, medical and pension schemes, housing assistance, generous leave and sick leave benefits, generous assistance with university education of employees dependants.

Further information on the vacancy, and application forms can be obtained from the Staff Officer, University of Fort Hare, Private Bag X1314, ALICE, Republic of Ciskei, South Africa. Completed applications should be lodged with the University by 10th March 1984. (W669)A

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF CHEMICAL PATHOLOGY AND HUMAN METABOLISM
RESEARCH ASSISTANT

Applications are invited for the above post which is to run for four years, to work with Dr H T Delves on the determination of lead and other trace elements in biological samples using modern atomic absorption spectrophotometric techniques.

Applicants should have a good degree in chemistry or Part II GRSC and have experience of, or interest in, analytical science.

Salary will be on the 1B scale for research assistants (£6,310 — £8,530). Applications (2 copies) with curriculum vitae and names of two referees should be sent to the Staffing Department, University of Southampton SO9 5NH. Previous candidates need not re-apply.

Further information may be obtained from Dr H T Delves, telephone 0703 777222 ext 3846. Please quote ref: 1279/R.

Closing date: 31 January 1984. (1029)A

THE UNIVERSITY OF WESTERN AUSTRALIA
Perth
CHAIR IN PHYSIOLOGY

Applications are invited for appointment to the Chair in Physiology to be vacated on the retirement of Professor W J Simmonds. There is one other Chair in the Department, a Personal Chair held by Professor E H Morgan.

Applicants must hold appropriate higher qualifications and must be able to demonstrate a substantial research background in human or mammalian physiology. The Department is strongly represented in cellular and neurophysiology and is therefore seeking applicants from other areas of physiology. However, applicants from all areas will be considered. Whilst preference will be given to medically qualified candidates, applications from persons with other appropriate qualifications will be welcome. previous experience in the teaching of Physiology to Medical and Science students at both undergraduate and graduate levels is essential.

The current salary for the appointment is $A48,997 per annum, plus a preclinical loading for medical graduates of $A4,386 per annum. Benefits include superannuation, fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave, eligibility for interestate travel grants and housing loan scheme. Further information including conditions of appointment may be obtained from the Staffing Officer, University of Western Australia, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications in duplicate stating full personal particulars, recent photograph, qualifications, experience and the names and addresses of three referees should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by 3rd March 1984. (W674)A

UNIVERSITY OF CALIFORNIA
Berkeley
POSTDOCTORAL RESEARCH

Applications are invited from persons having a PhD in Biochemistry or related fields for postdoctoral positions in the laboratories of Drs Bruce Ames, Lester Packer and Martyn Smith. These positions are sponsored by the National Foundation for Cancer Research. Research will be directed towards understanding the role of oxygen radicals in cancer and aging. Preference will be given to candidates with expertise in HPLC.

Please send curriculum vitae to Dr M T Smith, BEHS, School of Public Health, University of California, Berkeley, CA 94720. Telephone (415) 642-8770. The University of California is an Affirmative Action, Equal Opportunity Employer. (NW229)A

UNIVERSITY OF DUBLIN
Trinity College

LECTURESHIP IN GEOLOGY

Applications are invited for the above post in the Department of Geology at Trinity College, Dublin, tenable from 1st October, 1984.

Preference will be given to candidates with interests in structural geology or geochemistry. A commitment to field work is essential.

Salary Scale: IR£8,411 – £17,311 per annum.

Appointment will be made within the salary range IR£8,411 – £10,730 p.a. at a point to accord with qualifications and experience to date. Application forms and further particulars relating to this post may be obtained from:

Establishment Officer, Staff Office, Trinity College, Dublin 2. Ireland. Tel: Dublin 772941 ext 1775.

The closing date for receipt of completed applications will be 29th February, 1984. (1041)A

ST THOMAS'S HOSPITAL MEDICAL SCHOOL
(United Medical and Dental Schools)
London SE1 7EH

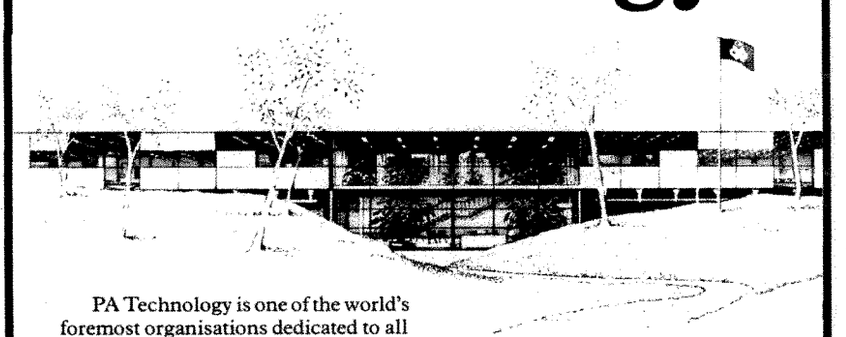
DEPARTMENT OF HISTOPATHOLOGY

ELECTRON MICROSCOPY UNIT JUNIOR MLSO

required in this Department, which provides research and diagnostic services in transmission and scanning electron microscopy for St Thomas's Hospital and Medical School. Previous experience an advantage. Salary on Whitley Council scale according to age, qualifications and experience.

Apply in writing, with names and addresses of two referees to Senior Chief MLSO from whom further information is available. (01-928 9292 ext 2549). (1040)A

Biotechnology



PA Technology is one of the world's foremost organisations dedicated to all aspects of new product and process development, placing emphasis on the strategic consequences of such development, and operating worldwide from laboratories in the UK, the USA, Belgium and Australia.

The continued expansion of our Cambridge Laboratory has created several new and challenging posts in our highly successful biotechnology group.

We are seeking high calibre scientists in a number of areas. In selecting appropriate candidates, emphasis will be placed on:

— proven scientific record
— creative/innovative skills
— commercial awareness
— ability to work in a multi-disciplinary environment
— good communicative skills

The posts are in:

- **genetic manipulation**
- **enzyme technology**
- **medical biosensors**
- **monoclonal antibody production**

Successful applicants for these posts will probably be educated to PhD level and subsequently have spent a number of years working towards commercially-directed goals. They will be authoritative within their own area of expertise and have a broad knowledge of commercial developments in the biotechnology area. They should have the ability to establish new project areas within our organisation and to direct these through to completion.

- **applied microbiology/biochemistry**
- **medical biosensors**

These are posts suitable for graduates which call for sound scientific ability coupled with commercial acumen. Applicants should have at least two years' experience in a commercial environment since graduating.

The salaries and other benefits for these posts will be compatible with the high quality of staff we are seeking and are unlikely to present a problem.

For further details about our company, and an application form, please write or phone our Personnel Department, PA Technology, Cambridge Laboratory, Melbourn, Royston, Herts SG8 6DP. Tel: Royston (0763) 61222.

PA Technology
Cambridge Laboratory

(1039)A

1979

CENTRE INTERNATIONAL DE RECHERCHES MEDICALES DE FRANCEVILLE – GABON
RECHERCHE

INGENIEUR DE LABORATOIRE RIA POUR SON LABORATOIRE D'ENDOCRINOLOGIE

Fonctions

- Supervision des dosages radioimmunologiques de routine et Organisation du Travail (Equipe de 5 techniciens)
- Mise au Point de Nouvelles Techniques.
- Participation aux Programmes de Recherched en Biologie de la reproduction.

Excellentes conditions de travail contrat debut Janvier 1984 pour 2 ans renouvelables. Conditions habituelles d'expatriation.

Candidatures àadresser à Monsieur le Directeur Général du CIRMF, 28 bd de Grenelle, 75015 Paris, avec curriculum vitae, références professionelles et prétentions. (W612)A

PUBLIC HEALTH LABORATORY SERVICE BOARD
PHLS CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH

A vacancy exists for a post doctoral molecular biologist to work jointly in the Molecular Genetics Laboratory and the Pathogenic Microbes Research Laboratory on a project to investigate the genetics of virulence and antigenicity in the bacterium *Bordetella pertussis,* the causative agent of whooping cough. This post is a fixed term contract, funded for three years by an MRC project grant, and is immediately available. The project will involve nucleic acid hybridisation, molecular cloning techniques and the use of transposon mutagenesis to study gene function. Experience in these techniques would be advantageous but not essential. Other applicants with experience in molecular biology or genetic manipulation are invited to apply and will receive favourable consideration.

The appointment will be made at the Senior Grade Microbiologist level £9,010 – £9,605 National Health Service terms and conditions will apply. Further information about this post can be obtained from Dr. C. Duggleby or Dr. A. Robinson on Idmiston (0980) 610391.

Applications, including curriculum vitae, and the names and addresses of three referees, should be sent to Mrs. Bushby, Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG.

Closing date: 3 February 1984. (1004)A

UNIVERSITY OF
SURREY

HEAD of the Department of Chemistry

Applications are invited for the post of Professor of Chemistry and Head of the Department.

The Department has a number of well-founded research activities with an applied orientation, and a strong commitment to the industrial year in the undergraduate course.

The successful candidate is likely to have wide experience as a chemist and to have a proven research record. Applications will be especially welcomed from candidates with a strong interest in the fields of macromolecular chemistry (including the synthesis of large molecules, and the study of their structure, function and reaction mechanisms) or the chemical effects of interfaces particularly, but not exclusively, in inorganic systems.

Further particulars are available from the Academic Registrar (LFG), University of Surrey, Guildford, Surrey GU2 5XH, or telephone Guildford 571281 ext 633. Applications giving the names and addresses of three referees, should be sent to the above by 29 February 1984 quoting reference 229/N. (999)A

UNIVERSITY OF BERGEN, NORWAY

UNIVERSITY LECTURERSHIP IN SEDIMENTOLOGY

A position as *university lecturer* is vacant at the Geological Institute (Dept. A), University of Bergen, Norway. Applicants must have an MSc, PhD or equivalent qualifications and competence in sedimentology (preferably clastic sedimentology). Experience in sedimentary mineralogy and geochemistry will be advantageous.

The vacant position is in association with the research group in sedimentology/petroleum geology which is composed, in addition to the advertised position, of a scientific staff of 5. The successful candidate must be able to take part in teaching at both undergraduate and postgraduate levels according to the requirements of the institute and follow the regulations which apply for the position. Applications from women are encouraged. Further particulars about the lectureship may be obtained from the Secretary of the Faculty of Sciences, PO Box 25, 5014 Bergen Universitet, Norway. The salary will be in the range NKr. 123.496 – NKr. 162.917.

Applications, with a complete curriculum vitae, copies of degrees and testimonials together with one set of scientific publications and a list of these in triplicate should be sent to the University of Bergen, Personnel Department, PO Box 25, 5014 Bergen – Universitet, Norway, by 20th March 1984. (W677)A

MCGILL UNIVERSITY
LADY DAVIS INSTITUTE FOR MEDICAL RESEARCH
RESEARCH IN AGING

A new unit for research in aging is being established at the Lady Davis Institute. Three positions are available immediately. These will carry appointments in the Faculty of Medicine, McGill University. One post is tenure-track, at the Associate Professor level; two are at the Assistant Professor level and may become tenure-track. It is anticipated that three more positions will be available over the next two to three years. Areas of major interest in relation to the aging process are molecular genetics, immunology and hormonal/neuropeptide changes of higher brain centers; however, other areas of research are not excluded.

Applications are invited from all; in accordance with Canadian immigration regulations applications from Canadian citizens and landed immigrants will be considered first. Review of applications will begin March 1 and will continue until suitable candidates are selected.

Send curriculum vitae, brief description of present and proposed research and names of three referees to Dr. Norman Kalant, Director, Lady Davis Institute for Medical Research, 3755 Cote St. Catherine Road, Montreal, Que. Canada H3T 1E2. (NW226)A

ZENTRUM FUER GENTECHNOLOGIE AN DER UNIVERSITAETMUENCHEN

SENIOR RESEARCH SCIENTIST ARBEITSGRUPPENLEITER

Applicants are invited for an independent research position in eucaryotic molecular biology. Candidates will be expected to develop and direct a vigorous research program in synthetic biology and/or gene structure and function. Positions will be filled for 5 years. Salary will be commensurate with experience and accomplishments.

Applications, including a curriculum, a list of publication and names of 2 references, should be sent by 29 February 1984 to Prof. E. L. Winnacker, Institut Fuer Biochemie, Karlstrae 23, D-8000 Muenchen 2. (W676)A

UNIVERSITY OF LONDON
KING'S COLLEGE LONDON
QUEEN ELIZABETH COLLEGE
CHELSEA COLLEGE

READERSHIP IN ANATOMY

The Senate invite applications for the above Readership tenable at King's College London, to be filled as soon as possible in 1984. Medical qualifications, although not essential, would be an advantage.

Applications (11 copies) should be submitted to the Academic Registrar (N) University of London, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained. Closing date for receipt of applications is 22 February 1984. (1021)A

NEWNHAM COLLEGE
CAMBRIDGE

DIRECTORSHIP OF STUDIES IN MEDICINE AND COLLEGE LECTURESHIP IN A MEDICAL SCIENCE

Applications are invited from men and women for the post of Director of Studies in Medicine, to be held in conjunction with a full-time or part-time College Lectureship in one of the following subjects: Anatomy, Pathology, Pharmacology or Physiology. The successful applicant will be expected to be actively engaged in research in the Biomedical Sciences, but need not necessarily hold a medical qualification. Separate appointment as Director of Studies only may be considered for a candidate already holding a full-time post in Cambridge. A woman appointed to a College Lectureship will be elected a Fellow of the College; under the College Statutes Fellowships are open to women only.

The appointment will be for five years and may be renewable. The salary for a full-time Lectureship is on the scale of Cambridge University Assistant Lecturers and Lecturers according to age and experience. The Directorship of Studies is separately remunerated. Further particulars may be obtained from the Principal, Newnham College, Cambridge CB3 9DF, to whom applications giving particulars of qualifications and experience and the names of three referees should be sent by 15th February 1984. (1020)A

ASSOCIATE Chief of Staff for Research position available at the Portland VA Medical Center, affiliated with the Oregon Health Sciences University. Responsibilities include administration of growing multidisciplinary research program, coordinating move to new hospital under construction, and maintaining liaison with affiliated programs. Candidates should have a record of nationally recognized ongoing research, background in basic and clinical sciences, and previous administrative experience. MD's preferred. Application deadline March 1, 1984. Send letter of interest, CV and list of 3 references to Daniel E Casey, MD, Chairman, ACOS/Research Search Committee, Psychiatry Service (116A) 3710 SW US Veterans Hospital Road, PO Box 1034, Portland, OR 97207. Equal Opportunity Employer. (NW187)A

THE FLINDERS UNIVERSITY OF SOUTH AUSTRALIA
SCHOOL OF MEDICINE

POST-DOCTORAL APPOINTMENT IN ELECTROPHYSIOLOGY

Applications are invited from suitably qualified persons with post-doctoral experience in intracellular microelectrode recording from nerve cells, preferably in mammalian ganglia. The appointee will be responsible for an investigation of the actions of opiates, in relation to their addictive properties, in enteric ganglia. This NH & MRC funded position, which is under the supervision of Drs M Costa and J B Furness in the Centre for Neuroscience, is available until December 1986 in the first instance.

Salary range: $A20,164 – $A30,733 depending on number of years of post doctoral experience.

Applications, including full curriculum vitae, details of academic record and publications, research interests and the names and addresses of three referees, should be lodged, in duplicate, with the Registrar, The Flinders University of South Australia, Bedford Park, South Australia, 5042, by 30 January 1984. Late applications may be accepted. Applicants should ask their referees to forward their confidential reports to the Registrar by the closing date. (W665)A

DUBLIN INSTITUTE FOR ADVANCED STUDIES
SCHOOL OF THEORETICAL PHYSICS

Applications are invited for a

RESEARCH POST AS ASSISTANT PROFESSOR

in the School, tenable for one or two academic years commencing 1 October 1984. Salary: IR£12,572 per annum.

Appplicants should send a *curriculum vitae* and list of publications, together with the names of two referees, so as to reach the undersigned not later than 12 March 1984. No application forms are issued.

The Registrar, Dublin Institute for Advanced Studies, 10 Burlington Road, Dublin 4, Ireland. (1027)A

UNIVERSITY OF CALIFORNIA, BERKELEY

DEPARTMENT OF ASTRONOMY AND ASTROPHYSICS

ADJUNCT ASSISTANT PROFESSORSHIP

Pending final budgetary approval, the Department of Astronomy of the University of California at Berkeley expects to make one non-tenure track appointment renewable up to five years in astronomy/astrophysics at the Adjunct Assistant Professor level, which can start as early as July 1, 1984. Applicants should send a curriculum vitae, bibliography and a brief statement of research interests, and should arrange to have three letters of recommendation sent to Jonathan Arons, Chairman, Search Committee, Department of Astronomy, University of California, 601 Campbell Hall, Berkeley, CA 94720. All materials should be received by February 1, 1984.

The University of California is an Equal Opportunity, Affirmative Action Employer. (NW181)A

DRUG DISPOSITION/DRUG DELIVERY

The School of Pharmacy, University of Wisconsin-Madison has an opening on the tenure-track faculty at the Assistant Professor level in the broad area of drug delivery or drug disposition. The successful applicant will be expected to initiate an independent research program. The research may be concerned with biological transport and binding mechanisms, chemical transformations, or other basic topics related to the physiological and biochemical phenomena involved in the delivery of drugs to their sites of action; and it may make use of physicochemical, biophysical, pharmacokinetic, biochemical, immunological, or other types of quantitative investigational approaches. An emphasis on the molecular or cellular level approaches is essential. Responsibilities also include participation in pharmaceutics teaching at the undergraduate and graduate levels. A PhD in an appropriate field is required.

Please submit curriculum vitae, including names of at least three references, to Dr Kenneth A Connors, School of Pharmacy, University of Wisconsin, 425 No Charter Street, Madison, Wisconsin 53706.

The University of Wisconsin is an Equal Opportunity/Affirmative Action Employer. (NW223)A

Exciting research opportunity in West Germany
Partake studies on
EPITHELIAL AND MEMBRANE TRANSPORT

using biochemical techniques. Ideal candidate should have MD and/or PhD (Postdoc or junior faculty) and should have experience in protein chemistry and preparative organic chemistry. In addition to the researcher's salary, this federally funded position includes salary for one technician, start-up funds and an appropriate supply budget. The research laboratories are part of the Max-Planck-Institut fuer System-physiologie in Dortmund, West Germany. Starting date: March, 1984.

Please send CV and references to Dr Rolf Kinne, Director, Max-Planck-Institut fuer System-physiologie, Rheinlanddamm 201, 4600 Dortmund 1, West Germany, Deadline for application: February 29, 1984. (W672)A

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

THE POST OF A GRADE III TECHNICIAN

renewable annually is available for suitable candidates in the above Department of Heath park, Cardiff. The successful applicant will be expected to assist in the organisation of a quality assessment scheme for drug measurement.

Preference will be given to candidates with experience in quality control procedures and drug assay techniques. Applicants should possess a Degree or equivalent qualification or appropriate practical experience.

Commencing salary will be dependent upon age on the Scale for University Technicians Grade III (£5,151 — £6,035).

Further particulars and application forms (quoting Ref No P8B) are available from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN (Tel: No. 0222/755944 ext 2296). Closing date for application 31st January, 1984. (1015)A

CSIRO
Postdoctoral Research Fellowship
$A24,344 — $A30,038
Division of Environmental Mechanics
Canberra ACT Australia

Field: Physics of Soil Erosion by Wind and Water.

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Environmental Mechanics is a member of the Institute of Physical Sciences.

Research in the Division of Environmental Mechanics is directed primarily at gaining a better understanding of the biological and physical processes of the soil-plant-atmosphere system with particular regard to the transport of energy, water, nutrients and other materials. Facilities include laboratories for soil physics, solar radiation, fluid mechanics, micrometeorology and physiology, as well as mobile laboratories for study of the field environment. The laboratory has a large and well instrumented boundary layer wind tunnel, and computer facilities for the collection and analysis of experimental data. The Division has a micrometeorology field site located 45 km from Canberra. Divisional staff at present includes 15 research scientists (biologists, physicists and mathematicians) together with experimental and technical staff, and is usually augmented by several visiting research workers.

The Division's research is being expanded to include a study of the problems of predicting and controlling erosion in the natural and agricultural environment. Applications are invited from suitably qualified persons to undertake theoretical and experimental investigations of the mechanisms involved in the movement of soil particles by wind and water. These investigations will interlock with work done by the Division on the relationship between rainfall intensity, soil physical properties and ponding; and work on the turbulence characteristics of air flows over rough and topographically variable terrain.

Applicants should have a PhD degree or equivalent, supported by evidence of proven research ability.

A fixed term appointment of three years within the classification of Research Scientist. An applicant with an outstanding research record may be offered appointment at a higher level. Australian Government superannuation benefits are available.

Applications stating full personal and professional details, the names of at least two scientific referees, and quoting No. A0456 should be directed to:

The Chief,
CSIRO Division of Environmental Mechanics,
GPO Box 821,
CANBERRA ACT 2601. AUSTRALIA.
By February 29, 1984. (W667)A

MERRELL DOW RESEARCH INSTITUTE
STRASBOURG CENTER — FRANCE

PHARMACOLOGIST

An opportunity has arisen for an experienced pharmacologist with cardiovascular interests to work in our research center. The successful applicant will be expected to contribute to research on the mechanisms of action of calcium antagonists and on their development as therapeutic agents.

Candidates should have a PhD or equivalent degree and relevant post-doctoral experience.

The post gives the opportunity of working in a challenging research field, in very pleasant surroundings.

Salary will be commensurate with experience.

Applications and CV to Personnel Manager, Gisela Nguyen-Dinh, 16, rue d'Ankara, 67084 Strasbourg Cédex, France. (W664)A

AGRICULTURAL BIOTECHNOLOGY

Shell Development Company is seeking scientists with significant records of achievement who are interested in applying their skills to agronomic research.

Positions are available for outstanding Ph.D.-level scientists with training in one of the following:

- Agricultural Microbiology
- Plant Molecular Biology
- Plant/Invertebrate Pathology
- Plant Cell and Tissue Culture

Shell Development Company at Modesto, California offers an outstanding research environment, a reasonable cost of living, and attractive salaries and benefits. Modesto is close to the San Francisco Bay Area and the Sierra Nevada Mountains. Shell's current staff includes a strong team of innovative scientists with a record of technical accomplishment and commercial success in agrochemicals research.

Applicants should be U. S. Citizens or have permanent resident visa. Interested applicants should send a résumé, in confidence, to: Manager, Employee Relations, Shell Development Company, Biological Sciences Research Center, P.O. Box 4248, Modesto, CA 95352.

Shell Development Company

An Equal Opportunity Employer M/F

(NW227)A

SENIOR ORGANIC GEOCHEMIST

Division Head Required for Interdisciplinary Exploration Team

Woodward-Clyde Oceaneering, an expanding International Geological consulting firm based in Aberdeen, invites applications from individuals with a PhD Degree and a minimum of five years experience in Petroleum Geochemistry. Responsibilities will include developing and managing geochemical hydrocarbon exploration programmes in frontier basins, both terrestrial and marine, utilising current theories in organic geochemistry. Knowledge of a variety of instrumental methods such as GC, GC/MS, Fluorescence, Pyrolysis and Isotope Ratio/MS is desirable.

This position provides the opportunity of working with a multi-disciplinary exploration team consisting of Geologists, Geophysicists and Engineers. Résumés will be treated confidentially. Please state availability. The successful candidate would be expected to assume duties in March/April 1984.

Salary will be negotiated within the range of £15,000 to £22,500, to be commensurate with experience. Interviews will be scheduled in London and Aberdeen during the week of 30 February 1984. Reply to Box No 925 c/o Nature, 4 Little Essex Street, London WC2R 3LF.

(925)A

TEXAS A&M UNIVERSITY

DEPARTMENT OF BIOLOGY

HEAD

Applications and nominations are invited for the position of Head of the Department of Biology at Texas A&M University.

The Department of Biology, with a faculty of 38, is an administrative unit of the College of Science offering BS, MS and PhD degrees in Biology, Botany, Microbiology and Zoology.

Candidates for the position must have an earned doctorate, a distinguished record in research and teaching, and other professional accomplishments. Candidates are sought who have demonstrated leadership abilities and a commitment to developing strong academic and research programs in Biology.

Candidates should be available to assume the position by September 1, 1984. Nominations and applications, with résumés and names and telephone numbers of references, will be accepted until February 15, 1984 or until a suitable candidate is identified. Materials should be sent to: Dr C J Maxson, Associate Dean, College of Science, Texas A&M University, College Station, Texas 77843. Phone (409) 845 7361.

Texas A&M is an Equal Opportunity Affirmative Action Employer.

(NW190)A

UNIVERSITY OF LONDON

READERSHIP IN PHARMACEUTICAL CHEMISTRY AT THE SCHOOL OF PHARMACY

The Senate invite applications for the above Readership from graduates in pharmacy, physical chemistry, chemical physics, physical biochemistry or biophysics. The duties will include teaching for the B Pharm and BSc (Pharmacology and Toxicology) Honours degrees.

Current physical research in the department involves fundamental and drug-related applications of light scattering and diffusion, nmr and mass spectroscopy, electrochemistry and electrochemical methods, macromolecular chemistry, and physical approaches to drug design.

Candidates should have an established record of published research. The successful candidate will take up the position as soon as possible after 1 June 1984. Salary will be in the range £13,515 — £16,925 plus London Weighting of £1,186.

Applications (ten copies) should be submitted to the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained. Closing date 28 February 1984. (997)A

POSTDOCTORAL POSITION available after January 1, 1984 in Genetic Sequence Analysis, to investigate problems in sequence pattern recognition and molecular evolution. Experience in Molecular biology and/or computer science required. Salary: $15,000 to $18,000 per annum. Send résumé and references to Dr T F Smith, Biophysics Laboratory, Northern Michigan University, Marquette, MI 49855. An Equal Opportunity Employer.

(NW219)A

ST MARY'S HOSPITAL MEDICAL SCHOOL

(University of London)

Norfolk Place,

London W2 1PG

DEPARTMENT OF CLINICAL PHARMACOLOGY

POST-DOCTORAL RESEARCH FELLOW (NON-CLINICAL) IN CARDIOVASCULAR PHARMACOLOGY

The appointment is for one year in the first instance, with a possible extension to a maximum of three years.

The research programme involves the investigation of drug actions on isolated human blood vessels. Applicants with previous experience in adrenergic and/or cardiovascular pharmacology will be preferred.

Salary range: £6,310 — £8,530 + £1,186 London Allowance.

Applications and names of two referees should be sent to the Assistant Secretary (Personnel) at above address no later than 3 February 1984. (998)A

QUEEN MARY COLLEGE
University of London
SCHOOL OF BIOLOGICAL SCIENCES

Applications are invited for the post of

RESEARCH ASSISTANT

for work funded by the Tropical Development and Research Institute on termite control by the inhibition of diuresis, under the supervision of Dr D E Bignell. Candidates should have a PhD, with experience in insect excretion, digestion or endocrinology and electron microscopy.

Initial appointment for 2 years from 1 February, with possible extension for a third year, with starting salary in range £8,376 — £9,716 per annum.

Please apply by letter, enclosing cv and names of 2 referees, to The Secretary, Queen Mary College, Mile End Road, London E1 4NS. (1030)A

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF ZOOLOGY

LECTURESHIP IN ANIMAL POPULATION ECOLOGY

Applications are invited for a Lectureship in the above Department, tenable from 1 April 1984. Within the general area there are no restrictions on specific field of interest except that the applicant must be involved in a computer-dependent research programme and have an active interest in theoretical aspects of the subject. Initial salary in the range £7,190 — £8,530 a year, on a scale rising to £14,125 a year. Expected age of candidates up to about 27 years but older candidates not precluded.

Particulars from the Registrar and Secretary (Staffing), the University, Sheffield S10 2TN, to whom applications (5 copies), including the names of three referees, should be sent by 10 February 1984. Quote ref: R27/G. (1008)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

TECHNICIAN

required to assist with teaching duties and endocrine biochemistry research work. Experience in RIA or HPLC and/or cell culture is desirable. Applicants must have at least two years previous working experience.

Salary on scale £5,491 — £7,747 plus £1,042 London Allowance. 37 hour week. 35 days leave including Bank Holidays and customary days.

Further details and application forms are available from the School Office, RFHSM, Rowland Hill Street, London NW3 2PF or telephone 01-794 0500 ext 4262 quoting reference BAC/T. Closing date 3 February 1984. (1028)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF MICROBIOLOGY

POST-DOCTORAL RESEARCH FELLOW

Applications are invited for a 3 year post-doctoral research position supported by the Medical Research Council. The project is to investigate the molecular basis of poliovirus neurovirulence using infectious cloned cDNAs. This investigation forms part of a larger programme concerned with the development of new prophylactic and therapeutic agents against medically important picornaviruses.

Previous experience of recombinant DNA techniques and/or of virology would be an advantage. Starting salary will be up to £8,530, depending on age and experience.

Applicants (PhD) should send a detailed curriculum vitae and the names of two referees to Dr J W Almond, University of Leicester, Department of Microbiology, University Road, Leicester LE1 7RH. (1016)A

CHURCHILL HOSPITAL
DEPARTMENT OF MEDICAL GENETICS

PRINCIPAL CYTOGENETICIST

We would like to appoint a scientist with considerable cytogenetic and theoretical experience preferably with PhD who would be able to assist in the management of all the various aspects of handling routine workloads under the guidance of the Head of the Laboratory.

Informal visits may be arranged by contacting Dr J Jonasson, Department of Medical Genetics, Churchill Hospital, Oxford.

Applications enclosing a full curriculum vitae and the names of two referees in writing to the Personnel Officer, Churchill Hospital, Headington, Oxford by 3rd February 1984. (1025)A

UNIVERSITY OF CAMBRIDGE

DEMONSTRATOR IN PLANT CYTOLOGY AND GENETICS

The appointments committee hope to be in a position to make an appointment to a University Demonstratorship in the Department of Botany as from 1 October 1984. The person appointed will be expected to teach plant cytology and genetics, and to assist in teaching an integrated cell biology or biology of organisms course. The applicant should have research interests in some aspect of plant cytology or genetics. The appointment will be in the first instance for 3 years, with a maximum tenure of 5 years. The salary is on an incremental scale rising from £7,630 to £9,425 per annum.

Applications with the names of two referees should be sent by 15 March 1984 to the Secretary of the Faculty of Biology 'A' Appointments Committee, Department of Zoology, Downing Street, Cambridge CB2 3EJ, from whom further details can be obtained. (1010)A

CSIRO
Research Scientist
(System Engineer)
$A24,344 — $A35,806
Division of Radiophysics
Epping NSW Australia

CSIRO conducts scientific and technological research in Laboratories throughout Australia and employs about 7,500 staff of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Radiophysics is a Division of the Institute of Physical Sciences.

General: The Division of Radiophysics conducts research in radiophysics and its application to community and industrial problems. The headquarters of the Division are located at Epping in Sydney, New South Wales. The Division also operates the Australian National Radioastronomy Observatory at Parkes, New South Wales. The Federal Government recently decided to fund the construction by the Division of a new radiotelescope array to be known as the Australia Telescope. The telescope will include an array of new antennas at Culgoora (near Narrabri) in NSW, a new antenna at Siding Springs (near Coonabarabran) in New South Wales and the existing 64 metre antenna at Parkes.

The appointee will be located at Epping but may be required to undertake trips to the Division's observatory.

Duties: The appointee will lead a group undertaking research into basic digital correlation techniques and design culminating in the production of a state of the art correlator for the Australia Telescope Project.

Qualifications: A PhD degree or equivalent qualifications in science or engineering and the ability to undertake independent scientific research. Broad experience in radioastronomy system design is essential. An understanding of VSLI design fabrication would be an advantage.

Tenure: Indefinite with Australian Government Superannuation benefits available.

Applications: Stating full personal and professional details, the names of at least two referees and quoting reference No A5083, should be directed to: The Chief, CSIRO Division of Radiophysics, PO Box 76, EPPING NSW 2121, AUSTRALIA. By February 17, 1984. (W668)A

UNIVERSITY OF EDINBURGH
DEPARTMENT OF PHYSICS

RESEARCH ASSOCIATE IN NEUTRON DIFFRACTION

Applications are invited for the post of research associate to take part in a programme of neutron diffraction experiments associated with the study of structural phase transitions. This project will mainly involve making very accurate high-resolution structure determinations, including anharmonic thermal motion, at various temperatures and pressures, usually with single-crystal samples. Experimental work will be done at the Institut Laue-Langevin, Grenoble, and on the new pulsed sources in the UK and USA.

The appointment, for up to three years, commences October 1984 at a salary according to age and experience, on scale 1A. Superannuation under USS. Applicants should have, or be about to obtain, a PhD.

Applications with the names of two referees and a statement of applicant's interests and career to date to Dr R J Nelmes, Department of Physics, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JZ, to arrive as soon as possible. Further details on request.

Please quote reference No 5145. (1046)A

Research Professorships in Biology

The University of Calgary, Department of Biology invites applications for research professorships in its four Divisions: Botany, Cell and Microbial Biology, Ecology, and Zoology. Successful applicants will be nominated for University Research Fellowships awarded in open competition by the **Natural Sciences and Engineering Research Council**, Canada. Appointment will be at the assistant professor level. Remuneration will be based on the salary scale at The University of Calgary (1983: $29,741-$46,359).

Applicants must hold the Ph.D. degree and have at least two years postdoctoral experience. Only those who have a demonstrated ability to carry out productive and independent research of a high calibre will be considered. NSERC Fellowships are open only to Canadian citizens or permanent residents of Canada.

Qualified individuals should send their curriculum vitae, a brief description of their research objectives and the names of three referees before April 15, 1984 to:

THE UNIVERSITY OF CALGARY

The Chairman
Scholarship and Fellowship Review Committee
Department of Biology
The University of Calgary
Calgary, Alberta, Canada
T2N 1N4

(NW224)A

Molecular Biology

CHIEF PROJECT ENGINEER
(c dna)

The research director of a world-wide leading pharmaceutic firm, is wanting a Chief Project engineer for his GENETIC RECOMBINATION UNIT. His main mission, within a team of biologists, shall be to build up a "c DNA EUCARYOTE" bank.

This man shall be 28 years old minimum, d.Sc., agronomic engineer, pharmacist, PHD... with previous successful experience in a laboratory of molecular biology.

Application possibilities are quite wide and this biologist will quickly see his research bear fruit.

Residence in a great French university town.

Please send your application file to Jacques TIXIER, ref. 333 N.

jacques tixier s.a.
7 rue de logelbach. 75017 paris

HAVAS CONTACT

(W678)A

membre de syntec

RESEARCH TECHNICIAN
required for project investigating intestinal mucous glycoprotein structure.
Salary scale £5,151 – £6,035 p.a. Application form from Assistant Secretary, Personnel Office, University of Birmingham, PO Box 363, Birmingham B15 2TT ref. 417/c/195. (1034)A

POST Doctoral Research Associate. Available immediately to study growth factors and protein kinase. Experience with handling of protein and tissue culture desirable. Send résumés and names of 3 references to: James Tam, Box 294, Rockefeller University, 1230 York Ave, New York City 10021. Equal Opportunity/Affirmation Action.
(NW183)A

UNIVERSITY COLLEGE DUBLIN
CHEMISTRY DEPARTMENT
NMR SPECTROSCOPY CENTRE

Applications are invited for a post carrying responsibility for the above unit. This centre will be an essential part of the Chemical Services Unit established by the Chemistry Department, University College Dublin with the aim of assisting Irish industry, especially with R&D. Instrumentation will comprise a JEOL GX270 SCM 90Q multinuclear spectrometer linked to a data system.

The person appointed will be responsible for the running of the centre including analysis of spectra and should have wide experience in NMR spectroscopy. Salary will be in the range: Ir£10,075 × 8 – Ir£12,675.

Applications giving a brief curriculum vitae and the names and addresses of two referees should be sent, as soon as possible, to Professor D. A. Brown, Department of Chemistry, University College, Belfield, Dublin 4, Ireland. (1003)A

MEMBRANE TRANSPORT POSTDOCTORAL AND RESEARCH ASSISTANT

positions are available immediately for individuals interested in pursuing studies on the structure and mechanism of the ion-translocating ATPases. We have recently demonstrated that the electrogenic proton-translocating ATPase in the plasma membrane of the eukaryotic microorganism, Neurospora operates via a mechanism that is quite similar to that of the Na^+/K^+ — and Ca^{2+} — translocating ATPases of animal cell origin.

Research will involve investigation of the biochemistry of this ATPase with the goal of elucidating the molecular mechanism of ion-translocating ATPases in general. Salaries are commensurate with experience.

Send curriculum vitae and names of three references to Dr Gene A Scarborough, Department of Pharmacology, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27514. Equal Opportunity Employer.
(NW228)A

UNIVERSITY OF TORONTO PLANT BIOLOGY

A limited term appointment (2 years with possible extension) will be made, effective July 1, 1984, at the Assistant Professor level. Applications are invited from botanists with a completed PhD and research expertise in any of: Cell Biology; Taxonomy and Evolution; Biochemistry.

The successful candidate will be required to pursue research and to teach in the undergraduate programme. Canadian immigration regulations require the University to assess applications from Canadian citizens and permanent residents of Canada before considering foreign applicants.

Applications should consist of a Curriculum Vitae, the names and addresses of three referees, and copies of graduate transcripts, submitted before March 31, 1984, to: Dr Joan Grusec, Chairman, Life Sciences Division, Scarborough Campus, University of Toronto, 1265 Military Trail, Scarborough, Ontario, Canada, M1C 1A4.
(NW222)A

Scientist
Division of Viral Products
Influenza Research

We have a position for a Post Doctoral Scientist to join a small group working on the molecular and biological aspects of antigenic variation in the influenza virus.

Candidates should have previous experience in molecular genetics of viruses and it would be advantageous to have an interest in viral immunology.

Salary is on a scale of £8,816 to £11,896 inclusive, dependent on experience, qualifications and age. The post is supported by external funds and is available initially for 3 years.

For further details please contact the Personnel Officer, NIBSC, Holly Hill, Hampstead, London NW3 6RB, tel 01-435 2232.
Informal enquiries may be made to Dr G.C. Schild. Please quote reference no. SU/VP/274.
(1049)A

Closing date for applications will be 10th February 1984.

NIBSC
National Institute for Biological Standards and Control

BIOTECHNOLOGISTS!

Are you reading Bio/Technology, the International Journal for Industrial Biology?

For a free sample copy of this new monthly Journal from the Publishers of *Nature*, please write or phone:

Mirta Sota
Bio/Technology
15 East 26th Street
New York 10010
USA
Tel: (212) 689 5900

Felicity Parker
Bio/Technology
Houndsmill Industrial Estate
Brunel Road, Basingstoke
Hampshire, UK
Tel.: (0256) 29242

HERIOT-WATT UNIVERSITY
DEPARTMENT OF PHYSICS

RESEARCH ASSOCIATES — SEMICONDUCTOR OPTICAL BISTABILITY

The bistability research team, under the direction of Professor S D Smith FRS, presently numbers fifteen scientists. Research objectives include studies of the nonlinearities that are the physical origin of semiconductor optical bistability, assessment of new materials for bistability in the infrared, near-infrared and visible spectral regions, investigations of bistable characteristics, and the development of novel devices. Achievement of the optically bistable switch has caused speculation of device applications ranging from fibre communication link repeater elements to optical parallel processing and optical signal computing. The group has a number of collaborative projects, both with Industry (through the Joint Optoelectronic Research Scheme) and with Universities throughout Europe (through the European Joint Optical Bistability Project).

Applications are invited from candidates with PhD experience in semiconductors/optical properties, optoelectronic devices, laser physics, or theory of optical properties of semiconductors.

Salaries will be in the range £7,190 to £11,615, according to age and experience.

Informal enquiries may be made to Dr B. S. Wherrett, Department of Physics — Tel: 031-449 5111 ext 2223.

Application forms are available from the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX (please quote ref. no. 3/84). (1043)A

MRC CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD
HARROW
MIDDX. HA1 3UJ
MEDICAL RESEARCH COUNCIL

HEAD OF DIVISION OF COMMUNICABLE DISEASES

Applications are invited for the post of HEAD OF THE DIVISION OF COMMUNICABLE DISEASES at the Medical Research Council's Clinical Research Centre in association with Northwick Park Hospital.

Preference will be given to medically qualified applicants for whom an honorary consultant contract will be arranged with the North West Thames Regional Health Authority. Current activities of the division include virology, hospital infection and applied immunochemistry. There are close links with the newly-formed division of sexually transmitted diseases (Dr. D. Taylor-Robinson) and with the MRC common cold unit: Salisbury (Dr. D. A. J. Tyrrell CBE FRS).

The appointee will have overall responsibility for the research direction of the division and will be expected to carry out a personal research programme. The clinical research centre may be visited by direct appointment and prospective candidates should discuss details of the post with the director (Sir Christopher Booth).

Further particulars can be obtained from the Director's Office, Clinical Research Centre, Watford Road, Harrow, HA1 3UJ. Telephone 01-864 5311 ext 2220. (1013)A

MEDICAL RESEARCH COUNCIL
MOLECULAR HAEMATOLOGY UNIT
John Radcliffe Hospital
Headington, Oxford
NUFFIELD DEPARTMENT OF CLINICAL MEDICINE

MEDICAL LABORATORY SCIENTIFIC OFFICER

A medical laboratory scientific officer is required to assist with a project concerned with the isolation and characterization of mammalian haemoglobin genes. Knowledge and experience of current recombinant DNA techniques for analysis of gene structure would be an advantage but is not essential.

Minimum qualifications: HNC in medical laboratory sciences or degree in related subjects.

Salary on the Whitley Council scale: £5,491 — £7,747 per annum: Salary on appointment according to age and experience.

Application form and job description from: Departmental Administrator, Nuffield Dept of Clinical Medicine, Room 5801 level 5, John Radcliffe Hospital, Headington, Oxford OX3 9DU.

Closing date: 17th February 1984. (1023)A

UNIVERSITY OF LONDON

CHAIR OF VETERINARY PATHOLOGY AT THE ROYAL VETERINARY COLLEGE

The Senate invite applications for the above Chair which became vacant on the retirement of Professor Ernest Cotchin.

Candidates must possess a registrable veterinary qualification and a PhD or other higher degree. They must have a good general knowledge and understanding of morbid anatomy in the broad sense and should have experience in teaching. They should also have a specialised knowledge and active research interest in one or more aspects of the subject.

Salary within the professorial range. The current minimum is £18,461 per annum including London Allowance.

Intending applicants should obtain further particulars from the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, before submitting applications (10 copies).

Closing date for applications: 28 March 1983. (996)A

UNIVERSITY OF EDINBURGH

DEPARTMENTS OF SURGERY AND CLINICAL CHEMISTRY

RESEARCH FELLOW 1A

Required for a three-year appointment to join a team engaged in the development of new assay methods for peptide hormones in serum. The person appointed would be required to supervise and participate in the laboratory's development and clinical evaluation of assays. Applicants should have a higher degree, and experience of immunoassay methods and their application in clinical endocrinology.

Salary on the scale £7,190 — £8,975 according to age and experience, with superannuation.

Further particulars are available from the Secretary's Office, University of Edinburgh, Old College, South Bridge, Edinburgh.

Applications (4 copies) including a cv and names and addresses of two referees should be returned to the Secretary's Office not later than 7th February 1984.

Please quote reference No 5144. (1014)A

KENNEDY INSTITUTE OF RHEUMATOLOGY

Applications are invited for 2 posts in the Division of Biochemistry as part of a group investigating transfer of lysosomal enzymes between cells.

A POST-GRADUATE RESEARCH ASSISTANT

is required for a 3 year project funded by MRC to purify lysosomal enzymes and to measure their uptake into a range of cells. Experience in cell culture and enzyme purification procedures is desirable. Salary in the range IB, (£6,310 — £8,530 + £1,186 LA), according to age and experience.

A POST-DOCTORAL RESEARCH ASSISTANT

is required for a period of 18 months to complete a project funded by Action Research. This involves production of species specific monoclonal antibodies and their use to localise transferred enzymes in recipient cells. Salary in the range IA (£7,190 — £8,975 + £1,186 LA), according to age and experience.

Applications, together with the names of 2 referees should be sent to: The General Secretary, Mathilda and Terence Kennedy Institute of Rheumatology, Bute Gardens, Hammersmith, London W6 7DW. (1033)A

MEDICAL RESEARCH COUNCIL

RADIOBIOLOGY UNIT
MAMMALIAN CYTOGENETICS
CAREER SCIENTIST

Applications are invited for a career non-clinical scientific post in the Genetics Division.

The successful candidate will join a group working on the mutagenic effects of radiation and chemicals in intact mammals, and on formal mouse genetics. Applicants should have considerable experience of a range of techniques for study of meiotic and mitotic chromosomes in laboratory animals, and of the use of these techniques in studies of the induction and interpretation of numerical and structural chromosome anomalies caused by mutagenic treatment.

Salary and conditions of appointment will be according to the MRC's scales for non-clinical scientific staff. Further information may be obtained from the address below.

Applications, including a full CV, publications list, and names of two professional referees, should be sent to Personnel Officer, MRC Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD. Tel: Abingdon (0235) 834393. Closing date 17th February 1984. (1037)A

CONFERENCES and COURSES

UNIVERSITY OF SURREY
DEPARTMENT OF BIOCHEMISTRY

MSc IN TOXICOLOGY

Applications are invited for a postgraduate course leading to an MSc degree in toxicology. The course, which may be attended either full-time or part-time is organised by the Department of Biochemistry, University of Surrey, with the MRC Toxicology Unit, Carshalton; the British Industrial Biological Research Association, Carshalton and Shell Toxicology (Tunstall) Laboratory, Sittingbourne. During the course instruction is given in these collaborative laboratories as well as at other major toxicological institutions. Entrance requirements are as for a PhD and a limited number of grants from the Medical Research Council and the Inveresk Research Foundation are available for suitable candidates. On the successful completion of the course, opportunities may be available for students to register for a PhD in toxicology, and to undertake a period of research training either in one of the above named organisations or in other laboratories approved by the University as being suitable for toxicology research.

The Medical Research Council, the Royal Society and the Royal College of Pathology have identified toxicology as a priority area for research training and therefore support the encouragement of first class students from a variety of disciplines to become involved in the science of toxicology. In modern society contact with potentially toxic chemicals is increasing. Problems may arise from the use of drugs for medical purposes, from occupational exposure to industrial products or intermediates and from environmental exposure to natural or synthetic substances.

The course, which was the first of its kind in Europe, is designed to provide graduates in science, and medical and veterinary graduates, with an understanding and appreciation of the many disciplines involved in toxicology. Particular emphasis is placed on aspects related to the biochemical mechanisms of chemically induced toxicity, and the assessment of toxicological hazard in the manufacture, use and disposal of new chemical compounds, pesticides and medicines. The course provides a broad biological education and is a suitable introduction for those intending to undertake research on the interaction of chemicals with biological systems.

There are numerous interesting opportunities for scientists, educated initially in different disciplines, who have acquired additional knowledge of toxicology. These may be in industry, government regulatory bodies, or research institutes with good career prospects in both laboratory work and administration.

Further information and application forms to be returned by either 1st March, 1984, (British and EEC applicants) or 1st June 1984 (Overseas applicants), can be obtained from: Dr. R. S. Jones, Department of Biochemistry, University of Surrey, GUILDFORD. Surrey GU25XH. (840)C

INTERNATIONAL HUMAN GENETICS SUMMER COURSE

GENETICS AND MALIGNANCY
July 16-27
University of Leuven — Belgium

LECTURES and DISCUSSION in english will cover the following topics: DNA structure and function, gene regulation, post-translational modification of proteins, mutation, chromosomes, malignant growth and behaviour, RNA and DNA tumor viruses, oncogenes, plant tumors, teratoma, plasmacytoma, genetics and cytogenetics of human tumors.

LECTURERS in addition to the organizers will include: R. Berger (Paris), W. Rombouts (Leuven), Van Montagu (Gent), Van Rooy (Gent), M. Van de Putte (Leuven), A. A. Sandberg (Roswell Park, N.Y.).

The course will be suitable for predoctoral students and postdoctoral workers.

Applications should be sent to J. J. Cassiman, Center for Human Genetics, Campus Gasthuisberg, O&N, Herestraat, B-3000 Leuven, Belgium, not later than April 15, 1984. They should contain a curriculum vitae, applicants scientific background and interests and one letter of recommendation. There is no special application form. The course is limited to 50 participants. An accomodation fee of US $200 includes room and board.

The organizing Committee: J. J. Cassiman — H. Van den Berghe (Leuven) J. Leroy (Gent), E. Meulepas (Leuven). (W670)C

Royal Postgraduate Medical School
(University of London)
at the
Hammersmith Hospital

Immunology for Clinicians

21-25 May 1984

This course is intended to bring clinicians up-to-date with current concepts in Immunology. It will consist of 10 units (2 per day):

Introduction to the Immune system
Regulation of the Immune system
The major histocompatibility complex
Receptors on lymphocytes
Viruses and the Immune response
Tumour Immunity
Complement
Autoimmunity
Hypersensitivity and DTH
Parasitology

Contributors include:

Dr. B. Askonas
Professor J. R. Batchelor
Dr. P. Beverley
Dr. L. Borysiewicz
Dr. A. Cook
Ms A. Fielder
Professor J. H. Humphrey
Dr. G. Klaus
Dr. J. Lamb
Dr. E. Liew
Dr. J. Mertin
Professor N. A. Mitchison
Dr. M. Owen
Dr. M. Parkhouse
Professor D. K. Peters
Professor R. Porter
Dr. M. A. Ritter
Professor I. Roitt
Dr. C. Sharrock
Dr. E. Simpson
Dr. P. Sissons
Dr. C. Spry
Dr. M. Walport

Course Fee: £150 including catering.

Course organisers: Dr Claire Sharrock and Professor J. R. Batchelor.

Details from: School Office (SSC), Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS. Telephone: 01-743 2030 ext 351. (1001)C

BIRKBECK COLLEGE
(University of London)

BIOMEDICAL NMR SPECTROSCOPY

Short Course

This 8 week course will be given on Tuesday and Thursday evenings (6-9 pm) starting on 24 April 1984 and covering the following topics: FT NMR, biopolymer conformation, enzymes, membranes, biosynthesis, drugs, cells and biofluids, living tissues and NMR imaging.

Details and application forms from The Secretary, Department of Chemistry, Birkbeck College, Malet Street, London WC1E 7HX. Telephone (01) 580 6622 ext 223. (1031)C

UNIVERSITY OF OXFORD
DEPARTMENT OF THEORETICAL PHYSICS

POSTDOCTORAL RESEARCH ASSISTANTSHIPS IN SOLID STATE THEORY

Applications are invited for Research Assistantships in the Solid State Theory Group. These are funded by the Science and Engineering Research Council to support an ongoing research programme on a wide range of topics in Solid State Theory. These temporary appointments are for one year from 1 October 1984 in the first instance but normally extendable for a further period. The salary is on the Research Staff Scale 1A, currently £7,190 — £11,615 pa (with USS membership).

Applications, with names of two referees, to Prof R J Elliott, Department of Theoretical Physics, 1 Keble Road, Oxford, OX1 3NP, by 15 February 1984. (1018)P

Assistantships cont page 14

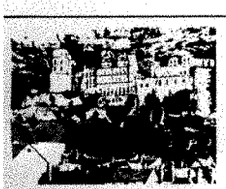
POSITIONS WANTED

APPOINTMENTS WANTED

advertise your qualities and qualifications through the most influential science weekly in the world at a special reduced cost of *25p a word.* (Personal Box Numbers £1).
Advertisements must be pre-paid and sent to: Nature Classified (Appts Wtd), 4 Little Essex Street, London WC2R 3LF. (890)B

VOUS en avez assez d'écrire vos articles en anglais? Ecrivez les en français; je vous en ferai la traduction. Highly qualified English scientist (PhD, London) presently at ENS, Paris, will undertake technical translations. Reasonable rates. References available. Box W655 c/o Nature, 4 Little Essex Street, London WC2R 3LF. (W655)B

WORKSHOP



EMBO-EMBL workshop on

Heidelberg
May 16-18, 1984

Molecular Approaches to Stem Cell Differentiation and Mouse Development

Programme

-In vitro differentiation studies (stem cell lines, teratocarcinomas, characterization of differentiation markers)
-Differentiation studies using viral model systems
-Genetics and mouse development
-Gene transfer into embryos and stem cells

Invited participants

D. Baltimore *(Cambridge, USA)*
P. Brûlet *(Paris)*
B. Cattanach *(Harwell)*
N. Copeland *(Cincinnati)*
F. Costantini *(New York)*
E. Eicher *(Bar Harbor)*
M. Evans *(Cambridge, UK)*
R. Gardner *(Oxford)*
B. Hogan *(London)*
R. Jänisch *(Hamburg)*
N. Jenkins *(Cincinnati)*
R. Kemler *(Tübingen)*
E. Lacy *(New York)*
H. Lehrach *(Heidelberg)*
E. Linney *(La Jolla)*
G. Martin *(San Francisco)*
B. Mintz *(Philadelphia)*
P. Rigby *(London)*
L. Silver *(Cold Spring Harbor)*
D. Solter *(Philadelphia)*
S. Strickland *(Stony Brook)*
S. Tilghman *(Philadelphia)*
M. Yaniv *(Paris)*

Applications

A letter of application containing research interests should be sent to the organizers before March 15th, 1984.
The total number of participants will be limited to 50.

Organizers

Erwin F. Wagner
European Molecular Biology Laboratory
EMBL
Meyerhofstraße 1
D-6900 Heidelberg, FRG

Peter Gruss
Zentrum für Molekulare Biologie
ZMBH
Im Neuenheimer Feld 364
D-6900 Heidelberg, FRG

(W679)V

Ninth International Herpesvirus Workshop, August 24-29, 1984
Seattle, Washington

Organizers: Dr Denise Galloway, Fred Hutchinson Cancer Research Center, Seattle: Dr Jack Stevens, University of California, Los Angeles

For information contact: Ms Tamara Moats, Conferences and Institutes, University of Washington DW-50, Seattle, WA 98195 (206) 543 5280.

(NW8)V

GRANTS and SCHOLARSHIPS

Mental Health Foundation
RESEARCH GRANTS

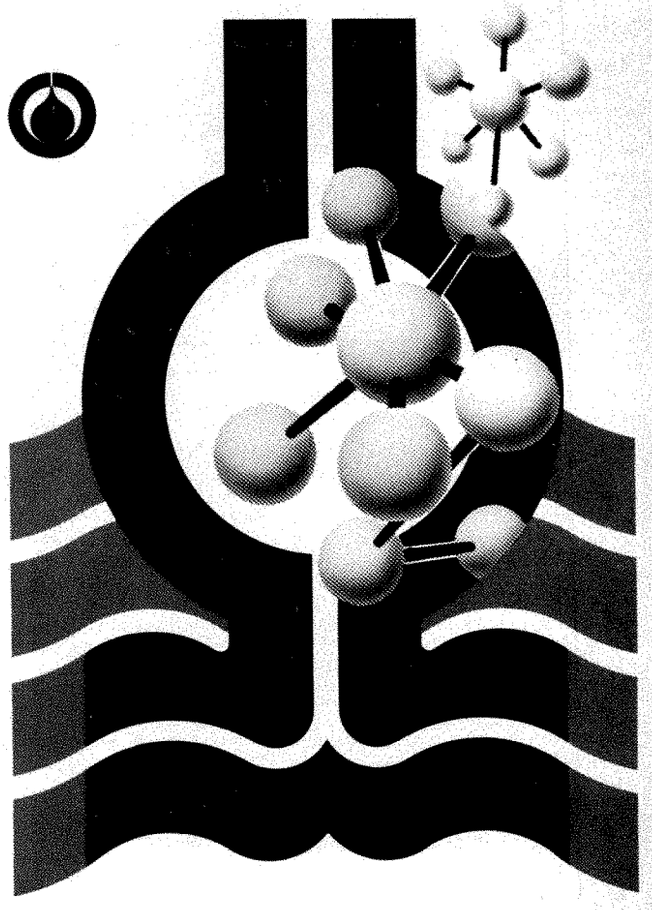
The Research Committee of the Foundation exists to foster and support research in all fields bearing on mental health, mental illness and disorders of mental development.
The Committee meets twice a year to award RESEARCH GRANTS TO PROVIDE SCIENTIFIC ASSISTANCE, EXPENSES or EQUIPMENT for research workers. Closing Dates: March 1st for Meeting in May, September 1st for Meeting in November.
For Grant application forms and further details apply in writing to: The Honorary Secretary, The Research Committee, Mental Health Foundation, 8 Hallam Street, London W1N 6DH.

(1006)H

CONGRESS

3rd Mediterranean Congress on Chemical Engineering

19 / 21 November 1984
Barcelona / Spain



INNOVATION ON DESIGN, PROCESSES AND TECHNOLOGIES
"C_1" Chemistry
Renewable resources
New technologies on classical processes
Fine chemistry
Catalysis and catalytic reactors

INFORMATIZATION
Simulation and Processes Control
Mechanization
Microprocessors

BIOTECHNOLOGY
Biochemistry engineering
Biomass resources
Waste treatment

OPTIMIZATION
Auditory and energetic savings
Processes and equipments

Correspondance: All correspondance should be addressed to:
3rd Mediterranean Congress on Chemical Engineering Expoquimia-84
Avda. Reina M.ª Cristina - Barcelona-4 - Tel. (93) 223 31 01
Telex 50458 FOIMB-E

Feria de Barcelona

(W598)Q

FELLOWSHIPS

EMBL

European Molecular Biology Laboratory

PREDOCTORAL FELLOWSHIPS

The European Molecular Biology Laboratory is to award a limited number (6-8) of predoctoral fellowships for graduates wishing to study for a PhD at EMBL Heidelberg or its outstations at DESY, Hamburg or ILL, Grenoble. The areas of research in Heidelberg include membrane biology, cellular differentiation, analysis of the structure of membrane proteins and protein nucleic acid complexes and the structure and regulation of genes in procaryotes and eucaryotes. At the EMBL outstations the research centres on the elucidation of biological structures using either synchrotron radiation (Hamburg) or neutron beams (Grenoble).

To be eligible, candidates must hold, or anticipate receiving in 1984, a first or upper second class honours degree, a masters degree or a diplom in biological sciences, physical sciences or mathematics. Preference will be given to candidates from the member states of EMBL and EMBC namely: Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom.

Short listed candidates will be asked to visit EMBL (travel expenses reimbursed) for an interview. Those awarded predoctoral fellowships will be expected to begin working at EMBL in September 1984. The normal duration of the fellowships will be 3 years subject to annual review.

The closing date for completed applications is February 1st 1984. Successful candidates will be notified by the end of April 1984.

Application forms and a catalogue giving further information about the research at EMBL and the groups participating in the Laboratory's predoctoral programme is available on request from Dr J. Tooze, Predoctoral Programme, EMBL, Postfach 10.2209, 6900 Heidelberg, F.R. Germany. (W511)E

DUBLIN INSTITUTE FOR ADVANCED STUDIES
SCHOOL OF THEORETICAL PHYSICS
Applications are invited for

RESEARCH SCHOLARSHIPS

Scholarships are awarded initially for a period not exceeding one year and will be renewed, subject to satisfactory performance, for a further year. The Governing Board may, for special reasons, extend the tenure of the Scholarship up to an inclusive maximum of three years. Commencing date will be 1 October 1984, and a limited number of stipends will be available. These awards are normally made at post-doctoral level.

For further particulars and Forms of Application candidates should apply immediately to The Registrar, Dublin Institute for Advanced Studies, 10 Burlington Road, Dublin 4, Ireland. Completed applications should reach the Registrar not later than 19 March 1984. (1026)E

E M B O

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1984 AWARDS

Next deadline: February 16, 1984

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is February 16, 1984. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on April 27, 1984.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany. (W531)E

THE WEIZMANN INSTITUTE OF SCIENCE REHOVOT, ISRAEL

Offers Postdoctoral Fellowships in the Life Sciences (Biology, Biochemistry, Biophysics), Chemistry (Physical, Organic, Geological and Biological), Mathematical Sciences, Physics and Science Teaching.

The fellowship provides a 12-month stipend (which is adjusted to the cost-of-living index), a relocation allowance and one-way air-fare. Fellowships which are renewed for a second year provide round-trip airfare.

Application forms and additional information may be obtained from the FEINBERG GRADUATE SCHOOL, of the WEIZMANN INSTITUTE OF SCIENCE, Rehovot 76-100, Israel.

The applications should reach the above address not later than May 15, 1984 for fellowships starting in September 1984 and not later than November 15, 1984 for fellowships starting in April 1985. (W671)E

THE ROYAL SOCIETY OF EDINBURGH RESEARCH FELLOWSHIPS

Applications are invited for post-doctoral Fellowships, tenable from 1 October 1984, at Scottish Universities, Colleges and Central Institutions.

Further details and application forms which should be completed and returned by 15 March 1984, may be obtained from the Executive Secretary, The Royal Society of Edinburgh, 22/24 George Street, Edinburgh EH2 2PQ. (1032)E

PATERSON LABORATORIES CHRISTIE HOSPITAL & HOLT RADIUM INSTITUTE MANCHESTER

DEPARTMENT OF MOLECULAR BIOLOGY

Two Cancer Research Campaign

POSTDOCTORAL RESEARCH FELLOWSHIPS

tenable for three years from 1 April 1984 are available in the new Department of Molecular Biology. The fellows will join a group investigating the molecular biology of Epstein-Barr virus with particular emphasis on the state and expression of the viral genome in EBV-associated tumour cells. Applicants should have a background in a relevant biological science and possess a keen interest in human tumour virology.

Requests for further details and applications, including the names and addresses of two referees should be sent as soon as possible to: Dr J R Arrand, Department of Molecular Biology, 2nd floor, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Withington, Manchester M20 9BX, Lancs. (814)E

1984 BEIT MEDICAL FELLOWSHIP

Applications are invited for a special BEIT Medical Fellowship to conduct research which will be of potential benefit to the inhabitants of Zimbabwe, Malawi or Zambia. The appointment will be for one year held jointly between an established unit in the United Kingdom and an appropriate agency in one of the three countries where the Fellow will work. Selection will made by the Advisory Board of the BEIT Trust on the basis of the joint applications. The salary will be in the range of a Clinical/Non-Clinical Lecturer, according to the age, previous experience and qualifications of the applicant. There will be the usual university superannuation contributions and benefits. In conformity with the conditions under which the Fellowships were first established, every Fellow shall be a man or woman of any nationality whatsoever who at the date of election shall have taken a degree in any faculty in any University approved by the Trustees in the UK or in any country which is or has been since 1910 a Dominion, Protectorate or Mandated Territory of the Crown. Elections to this Fellowship will rarely be made above the age of 35 years. Applications from candidates must be received not later than 16 march 1984. Elections will take place in May 1984 and the successful applicant will begin work on 1 October 1984. Candidates must supply supporting statements for the programme from the Heads of the two Departments.

Forms of application and all information may be obtained from Miss D. Billington, Administrative Secretary, BEIT Medical Fellowships, Histopathology Dept., St Bartholomew's Hospital, London EC1A 7BE. (1044)E

FELLOWSHIPS

THE QUEEN'S UNIVERSITY OF BELFAST

Department of Pure and Applied Physics

POSTDOCTORAL ELLOWSHIP IN ATOMIC AND MOLECULAR PHYSICS

his SERC Postdoctoral Research llowship is tenable from 1st June 84 until 31st May 1987. The ccessful applicant will join an isting programme concerned ith the study of collision ocesses involving state selected omic and molecular photoions ing a technique based on photo-ectron-photoion coincidence ectroscopy.

ndidates should preferably hold PhD degree and have appropriate perimental experience. Initial lary in the range £7,191 – £7,632 r annum with USS. Closing date: th February 1984.

Department of Applied Mathematics and Theoretical Physics

EARCH ASSISTANTS

osts are available, funded by NSERC to carry out theoretical search in (1) low energy attering of electrons by heavy oms and ions where relativistic fects are important, and (2) low ergy scattering of electrons by olecules. The research will make tensive use of the University's mputers including a partmental VAX 11/750 as well the NAS 7000 at the Daresbury boratory and the GRAY-1 at the iversity of London via a depart-ntal link.

plicants must have a good nours degree, and preferably a D, in Physics of Applied athematics and research perience in the development and e of large scientific computer ogram packages. A knowledge of mic and molecular processes uld be an advantage.

ary range £7,191 – £8,532, placing depending on age and rience. Closing date: 1st rch 1984.

plications including curriculum ae and the names and addresses two referees should be sent to Personnel Officer, The Queen's iversity of Belfast, Northern and BT7 1NN. (1035)E

TDOCTORAL Fellowship avail-immediately to study the role of l experience on development of ections in the visual system of nibians. Experience in electro-ological, anatomical (light and on microscope) and/or tissue e techniques is desirable but not tial. Two-year position available 1, 1984. Send curriculum vitae names of three references to: Dr n Udin; Division of Neuro-gy, Department of Physiology; University of New York; alo, NY; 14214. The State ersity of New York at Buffalo is ffirmative Action/Equal Opport-Employer. (NW230)E

Atlas Research Fellowship

St. Cross College, Oxford University

The Rutherford Appleton Laboratory of the Science and Engineering Research Council in conjunction with St. Cross College, invite applications for a joint research fellowship. The aim of the Atlas Fellowship is to encourage studies, particularly those involving computers, in an area of research supported by the Rutherford Appleton Laboratory.

Topics include the following: study of condensed matter by scattering of neutron beams; some aspect of information technology including software engineering, IKBS, use of electron beam lithography in the fabrication of semi-conductor devices, image processing, computer aided design including electrical circuit design; finite element calculations; electro-magnetic systems; theoretical studies of the atmsophere and climate; remote sounding from space; image processing related to space astrophysics data reduction.

The successful candidate will be expected to undertake research work and have ideas on the programme he/she wishes to follow. Fellows have the rights and privileges of research fellows of St. Cross College, and will be expected to work on some aspect of the programme of the Rutherford Appleton Laboratory.

There will be access to exceptionally powerful computer facilities, and our involvement with numerous university research groups gives an opportunity for contact with others engaged on a wide range of exciting research programmes.

The fellowship will be tenable for a period of three years and may be extended up to a period not exceeding five years. The stipend will depend upon the age and experience of the candidates selected, within the broad range £9,500 to £14,800.

For details of research fellows' rights at St. Cross College and application forms, write to Recruitment Office Personnel Group, Rutherford Appleton Laboratory, Chilton, Didcot, Oxon OX11 0QX, or telephone Abingdon 0235 445435 quoting reference No: VN 201.

The closing date for applications is: 10th February 1984 (1009)E

serc Rutherford Appleton Laboratory

UNIVERSITY OF ABERDEEN

DEPARTMENT OF MICROBIOLOGY

TWO POSTDOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for two postdoctoral Research Fellowships to join the group led by Professor W A Hamilton working on the role of sulphate reducing bacteria.

One post (ref Mic 1) financed by the SERC Marine Technology Directorate will be concerned with a study of corrosion processes associated with steel structures in the offshore oil industry, and will attempt to relate microbiological activities of biofilms with the electrical polarisation characterisation of naturally fouled steel surfaces.

A second post (ref Mic 2) is financed by BP Petroleum Development and is designed to strengthen basic continuous culture studies on the enzymology and physiology of growth of sulphate reducing bacteria on hydrogen.

Appointments will be from 1 March 1984 and will be for a minimum of one year at this stage.

Salary within range £7,190 to £8,530 per annum, with appropriate placing, within IA Scale for Research and Analogous Staff.

Further particulars and application forms from The Secretary. The University, Aberdeen, with whom applications (2 copies), specifying references no(s), should be lodged by 10 February 1984. (1019)E

MENTAL HEALTH FOUNDATION

FELLOWSHIPS

Applications are invited for **Mental Health Foundation Fellowships** from suitably qualified persons wishing to pursue **Full-time Research Work** bearing on problems of mental health, mental illness or mental handicap, whether in clinical psychiatry or in one of its supporting sciences.

Both Junior and Senior Fellowships are offered in the salary ranges of Junior/Senior Lecturer, Junior/Senior Registrar, according to seniority and experience, plus superannuation. However, Senior Fellowships are only awarded under exceptional circumstances, e.g., as a temporary measure prior to the Fellow taking up a permanent career post. The appointments, which are also open to candidates from overseas, will be for up to **three years** in the first instance and in exceptional circumstances may be extended to **five years.** Interviews will be held in May in London and the Fellowships will be taken up between the following July 1st and March 31st. Overseas candidates, if shortlisted, must attend for interview at their own expense. **Closing date: March 1st.**

Application forms and further information may be obtained from The Secretary, The Research Committee, Mental Health Foundation, 8 Hallam Street, London W1N 6DH. (1007)E

Fellowships cont p. 14

SEMINARS and SYMPOSIA

6th INTERNATIONAL SYMPOSIUM
on
MICROSOMES AND DRUG OXIDATIONS
5th-10th August 1984
Brighton, United Kingdom

The Symposium is organized with plenary sessions, posters and oral communications. Themes to be covered include:
1. Chemistry and physics of cytochrome P-450.
2. Cytochrome P-450 and the metabolism of endogenous substrates.
3. Molecular biological studies of cytochrome P-450.
4. Post-oxidation enzymes.
5. Cytochrome P-450; role in toxicity and carcinogenicity.
6. Drug oxidations in man.

Abstract should be submitted by February 29, 1984.

For further information and registration forms contact:
Secretariat
6th International Symposium on Microsomes and Drug Oxidations
Caroline Roney Medical Conference Organizer
100 Park Road
London NW1 4RN
United Kingdom
Telephone: 01-723 6722 (1048)M

CHROMATOGRAPHY DISCUSSION GROUP
One-day Symposium
University of Newcastle upon Tyne
28th March 1984
PYROLYSIS-GC/MS

Lectures to be given by W. J. Irwin, University of Aston; J. M. Hindmarch & J. Magee, Royal Hallamshire Hospital, Sheffield; B. B. Wheals, Metropolitan Police Forensic Science Laboratory; C. S. Gutteridge, Lord Zuckerman Research Centre, University of Reading; J. M. Bracewell, Macaulay Institute of Soil Research, Aberdeen; S. T. Jones, BP Research Centre, Sunbury; W. J. Criddle, University of Wales Institute of Science and Technology.

Information from the Executive Secretary, CDG, Trent Polytechnic, Nottingham NG1 4BU. Tel: 0602-418248. (1024)M

FELLOWSHIPS

UNIVERSITY OF YORK
DEPARTMENT OF BIOLOGY
POSTDOCTORAL RESEARCH FELLOWSHIP

A self-motivated immunologist is required to join a well-equipped group whose prime interest is the pathogenesis of relapse in chronic relapsing experimental allergic encephalomyelitis. The position, funded by the Wellcome Trust, is available for three years and would be concerned particularly with the role of soluble factors derived from immune cells in the CNS and the CSF. Applicants must be under the age of 32 and have worked continuously for the last three years in a university or hospital in the UK.

Starting salary: £7,630, with USS, within Range IA of the salary scales for Research and Analogous staff. The starting date is flexible.

Two copies of applications, with curriculum vitae and naming two referees, should be sent as soon as possible to Dr A J Suckling, Department of Biology, University of York, Heslington, York YO1 5DD, to whom informal enquiries can be made. (Tel: 0904 59861 ext 5839). Please quote reference number NA/6188. (1038)E

STUDENTSHIPS

KING'S COLLEGE LONDON

RESEARCH STUDENTSHIPS

Applications are invited now for research studentships leading to the PhD degree in Biophysics for the year beginning in October 1984. The Biophysics Laboratories, comprising the King's College Department of Biophysics and the MRC Cell Biophysics Unit, offer five main areas of research: Macromolecular studies of contractile systems; Cellular and developmental neurobiology; DNA and molecular genetics; Cell behaviour and motility; Crystallographic and computer graphics studies of biological molecules.

Applicants need a good honours degree, most likely in biochemistry, biophysics, biological sciences or molecular biology, chemistry or physics. A full list of research topics and supervisors can be obtained from Mr D M Drummie, King's College London, 26-29 Drury Lane, London WC2B 5RL, or by telephoning 01-836 8851 and asking for Mrs Crick. (971)F

STUDENTSHIPS

FOREST RESEARCH STATION PATHOLOGY BRANCH RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship (for postgraduate study to PhD) on the genetics of pathogenicity and population structure in Nectria cinnabarina (Ascomycetes). Financial support (equivalent to NERC rates) is available immediately. Applicants should hold a first or upper second class honours degree in an appropriate biological subject. Preference may be given to applicants with a strong background in microbiology/mycology, plant pathology and genetics.

Applications, including a curriculum vitae citing details of University course work and names and addresses of two academic referees, should be sent to Dr C M Brasier, Pathology Branch, Forestry Commission Research Station, Farnham, Surrey GU10 4LH by 6th/13th February 1984. (1036)F

UNIVERSITY OF ABERDEEN MEDICAL ENDOWMENTS RESEARCH STUDENTSHIP

Applications are invited for a PhD Studentship for research in one of a wide range of biomedical subjects in Departments of the Faculty of Medicine. The value of the studentship is similar to those of the research councils.

Application forms and further details are obtainable from Mr A B Buckley, Clerk to the Faculty of Medicine, University Office, Regent Walk, Old Aberdeen AB9 1FX. Closing date for receipt of applications: 21 February 1984. (1042)F

UNIVERSITY OF SOUTHAMPT
LYMPHOMA RESEA
UNIT

Lymphoma Research Unit, Laboratory, General H Southampton SO9 4XY. T studentships will become ava 1 October 1984. The fields are (1) the interaction antibody and tumour cells largely at the molecular level the therapeutic application i models of human lymph phenotypic studies of lymphoma cells, including of immunoglobulin synth markers detected by mo antibodies.

Remuneration and conditions as for Research grants for PhD students.

Enquiries to Profes Stevenson or Dr J L Smi above address. (

EAST MALLIN
RESEARCH STAT
CROP PRODUCTIO
PLANT SCIENCE DI
PhD RESEARC
STUDENTSHI

Applications are invite Forestry Commission studentship, from graduat First or Upper Second Class Degree in Botany, Forestry subject.

The three year project w an investigation of the an hormonal and other phy factors important in epicc emergence in oak.

Application form an details from the Deputy East Malling Research Maidstone, Kent ME19 6B

Closing date for ap 2/2/84.

ASSISTANTSHIPS

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHARMACY

POSTDOCTORAL RESEARCH ASSISTANTSHIP IN MICROBIOLOGICAL CHEMISTRY

Applications are invited from suitably qualified chemists or biochemists to investigate the biological chemistry associated with the formation of the carbapenem antibiotics and related compounds. The post is tenable for two years with a salary in the IA range (£7,190 — £8,530) plus superannuation.

Informal enquiries to Professor B W Bycroft, telephone Nottingham 56101 ext 3218.

Applications including the names and addresses to two referees should be sent to the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref: No 912. (1000)P

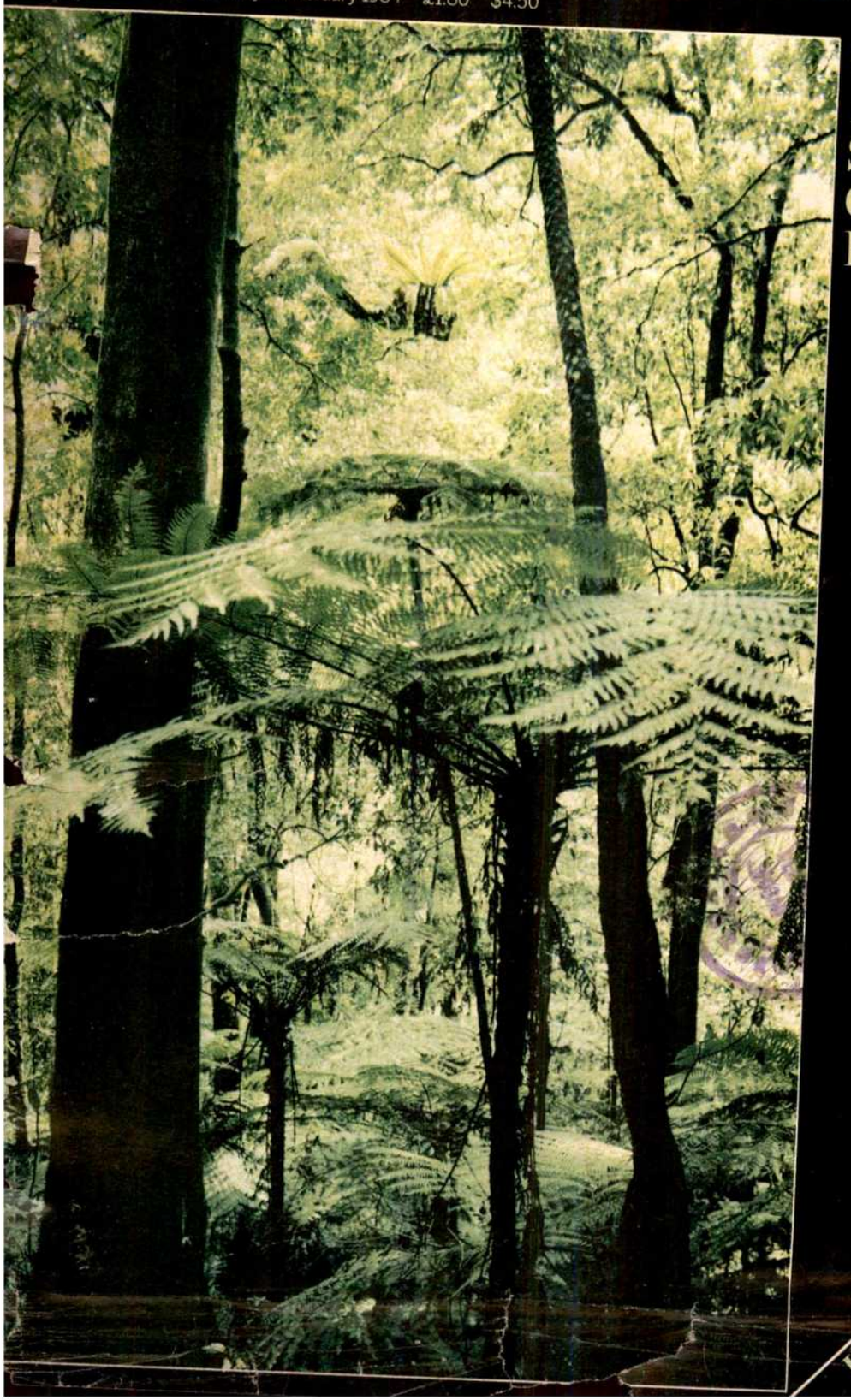
UNIVERSITY OF K
POSTDOCTORA
RESEARCH
ASSISTANTSHI
ASTROPHYSI

Applications are invite SERC-supported post research assistantship physics in the Department The successful applic collaborate with Dr A Ev investigation of grain form destruction in nova win particular their dependenc chemistry and environm appointment, which is point 3 of the RAIA scal available for up to three commences on 1st January soon as possible thereafte

Applicants should subr of application, together wi curriculum vitae and the two referees, who should write directly to Dr Ev address below. Applica references should undersigned by Friday 3rd 1984. Dr A Evans, Dep Physics, University of Ke Staffordshire, ST5 5BG.

Typesetting: J.W. Arrowsmith Ltd, Bristol and Outline Graphics Ltd, London EC4Y 0HP. Printing: Kingsdale Press Ltd, Reading, Berks, in association with Godfrey Lang Ltd, London W and The William Byrd Press Inc., Richmond, Virginia, USA Published by MACMILLAN JOURNALS LTD, 4 Little Essex Street, London WC2R 3LF. — 19 January 1984

nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Vol 307 No 5949 26 January - 1 February 1984 £1.80 $4.50



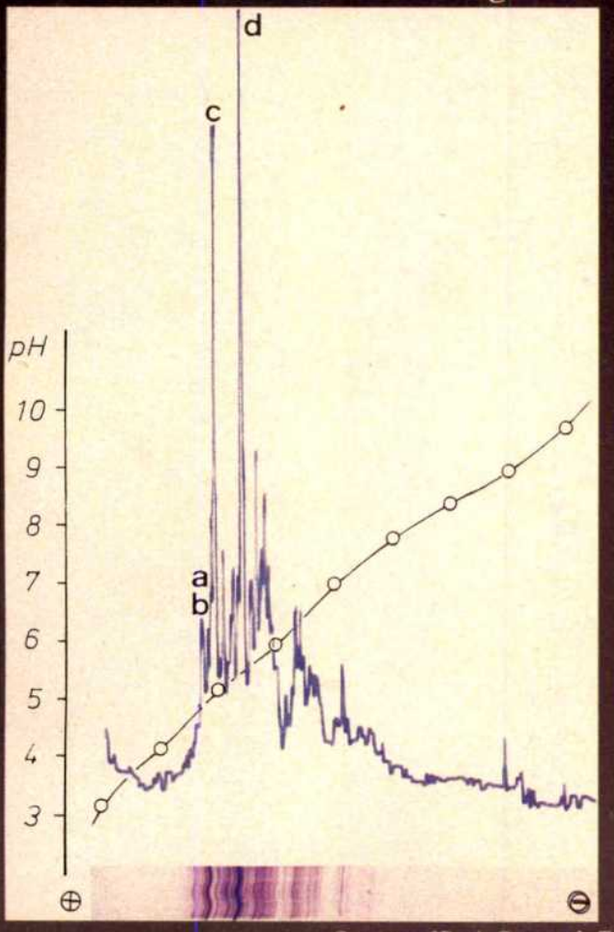
COMPLEXITY AND STABILITY OF ECOSYSTEMS

IMAGE ANALYSIS
product review

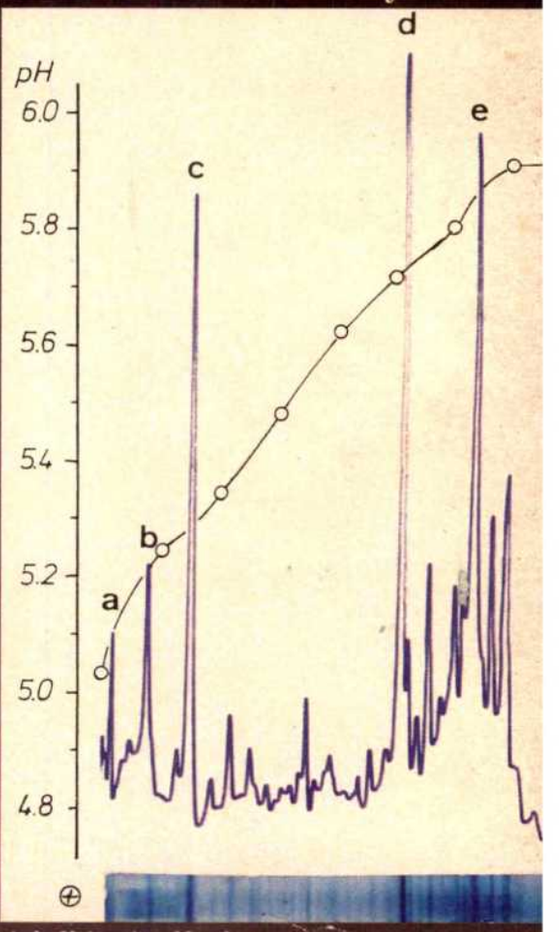
electrofocusing resolution barrier

LKB's new Immobiline System!

Traditional electrofocusing



LKB Immobiline System



Courtesy of Dr. A. Görg et al., Technische Universität, München.

LKB's revolutionary Immobiline System smashes the resolution barrier in electrofocusing by replacing conventional carrier ampholytes with immobilized pH gradients.

Since gradient drift and conductivity gaps are eliminated, you can perform electrofocusing in pH gradients as narrow as 0.1 pH units, to obtain resolution previously considered impossible. Perfectly straight bands of focused proteins are obtained, even with high salt loads.

You can load 5—10 times more sample per run, for trace component analysis, or up to 100 mg protein on a 4 mm-thick Immobiline gel for extremely high resolution purification; there are no carrier ampholytes to remove afterwards.

Now, you can analyse peptides without using special staining techniques, and you can zoom-in on the fine detail of a 2-D map.

You can prepare and run Immobiline gels with your present Multiphor or Ultrophor system and the Multiphor Gradient Gel Kit. Discover for yourself the full capabilities of the Immobiline System by ordering your starter kit today!

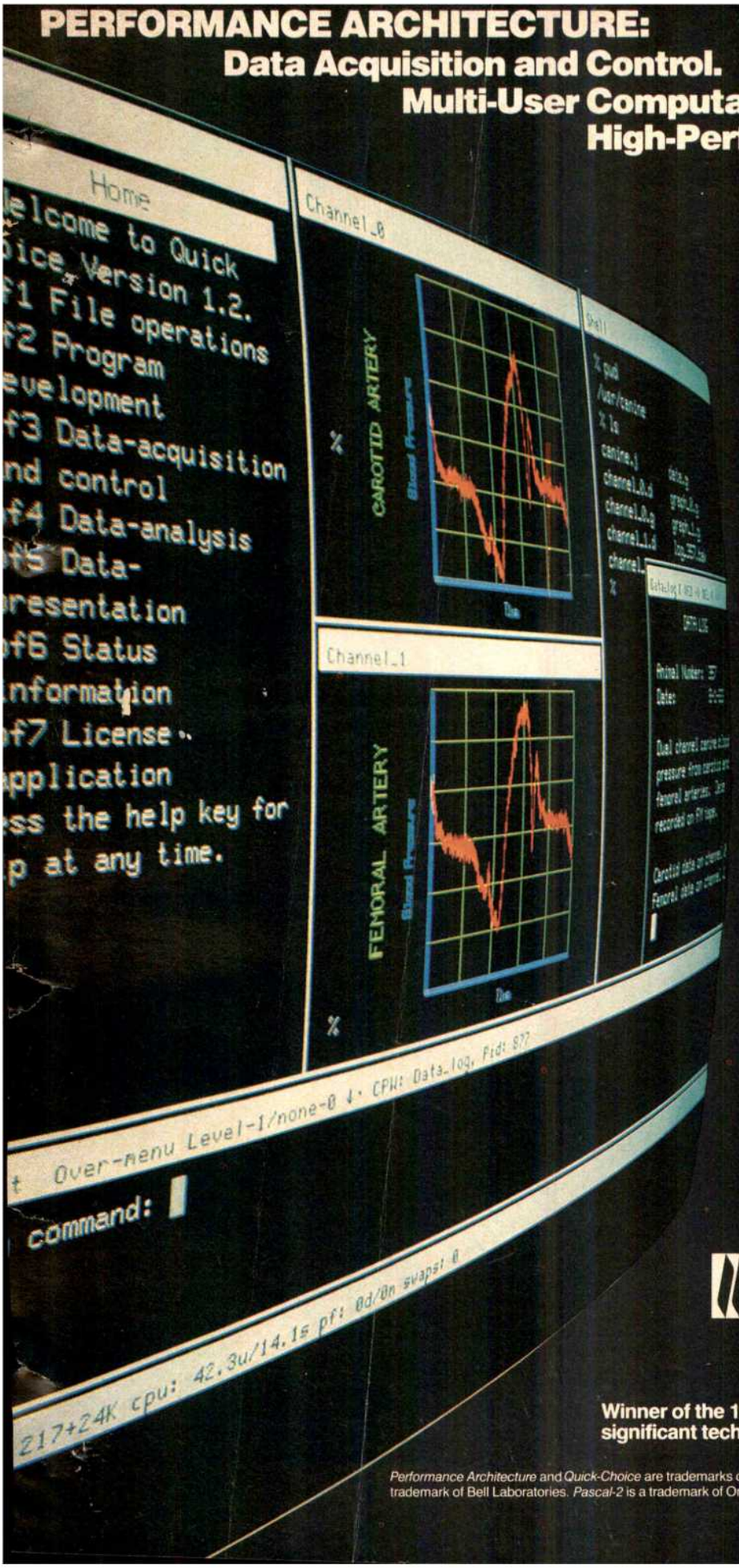


LKB
BROMMA

Circle No.40 on Reader Service Card.

Head office: LKB-Produkter AB, Box 305, S-161 26 Bromma, Sweden. Tel. 08-98 00 40, telex 10492
Main US sales office: LKB Instruments, Inc. 9319 Gaither Road, Gaithersburg, Maryland 20877. Tel. 301-963-3200, telex 909870 (dom.), 64634 (int
UK sales office: LKB Instruments Ltd., 232 Addington Road, S. Croydon, Surrey CR2 8YD, England. Tel. 01-657 8822, telex 264414
Other sales offices in: Athens (for Middle East), Co[illegible] Ghent, Zoetermeer, Hong Kong, Luzern, Munich, Paris, Rome, Turku, Vienna

PERFORMANCE ARCHITECTURE:
Data Acquisition and Control.
Multi-User Computation.
High-Performance Graphics.
All Simultaneously.



Masscomp's unique triple-bus Performance Architecture™ delivers unequalled system price/performance. With its dual-processor CPU and up to 7 megabytes ECC memory, the Masscomp MC-500 offers scientists and engineers outstanding computation power. With the addition of Masscomp-designed floating-point and array processors, throughput is even further enhanced.

The MC-500 runs a virtual memory, real-time UNIX™ operating system with Ethernet™ support. Supported languages include C, FORTRAN 77, and Pascal-2.™ The Quick-Choice™ multiple-window user interface offers menu-driven access to system functions.

Key system capabilities include:

DATA ACQUISITION AND CONTROL
- Bit-slice Data Acquisition and Control Processor
- One million 12-bit analog samples/second
- One microsecond external-even response

MULTI-USER COMPUTATION
- 32-bit CPU with 4K byte cache
- 16 megabyte virtual address space
- 7 millisecond 1024-point comple FFT

HIGH-PERFORMANCE GRAPHICS
- Independent 32-bit graphics processors
- 1024 x 800 x 2 x 1 pixel mono-chrome graphics
- 832 x 600 x 2 x 10 pixel color graphics

For more information on how the MC-500 is delivering results in industrial, university, government, and medical applications, call 1-800-451-1824.

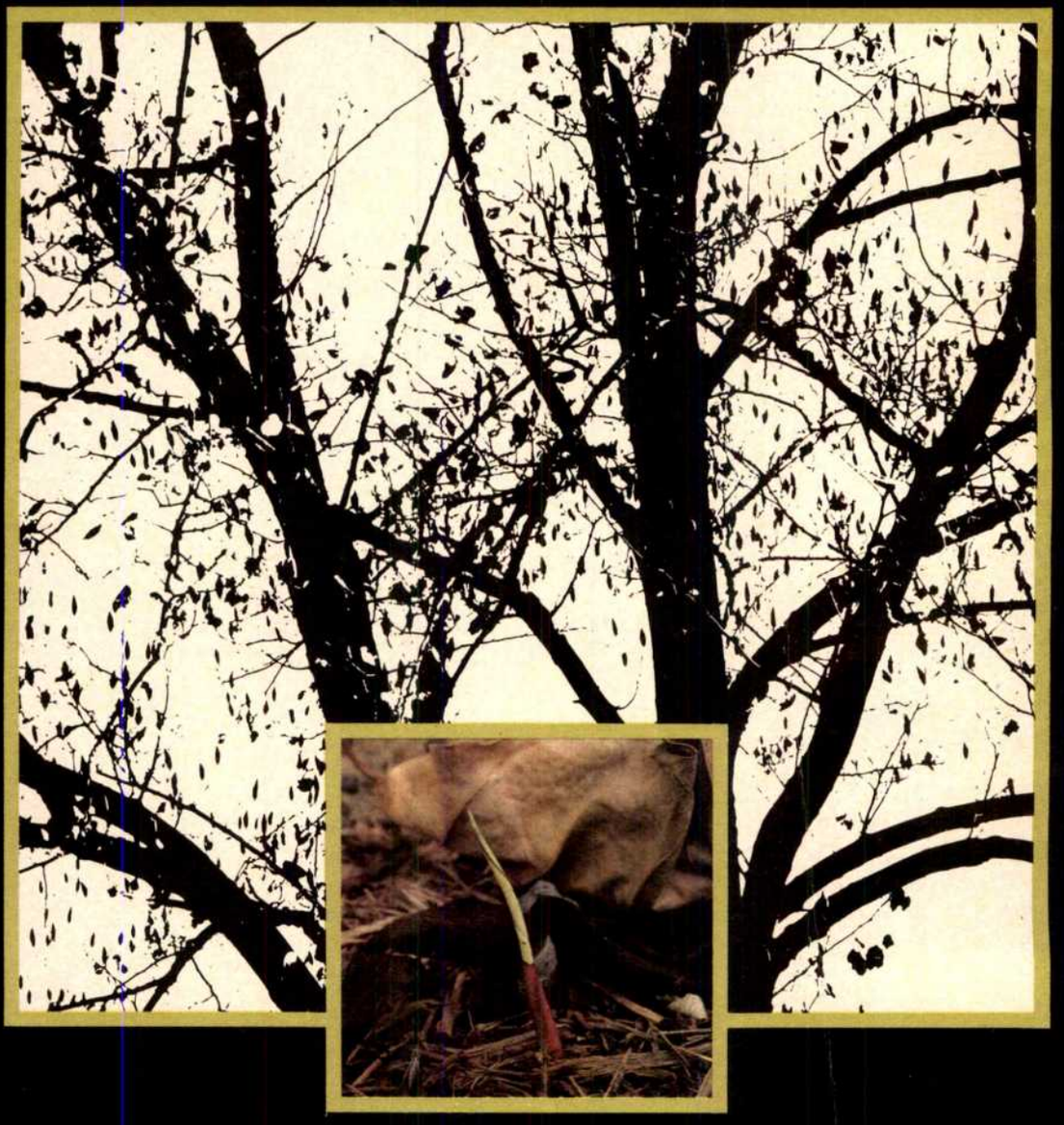
MASSCOMP
One Technology Park
Westford, Massachusetts 01886
TWX: ESL 196520
Telex: 704353
Cable: *MASSCOMP*

Winner of the 1983 I-R 100 Award as one of the most significant technical products of the year.

Performance Architecture and *Quick-Choice* are trademarks of the Massachusetts Computer Corp. *UNIX* is a trademark of Bell Laboratories. *Pascal-2* is a trademark of Oregon Software. *Ethernet* is a trademark of the Xerox Corp

Circle No.07 on Reader Service Car

More Than a Merger . . .



an Emerging Technology

A powerful technology is emerging. Its seeds are in olecular and cell biology, microbiology, biochemistry and mmunology. Rooted in the experience and knowledge of ese many disciplines, this new technology embraces adnces in every area of life science research.

Today, in the search for a fuller understanding of the echanisms of life, researchers are using tools and methods veloped in many areas of science, lessons learned and oven through years - sometimes decades - of experience. hese time-tested lessons form the core of an evolving chnology that will live through many generations, proding answers that otherwise would have been out of reach.

As this integrated technology takes shape, a new company, lidly based in experience, is emerging to serve it:

Life Technologies, Inc. At the company's GIBCO/BRL Division, we bring to the life scientist years of expertise in our respective fields, GIBCO in cell and tissue culture and microbiology, BRL in molecular biology. Together, we provide the firm, fertile ground on which science can grow: the most comprehensive selection of life science research products available.

To learn more about GIBCO/BRL and how we are helping a powerful new technology grow, ask for a copy of the booklet, "GIBCO/BRL: Products for an emerging technology."

GIBCO/BRL

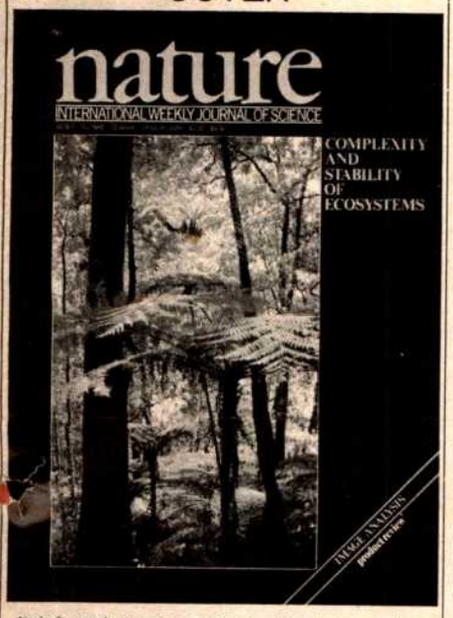
GIBCO/BRL is a division of LIFE TECHNOLOGIES IN

Circle No. 21 on Reader Service Card

nature

NATURE VOL. 307 26 JANUARY 1984

COVER



Rainforest in Lamington National Park, Queensland, Australia. The relationship between complexity and stability in this and other ecosystems is the subject of the review article on page 321.

OPINION

European research collaboration 303
Breaking all the rules 304
West German reform

NEWS

US nuclear power 305
West German universities
Antisatellite weapons 306
Human rights
NIH curbs drive out researcher
UK nuclear power 307
Indian universities
Halley's comet 308
European pollution
Afforestation 309
UK research 310
Information technology
East German chemicals 311
US–Soviet space research
UK observatories reprieved

CORRESPONDENCE

The merits of Indian science/Fetus not 'previable'/Making-up words/etc. 312

NEWS AND VIEWS

Dispute over scale of Universe
John Maddox 313
Clues to recent human evolution from specialized technologies?
Marek Zvelebil 314
Towards the optical computer
S D Smith 315
Mechanism of T-cell tolerance
Roger B Taylor 317
Latest view of Mt St Helens 317
Genetic noise in evolution?
Darryl Reanney 318
Plants under strain
Clive Lloyd 319
IRAS circular 6 320

BOOK REVIEWS

Consciousness Regained *by N Humphrey*
Stuart Sutherland 389
Superspace or One Thousand and One Lessons in Supersymmetry
by S J Gates Jr et al.
M J Duff 390
Letters of Edward Jenner: And Other Documents Concerning the Early History of Vaccination *G Miller ed.*
Donald R Hopkins
McGraw-Hill Encyclopedia of Physics
S P Parker ed.
R H Dalitz 391
The Left Hand of Creation: The Origin and Evolution of the Expanding Universe
by J D Barrow and J Silk
Geoffrey Bath 392
Yeasts: Characteristics and Identification
by J A Barnett et al.
D R Harper

REVIEW ARTICLE

The complexity and stability of ecosystems
S L Pimm 321

ARTICLES

The Hubble constant as derived from 21 cm linewidths
A Sandage & G A Tammann 326

A helix stop signal in the isolated S-peptide of ribonuclease A
P S Kim & R L Baldwin 329

Activation of a translocated human c-*myc* gene by an enhancer in the immunoglobulin heavy-chain locus
A C Hayday, S D Gillies, H Saito, C Wood, K Wiman, W S Hayward & S Tonegawa 334

LETTERS TO NATURE

A lower limit for the mass of the compact object in SS433 — a black hole?
E M Leibowitz, T Mazeh & H Mendelson 341

Continuing formation of the central star cluster in M87
A C Fabian, H Itoh, G C Stewart, C R Canizares & P E J Nulsen 343

Mystery cloud of AD 536
R B Stothers 344

Scattered UV solar radiation within the clouds of Venus
A P Ekonomov, V I Moroz, B E Moshkin, V I Gnedykh, Yu M Golovin & A V Crigoryev 345

Analysis for rare earth elements in accessory minerals by specimen isolated secondary ion mass spectrometry
J B Metson, G M Bancroft, H W Nesbitt & R G Jonasson 347

Evolution of the geomagnetic secular variation field from the beginning of the century
C Gire, J L Le Mouel & J Ducruix 349

Oldest reliable $^{40}Ar/^{39}Ar$ ages for terrestrial rocks: Barberton Mountain komatiites
M L Martinez, D York, C M Hall & J A Hanes 352

Dating diagenesis in a petroleum basin, a new fluid inclusion method
R S Haszeldine, I M Samson & C Cornford 354

Did the Dalradian slides originate as extensional faults?
N J Soper & R Anderton 357

The gradual decline of the dinosaurs — fact or fallacy?
D A Russell 360

Courtship feeding increases female reproductive success in bushcrickets
D T Gwynne 361

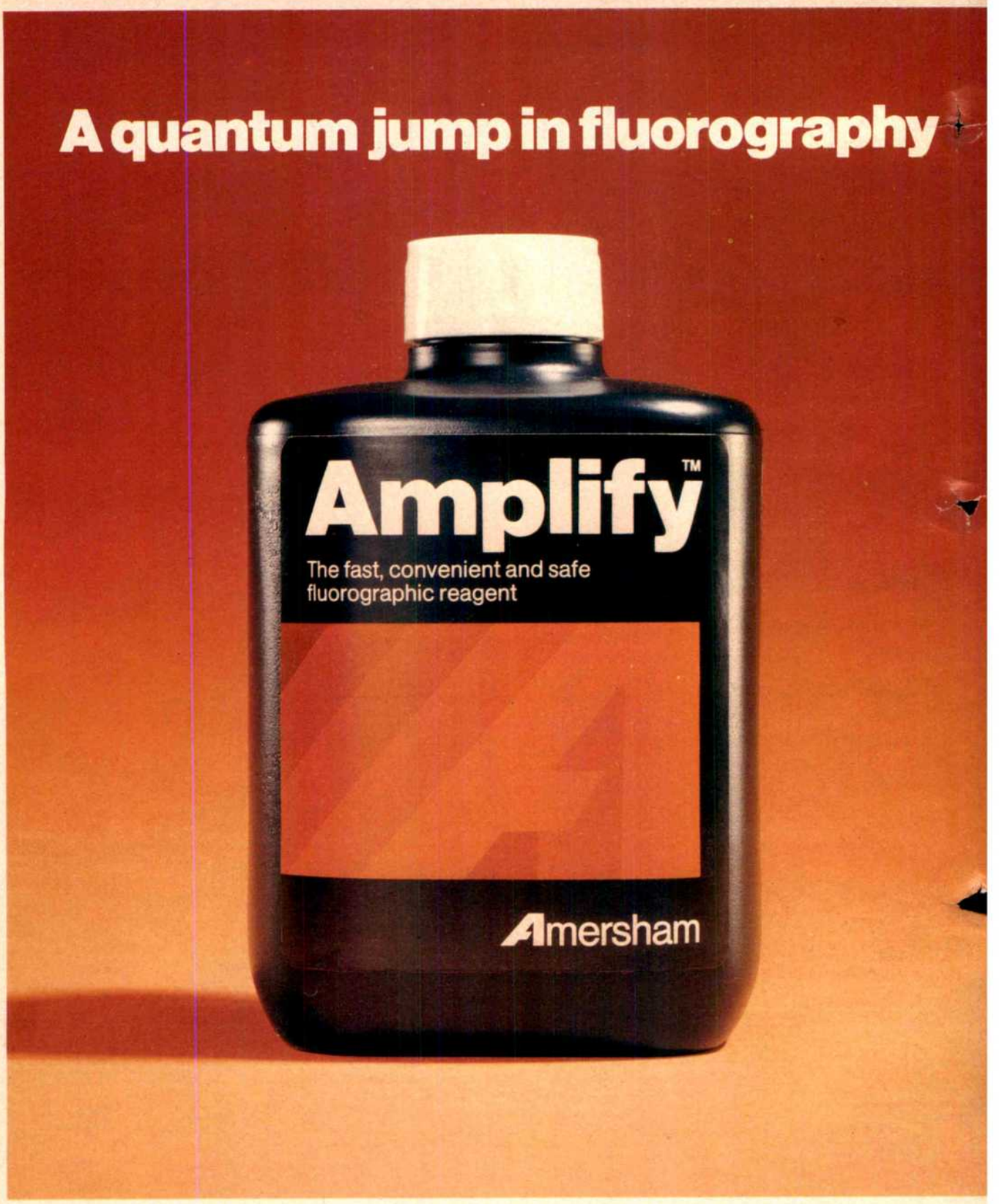
Stress-induced alignment of division plane in plant tissues grown *in vitro*
P M Lintilhac & T B Vesecky 363

Contents continued overleaf

Nature® (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Annual Index (mailed in February). Annual subscription for USA and Canada US $230 (for subscription prices elsewhere, see next page). USA and Canadian orders only to: *Nature*, Subscription Dept PO Box 1018, Manasquan, New Jersey 08736, USA. All other orders to *Nature*, Brunel Road Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY10010 and additional mailing offices. US Postmaster send form for 3579 to: *Nature*, 15 East 26 Street, New York, NY 10010. Published in Japan by Macmillan Shuppan K.K., 5th FloorEikow Building, 1-10-9 Hongo, Bunkyo-ku, Tokyo, Japan.

© 1984 Macmillan Journals Ltd.

A quantum jump in fluorography



Please contact Amersham or your local representative for further details.

Amersham International plc
Amersham England HP7 9LL
telephone Little Chalfont (024 04) 4444

Amersham

Amersham Australia PTY Limited Sydney **Amersham Belgium SA/NV** Brussels **Amersham Buchler GmbH & Co. KG** Braunschweig W Germany
Amersham Corporation Arlington Heights USA **Amersham Denmark ApS** Birkerød **Amersham France SA** Paris **Amersham Japan** Tokyo **Amersham Nederland BV** Utrecht

Circle No.50 on Reader Service Card.

CONTENTS

Keep your copies of *Nature* protected in these tough black PVC binders



- Each binder is supplied with a set of wires for the easiest possible loading
- *Nature* logo blocked in gold on spine
- Transparent plastic pocket provided for your own reference
- Each binder takes two volumes of *Nature*; three binders will serve for a year
- Prices and address for orders are set out below

Annual Subscription Prices

UK & Irish Republic		£98
USA & Canada		US$230
Australia & NZ	Airspeed	£150
Continental Europe	Airspeed	£120
India	Airspeed	£115
Japan	Airspeed	Y85000
Rest of World*	Surface	£120
Rest of World*	Airmail	£180

(not USA, Canada, Europe & Japan)

Orders (with remittance) to:

USA & Canada
Nature
Subscription Dept
PO Box 1018
Manasquan
NJ 08736
USA

UK & Rest of World
Nature
Circulation Dept
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to:
Japan Publications Trading Company Ltd
2-1 Sarugaku-cho 1-chome
Chiyoda-ku, Tokyo, Japan
Tel: 03-292-3755

Personal subscription rates
These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
15 East 26 Street
New York,
NY 10010, USA
Tel: (212) 689-5900

UK & Europe
Felicity Parker, Nature
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

Credit card orders only (in USA):
Call toll-free: (800) 824-7888 (Operator 246)
In California: (800) 852-7777 (Operator 246)

Back issues
UK, £2.50; USA & Canada, US$6.00 (surface), US$9.00 (air); Rest of World, £3.00 (surface), £4.00 (air)

Binders
Single Binders: UK, £4.50; Rest of World, $10.00
Set(s) of 3 Binders: UK, £12.00; Rest of World, $25.00

Annual indexes (1971–1982)
UK, £5.00 each; Rest of World, $10.00

Nature First Issue Facsimile
UK, 75p; Rest of World (surface), $1.50; (air) $2.00

Nature in microform
For information:
University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

Prevention of gastrulation but not neurulation by antibodies to fibronectin in amphibian embryos
J C Boucaut, T Darribère, H Boulekbache & J P Thiery **364**

Cultivation of the liver forms of *Plasmodium vivax* in human hepatocytes
D Mazier, I Landau, P Druilhe, F Miltgen, C Guguen-Guillouzo, D Baccam, J Baxter, J-P Chigot & M Gentilini **367**

Endogenous ouabain-like compound increases heart muscle contractility
Y Shimoni, M Gotsman, J Deutsch, S Kachalsky & D Lichtstein **369**

ß-Adrenergic modulation of calcium channels in frog ventricular heart cells
B P Bean, M C Nowycky & R W Tsien **371**

Reversible shape change of Triton-treated erythrocyte ghosts induced by Ca^{2+} and Mg-ATP
Y Jinbu, S Sato & M Nakao **376**

Viscoelastic properties of erythrocyte membranes in high-frequency electric fields
H Engelhardt, H Gaub & E Sackmann **378**

Effect of IFN-γ on the immune response *in vivo* and on gene expression *in vitro*
M Nakamura, T Manser, G D N Pearson, M J Daley & M L Gefter **381**

Surface dynamics of the integral membrane protein bacteriorhodopsin
M A Keniry, H S Gutowsky & E Oldfield **383**

Preferential integration of yeast transposable element Ty into a promoter region
H Eibel & P Philippsen **386**

MATTERS ARISING

Solution contact charging with respect to earthquake lights
C Pounder **389**

Carnivore social behaviour — does it need patches?
T von Schantz
Reply: D W Macdonald **389**

111-day periodicity of X-ray transient A0535 + 26
F Giovannelli
Reply: W C Priedhorsky & J Terrell **390**

MISCELLANY

100 years ago **316**
Books received **395**
Product review:
Image analysis **397**

NATURE CLASSIFIED

Professional appointments — Research posts — Studentships — Fellowships — Conferences — Courses — Seminars — Symposia: **Back pages**

Next week in *Nature*:

- Archaeology of Awash Valley (and more hominids)
- Origin of Mediterranean climate
- Incomplete histocompatibility antigen
- Action of botulinum toxin
- Genetic origins of retinoblastoma
- Thermodynamics of left–right DNA conversion
- Warming of deep Atlantic water
- Dust around Vega
- Lord Ashby reviews a recent publication on the higher education system

GUIDE TO AUTHORS

Authors should be aware of the diversity of *Nature*'s readership and should strive to be as widely understood as possible.

Review articles should be accessible to the whole readership. Most are commissioned, but unsolicited reviews are welcome (in which case prior consultation with the office is desirable).

Scientific articles are research reports whose conclusions are of general interest or which represent substantial advances of understanding. The text should not exceed 3,000 words and six displayed items (figures plus tables). The article should include an abstract of about 50 words.

Letters to *Nature* are ordinarily 1,000 words long with no more than four displayed items. The first paragraph (not exceeding 150 words) should say what the letter is about, why the study it reports was undertaken and what the conclusions are.

Matters arising are brief comments (up to 500 words) on articles and letters recently published in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted.

Manuscripts may be submitted either to London or Washington (decisions being made only in London). Manuscripts should be typed (double spacing) on one side of the paper only. Three copies are required, each accompanied by copies of lettered artwork. No title should exceed 80 characters in length. Reference lists, figure legends, etc. should be on separate sheets, all of which should be numbered. Abbreviations, symbols, units, etc. should be identified on one copy of the manuscript at their first appearance.

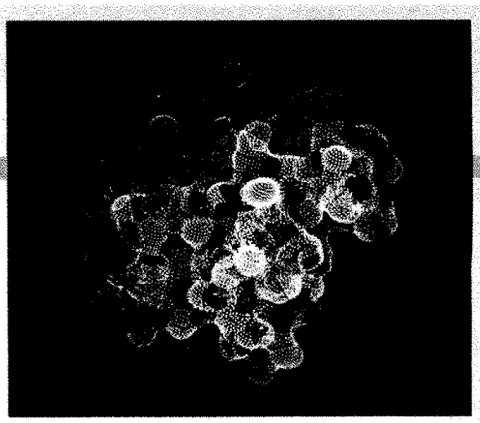
References should appear sequentially indicated by superscripts in the text and should be abbreviated according to the *World List of Scientific Periodicals*, fourth edition (Butterworth 1963–65). The first and last page numbers of each reference should be cited. References to books should clearly indicate the publisher and the date and place of publication. Unpublished articles should not be formally referred to unless accepted or submitted for publication, but may be mentioned in the text.

Each piece of artwork should be clearly marked with the author's name and the figure number. Original artwork should be unlettered. Suggestions for cover illustrations are welcome. Original artwork (and one copy of the manuscript) will be returned when manuscripts cannot be published.

Requests for permission to reproduce material from Nature *should be accompanied by a self-addressed (and, in the case of the United Kingdom and United States, stamped) envelope.*

Applications Report No. 2

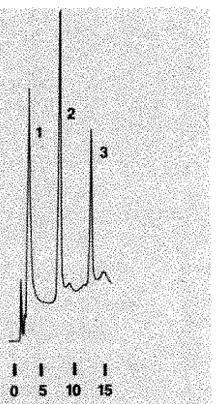
Hydrophobic Chromatography



Hydrophobic Chromatography

Conditions
Column: Bio-Rad TSK Phenyl 5PW
Eluant: A. 1.7 M $(NH_4)_2 SO_4$, 0.1 M Sodium Phosphate, pH 7.0
B. 0.1 M sodium phosphate, pH 7.0
0-100% B, linear gradient.
Flow Rate: 1.0 ml/min.
Detection: Model 1305 UV at 280 nm
0.16 A.U.F.S.

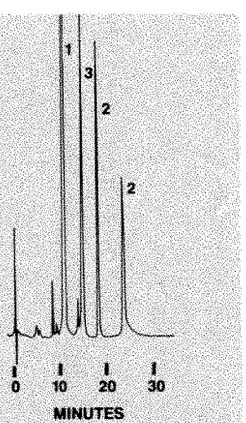
1 = Cytochrome c
2 = Myoglobin
3 = Lysozyme



Reverse Phase Chromatography

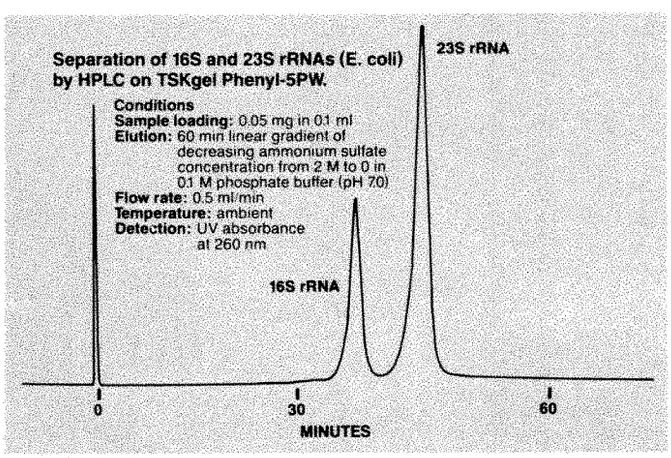
Conditions
Column: Bio-Rad Reverse Phase (RP-304) Hi-Pore 250x4.6mm
Eluant: A. 0.1% TFA
B. 0.1% TFA, 95% Acetonitrile
25-50% B linear gradient
Flow Rate: 1.5 ml/min
Detection: Model 1305 UV at 280nm
0.08 A.U.F.S.

1 = Cytochrome c
2 = Myoglobin
3 = Lysozyme



Separation of 16S and 23S rRNAs (E. coli) by HPLC on TSKgel Phenyl-5PW.

Conditions
Sample loading: 0.05 mg in 0.1 ml
Elution: 60 min linear gradient of decreasing ammonium sulfate concentration from 2 M to 0 in 0.1 M phosphate buffer (pH 7.0)
Flow rate: 0.5 ml/min
Temperature: ambient
Detection: UV absorbance at 260 nm



Circle No.20 on Reader Service Card.

Fast, sharp protein separations with high activity retained!

If you've been using a reverse phase column to separate proteins and enzymes, you may have noticed a substantial loss of activity in the sample after analysis. The separation is good, but there's low recovery of activity because the organic solvents required for sample elution often cause proteins or enzymes to unfold and denature.

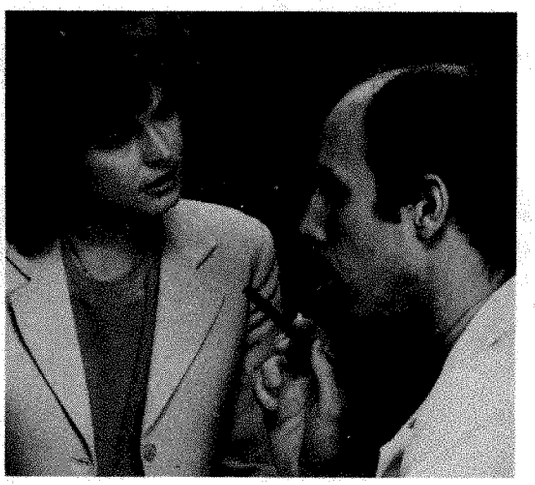
That problem is overcome with the new Bio-Gel® TSK Phenyl 5PW HPLC column for hydrophobic chromatography. It requires only a **salt gradient** for elution. For example, 5 enzymes analyzed on this hydrophobic column retained at least 80% of their original activity. As you can see at left, this new alternative for HPLC separation of proteins and enzymes is fast—and just as easy and reliable—as reverse phase. Give it a try!

As always, Bio-Rad is prepared to assist you in plotting an effective strategy for your protein separations, using a growing number of techniques. Give our applications staff a call at (800) 4 BIO-RAD, or contact us at:

BIO-RAD

Bio-Rad Laboratories—Chemical Division
2200 Wright Avenue
Richmond, CA 94804
(415) 234-4130

Also in Rockville Centre, N.Y., Australia, Austria, Canada, England, Germany, Italy, Japan, The Netherlands, and Switzerland.



Sometimes a good idea begins with a product or service you didn't know about. That's where our technical representatives can help.

There's always a possibility that something you're doing could be accomplished faster, or better, or more efficiently if you had the needed information.

Often enough your NEN representative has that information—whether it be a new method, a better product, or one of NEN's extra services.

Just ask. The answer will come from solid training and experience. More detailed technical answers are quickly available, too. Our representative will see that the question is handled by the most appropriate of several sources at NEN, such as our Technical Services Group, who specialize in customer assistance.

But first talk with the person who represents it all. A good idea may be only a conversation away.

NEN New England Nuclear®

DUPONT

REG U.S. PAT. & TM. OFF.

Circle No. 10 on Reader Service Card.



Separation power

Preparative fractionation of a 10 component mixture of proteins in 15 minutes is typical of the performance obtained with the FPLC System from Pharmacia Fine Chemicals.

High performance preparative and analytical chromatography of enzymes, monoclonal antibodies, synthetic polynucleotides and other biopolymers demands an uncompromising approach.

The FPLC System is not a compromise. New media and methodologies have been developed specifically to give the highest resolution of complex biological samples with high yields and separation times of 30 minutes or less.

Electrophoretically pure, native enzymes are typically purified from a crude tissue extract in one or two chromatography steps with a total separation time of 30–40 minutes and an overall yield of enzyme activity of 80%.

Other highly purified proteins, peptides and oligonucleotides are equally easy to obtain even in the presence of urea, non-ionic detergents or organic solvents.

The following list represents a small selection of the substances which have already been studied using the FPLC System.

Bacitracin	Alcohol dehydrogenase isoenzymes
Monoclonal antibodies	Glucose 6-phosphate dehydrogenase
Serum proteins	NADP
Urine proteins	Snake venoms
Transferrins	Bee venoms
Haemoglobins	5'-nucleoside phosphates
Synthetic oligonucleotides	Pollen extracts
Viral proteins	L-amino oxidase isoenzymes
Membrane proteins	Ovalbumin
Lactate dehydrogenase	Pyridoxalated haemoglobins
Cellulase	Gc-globulin
Tryptic peptides	Yeast proteins
Haemoglobin chains	Citrate synthetase
Insulins	Placental homogenate
Triose phosphate isomerase	2,4-dienoyl-CoA reductase

Find out how the separation power of the FPLC System can solve the separation problems in your laboratory by contacting your local representative of Pharmacia Fine Chemicals.

Pharmacia Fine Chemicals AB
Box 175
S-751 04 Uppsala 1
Sweden

Pharmacia Fine Chemicals

Circle No.25 on Reader Service Card.

BIOTECHNOLOGY RESEARCH OPPORTUNITIES

THE BIOTECHNOLOGY RESEARCH INSTITUTE OF THE NATIONAL RESEARCH COUNCIL OF CANADA OFFERS A CHALLENGING AND INTELLECTUALLY STIMULATING ENVIRONMENT, ACCESS TO WORLD CLASS FACILITIES, EQUIPMENT AND EXPERTISE, AND THE INTANGIBLE BENEFITS OF A GREATER MEASURE OF INTELLECTUAL FREEDOM THAT CAN BE FOUND IN MOST OTHER RESEARCH INSTITUTIONS.

The National Research Council is establishing a new biotechnology research institute in Montreal, Canada. This new research institute will emphasize the industrial applications of biological sciences and focus on the development of new processes and products. It will foster mission-oriented research and technology transfer to industry and promote communication and collaboration amongst industrial, university, federal and provincial government laboratories.

The main areas of research will include

- **Fermentation and biochemical and process engineering**
- **Genetic engineering**
- **Cell fusion**
- **Enzyme technology.**

A major large scale fermentation facility will allow pilot scale experimentation with new fermentation methods and economic evaluation. The R & D will focus on agro-food, energy, pharmaceuticals, health care and forestry.

RESEARCH SCIENTISTS

The Biotechnology Research Institute needs researchers in the following disciplines: microbiology, biochemistry, molecular biology, enzymology, microbial physiology, genetics, cell biology, immunology, protein and nucleic acid chemistry, biochemical engineering, process and systems engineering. Applicants should have experience in the main areas of research mentioned above.

QUALIFICATIONS

Candidates should have at least 2 years relevant post-doctoral research experience. Many of the positions are at the level of section leaders and project leaders and require additional in depth knowledge of the subject area and several years of research experience.

LOCATION

The Biotechnology Research Institute will be located in downtown Montreal in a new $60 million, 18,000 m^2 complex. It will employ 220 people and will host up to 80 researchers from industries, universities and other research organizations. It will have an annual budget of $35 million.

LANGUAGES

Both English and French will be working languages at the new Institute.

BENEFITS are fully competitive and SALARY will be commensurate with qualifications and experience.

PLEASE SEND DETAILED CURRICULUM VITAE IN CONFIDENCE TO:
The Employment Office
National Research Council
OTTAWA, Canada
K1A 0R6

National Research Council Canada | Conseil national de recherches Canada

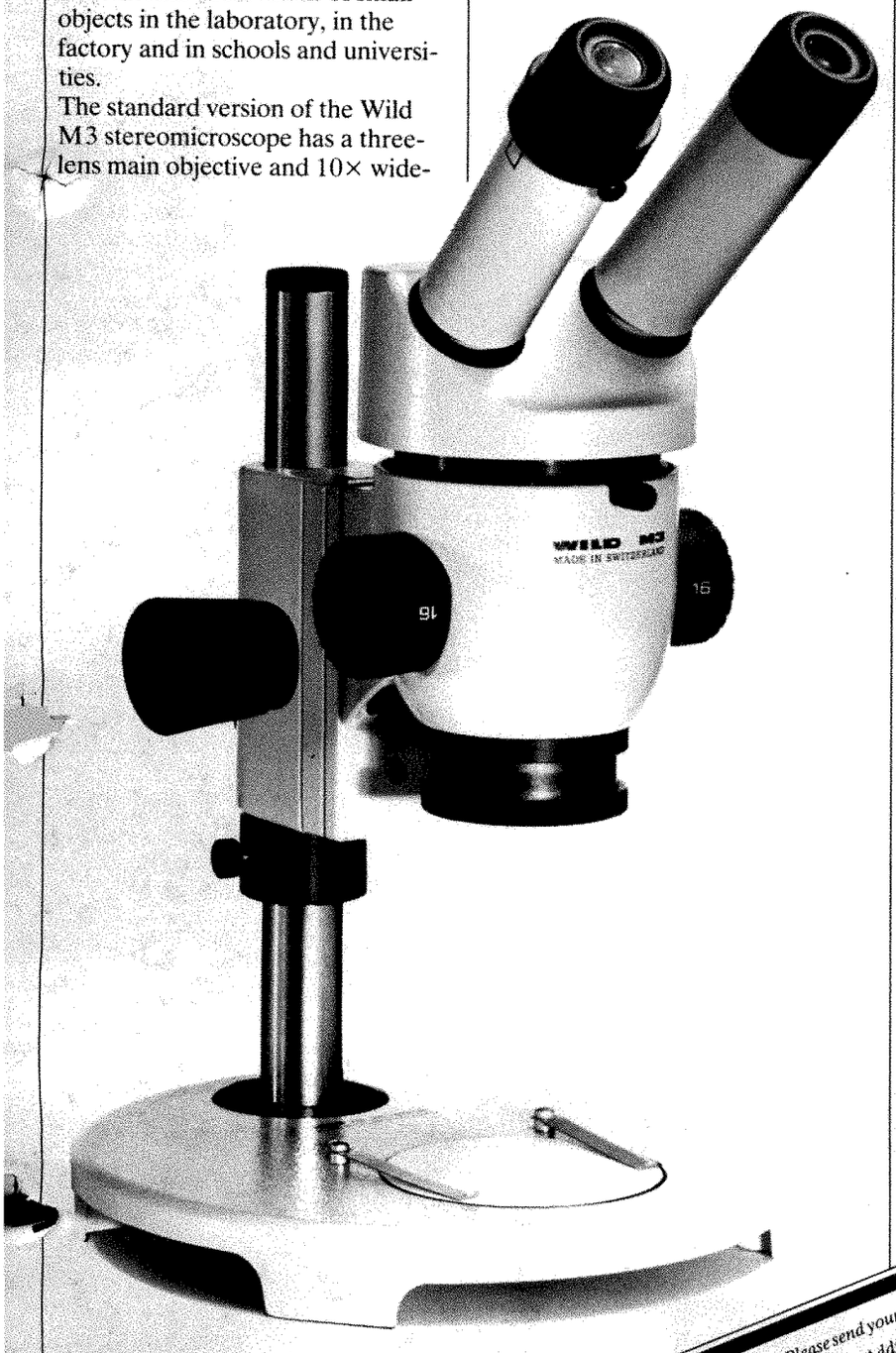
Canada

Circle No.14 on Reader Service Card.

The polyvalent

Wild M3 Stereomicroscopes
... those with the comprehensive accessories.

High-quality optics, high operator comfort and its comprehensive modular system mark the Wild M3 stereomicroscope as the universal routine instrument for the three-dimensional observation of small objects in the laboratory, in the factory and in schools and universities.
The standard version of the Wild M3 stereomicroscope has a three-lens main objective and 10× wide-field eyepieces. Even in this combination, the three-step magnification changer shows the object as required at a magnification of 6.4×, 16× or 40×. Interchangeable eyepieces and supplementary front lenses are available for extending the magnification range. These permit a magnification from 1.5× to 160×. New inclined tubes at various angles provide the operator with comfortable, fatigue-free viewing in practically any working position of the stereomicroscope and in any posture. Interchangeable stands, lighting units and accessory equipment such as the MPS photomicrography-system, coaxial incident-light housing, drawing tube, double-iris diaphragm etc can be used according to the task in question. The Wild M3 thus becomes a universal instrument capable of performing any task.
Ask for detailed documentation ■





Colorado beetle, scale 6:1

Wild Heerbrugg and Leitz Wetzlar: World leaders in macroscopy and microscopy

Circle No.19 on Reader Service Card.

WILD HEERBRUGG

NAT-883

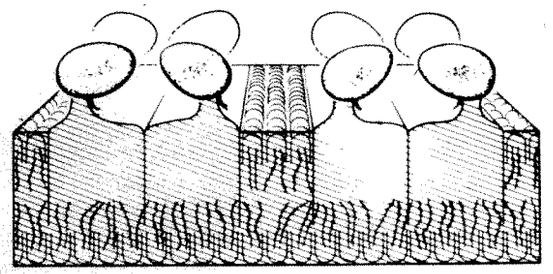
Please send your technical literature on the 'Wild M3'

Address

Send to your nearest Wild representative or to Wild Heerbrugg Ltd CH-9435 Heerbrugg Switzerland

Attention all biophysicists, molecular biologists and biochemists!

Biomembrane Structure and Function



Edited by
DENNIS CHAPMAN
Professor of Biophysical Chemistry
Royal Free Hospital
School of Medicine, London

Volume 4 in the TOPICS IN MOLECULAR AND STRUCTURAL BIOLOGY Series
Series editors:
Professor Watson Fuller, Dr Stephen Neidle and Professor Tony Atkinson

The application of physical techniques – such as X-ray diffraction and Raman spectroscopy – has revolutionised much of our thinking on the structure and function of membranes. In this important new study, contributors of international standing review a topical selection of approaches to the elucidation of **biomembrane structure and function.** The emphasis, throughout, is on the in-depth analysis of recent experimental work.

Published January 1984 Price £50.00 432pp
ISBN 0 333 31869 2

Contents

Calorimetric studies of model and natural biomembranes – *Diana Bach*, The Weizman Institute of Science, Israel; X-ray diffraction and electron microscope studies of the molecular structure of biological membranes – *Lee Makowski and Jade Li*, Columbia University, USA; Raman spectroscopy of lipids and biomembranes – *Surendra P. Verma and Donald F.H. Wallach*, Tufts-New England Medical Centre, USA; Infrared spectroscopic studies of model and natural biomembranes – *Ralph L. Arney*, Occidental College, USA and *Dennis Chapman*; Rotational and lateral diffusion of membrane proteins as determined by laser techniques – *Winfried Hoffman*, NUKEM GmbH, E.Germany, and *Colin J. Restall*, Royal Free Hospital, London; Theoretical models of monolayers, bilayers and biological membranes – *David A. Pink*, St Francis Xavier University, Canada; The Ca^{2+} dependent ATPase of sarcoplasmic reticulum – *G. Inesi and M. Kurzmack*, University of Maryland, USA.

Please send orders and requests for information to:
Carolyn Leuw, Product Manager,
Scientific and Medical Division,
The Macmillan Press Ltd.,
Houndmills, Basingstoke, Hants RG21 2XS, England

SCIENTIFIC & MEDICAL | MACMILLAN PRESS LTD

Circle No.64 on Reader Service Card.

EDITORIAL OFFICES

London Office
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024

Washington Office
1134 National Press Building, DC 20045
Telephone: (202) 737-2355

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff: Alun Anderson, Tim Beardsley, Philip Campbell, Isobel Collins, Peter Gambles, Melanie Kee, Tim Lincoln, Naomi Molson, Sara Nash, Geoffrey North, Peta Pickering, Miranda Robertson, Robert Walgate, Charles Wenz, Nigel Williams, Guil Winchester

Washington Editor: Peter David
Washington Correspondent: Stephen Budiansky

Art and Design: Joan Godwin, Joel Chernin, Theresa George, Paul Norris, Marie Xeridat

Administration: Mary Sheehan, Jacqueline Cole, Ruth Cutts, Joanna Czechowska, Ruth Goldenberg, Sharon Isaacs and Penny Walker (Washington)

Publisher: Elizabeth Hughes

Marketing Director: Ray Barker

Promotion Manager: Felicity Parker

International Advertising Manager: Andy Sutherland
Features Advertising Manager: Marion Delaney

Group Classified Advertising Manager: John Barnes — Telephone: (01) 240 1101

Nature New York Office
15 East 26 Street, New York, NY 10010
Telephone: (212) 689-5900 Telex: 668497

General Manager: Gary Rekstad

American Advertising Manager: Henry Dale

Classified Advertising Manager: Miss Gene Fein

Circulation Manager: Mirta Soto

Canadian Display and Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1—(416) 690-2423

Display Advertising Representatives

New York: Nature Office

Chicago: Didier & Broderick, Inc.—(312) 498-4520

San Francisco: Jobson/Jordan/Harrison/Schulz, Inc.—(415) 392-6794

Los Angeles & Pasadena: Jobson/Jordan/Harrison/Schulz, Inc.—(213) 796-9200

Philadelphia: Daniel Adams Associates, Inc.—(215) 353-6191

Boston: CEL Associates—(617) 848-9306

Fort Lauderdale: Brinker & Brinker—(305) 771-0064

New Jersey: Mary Grunmeier—(201) 572 9018

Dalton, Pennsylvania: Donald E. Northup Associates—(717) 563 2572

West Germany and Austria: Franz Schrecklinger TMW Top Media Werbegesellschaft GmbH Frankfurt—(611) 726046

Holland and Belgium: Adele Struyck G. Arnold Teesing BV Amsterdam—(020) 2636 15

Switzerland: Werner Stähli Agentur Iff AG Schaffhausen—(053) 45821

Scandinavia: Andrew Karnig Andrew Karnig & Associates AB Stockholm—(08) 51 68 70

Japan: Mashy Yoshikawa Orient Echo Inc Tokyo—541-4923

Australia: Gus Bartel & Associates PO Box 1755, Southport, Queensland 4215 (075) 466 577

Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office
1984 Macmillan Journals Ltd
Vol. 307 No. 5949 26 January 1984

nature

European research collaboration

The British will not be pulling out of the molecular biology laboratory at Heidelberg, but Europe needs urgently to explore ways of collaboration in research other than international laboratories.

THE British, who have earned themselves the unenviable reputation of not being reconciled to the notion that Britain is a part of Europe, have been making heavy weather in the past few years over their continued membership of the European Molecular Biology Laboratory, the international laboratory at Heidelberg in West Germany which is, confusingly, an offshoot of the European Molecular Biology Organization but constitutionally separate therefrom. The difficulties are several. When the foundation of a collaborative laboratory was first canvassed more than a decade ago, largely on the grounds that there was a need for an institution that would enliven European molecular biology as CERN at Geneva had enlivened European high-energy physics, the British Medical Research Council was one of the chief sceptics. In the pragmatic British tradition, the council argued that the analogy with CERN could be misleading, for molecular biology does not depend on the construction of huge central facilities, and that the mere existence of a central laboratory, by attracting bright people, might serve in fact to weaken national efforts in this important field.

In the event, the council's misgivings were stilled by political considerations. Ten countries (including Switzerland and Israel) signed a treaty setting up the laboratory in May 1973, and the Medical Research Council has since been the channel through which the annual British contribution to the laboratory is paid. But in that role, the council seems never to have exorcised its original doubts. The council's latest formal inquiry into the value of the work done at Heidelberg (see *Nature* 19 January, p.203) is the third occasion in ten years on which the council has thought it appropriate solemnly to appraise the value of the laboratory's scientific programme, although on this occasion it was on formal instructions from the Advisory Board on the Research Councils that continued membership of the laboratory was reviewed.

British blowing hot and cold in this way has had a predictably undermining effect at Heidelberg and among other members of the laboratory. It seems to have been widely understood that if the British chose to withdraw from the laboratory, others would follow. It has been especially disconcerting for the new director of the laboratory, Dr Lennart Philipson, that one of his chief sources of financial support (the British contribution to the laboratory is now nearly 17 per cent of the total) should be put in hazard within a few months of his appointment. Understandably, if wrongly, there have been complaints that the British were content with continuing membership only so long as a British national (Sir John Kendrew) was the director of the laboratory, and that they have seized on his departure (half-way through 1982) as the occasion for pulling out.

Moratorium

These are some of the reasons why the report of the committee of inquiry, now unexpectedly published, is so warmly to be welcomed. Its recommendation, that there should be a once and for all decision (to belong or to pull out) and then a moratorium on further introspection about membership of the laboratory, has been accepted by the research council. It is unthinkable that the Advisory Board on the Research Councils will not follow suit when it considers the matter this week.

So has the time come when people in Heidelberg can relax? Not quite. The latest British inquiry has been chiefly concerned with the laboratory's record and with its programme for the years ahead. Its report acknowledges that the laboratory has provided distinctive facilities at its two outstations (neutron diffraction at Grenoble, synchrotron radiation at Hamburg), that some of the groups at the Heidelberg laboratory are working in fields which are comparatively neglected in Britain, that many of these projects offer the chance that collaboration will yield an effort greater than the sum of its parts and that there are, indeed, opportunities for creating central pools of expertise unlikely to be replicated in the laboratories of member states. The committee of inquiry also considered the relatively high cost of the Heidelberg laboratory and offered its advice that the extra price is worth paying for the sake of the benefits of collaboration. But the inquiry has not taken up the more important question for the future of European collaboration, that of the utility of the Heidelberg as a model for collaboration in other fields.

Scepticism

The truth is that some of the original scepticism about the need for a central laboratory can still be justified. Molecular biology is not, after all, high-energy physics and the case for the Heidelberg laboratory differs in important ways from that for CERN. That, at least, is the present position, although the founders of CERN were diligent advocates of the view that central facilities at Geneva should not be regarded as a substitute for national accelerators but, rather, as a spur to member states to build big facilities of their own. That doctrine of calculated extravagance has now mercifully been dropped and CERN's justification, for the time being, is that it has, or is building machines that no member state could afford. Its worry is (or should be) that even collaboration on a European scale may be insufficient to meet the cost of the next generation of particle accelerators and that the building of large accelerators may not even be the best way of stimulating high-energy physics (see *Nature* 1 December, p.421).

The case for the Heidelberg laboratory, originally cast in similar language, is now recognized to be intrinsically different. Although there are facilities not to be found elsewhere in Europe, their provision does not hang on the existence of a central laboratory, whose justification then becomes its capacity to attract distinctive people and problems. The unanswered question is whether the same benefits might be accomplished by other means, perhaps at less cost. If, for example, there were a grant-making body operating on a European scale in the support of molecular biology, the chances are that its funds would go on the support of projects not very different from those being assembled at Heidelberg. Similarly, if national research councils were more ready than they are at present to spend money on collaborative projects, and if their governments would let them follow such an enlightened policy, much the same might be accomplished. Collaboration along lines like these could, in the long run, be cheaper, more flexible and more productive. So the case for central laboratories on a European scale is largely psychological — they entail a visible commitment by member states which cannot easily be shuffled off.

There are several practical lessons to be drawn from this argument. On the Heidelberg laboratory, the British institutions which have found it impossible to bury their doubts about the wisdom of collaboration should do what the latest inquiry

0028-0836/84/040303-01$01.00

© 1984 Macmillan Journals Ltd

recommends — pay up and do what they can to make a success of the venture. And because the venture implies a commitment not just by the Medical Research Council but by the British government, steps should be taken to see that the funds available for supporting academic research in the United Kingdom do not fluctuate with each variation of the international value of sterling or the relative economic growth of the partners in these collaborations. But none of this should be taken as a sign that European collaboration (of which there is too little) should always be organized around central laboratories. Indeed, there is the strongest case for deliberate experiments in the support of European research from central funds along the lines on which research councils function nationally. The European Molecular Biology Organization, which has won a high reputation in its administration of fellowships and training programmes, is ironically the most obvious vehicle for such an experiment. Is it outrageous to suggest that when the issue of the laboratory is settled, national research councils should bite harder on the bullet and set aside further modest funds for supporting less formal kinds of collaboration? □

Breaking all the rules

The US accuses the Soviet Union of violating arms agreements, but is breaking other rules.

THOSE who worry about the growing tension between the superpowers should remember that one of the earliest and most successful arms control agreements ever negotiated by the United States is already 167 years old. That agreement was not, of course, with the Soviet Union but with Great Britain. The Rush-Bagot agreement which, in 1817, demilitarized the Great Lakes is still regarded by historians as an outstanding instance of hostile powers entering into sensible arrangements to reduce the military friction between them. Yet for the first hundred years of its life, the treaty was the subject of heated accusations by both sides. It is conceded that its technical provisions have been violated countless times.

The US Congress would do well to keep in mind Rush-Bagot when it ponders the report it has now received from the Reagan Administration alleging that the Soviet Union is guilty of several violations of the fragile arms control regime now in place. Regrettable as they are, technical violations need not unmake a useful treaty unless the signatories choose to let them. That is why it is unfortunate that at least some members of the Reagan Administration have created the impression that they would like the still unpublished report to destroy what remains of American confidence in the trustworthiness of the Soviet Union, perhaps even to undermine the notion of arms control as such.

Why else should the administration have chosen to raise a public stink about the alleged violations before exhausting the opportunities that exist in the private Standing Consultative Commission (SCC) set up under Salt I and Salt II expressly to deal with problems of this kind? SCC has already proved itself a successful (but slow) mechanism. Between 1973 and 1977, discussions in SCC persuaded the United States that silos being constructed in the Soviet Union were indeed intended to house launch-control facilities and not new missiles. And in 1975, when radioactive material was vented into the atmosphere after a Soviet nuclear test in violation of the 1963 Limited Test Ban Treaty, private bilateral talks persuaded the Carter Administration that the technical violation had not undermined the value of that agreement.

The decision to complain in public would be more understandable if the administration had clear proof of the alleged violations, and if they were of serious military significance. Yet the United States concedes that only two of the seven violations have been confirmed to its own satisfaction and that neither of those — the alleged use of chemical and biological weapons in Afghanistan and a breach of the 1975 agreement on notification of military manoeuvres in Europe — is part of the bilateral nuclear agreements between the superpowers. The other allegations are more tentative. In some cases, such as the allegation that the Soviets have tested above the 150 kiloton threshold set by the unratified Threshold Test Ban Treaty, the technical evidence is ambiguous. In others, like the encryption of telemetry during missile tests, the superpowers differ over the legal interpretation of the Salt II agreement.

Why, then, has the administration chosen this moment to raise public questions about the value of the treaties it has signed with the Soviet Union? One plausible though perhaps uncharitable explanation is that the administration is preparing public opinion for an abrogation of the Anti-Ballistic Missile (ABM) Treaty so that it can proceed more quickly with development of the "star wars" missile defence system proposed by President Reagan last March. There are signs enough for the congenitally suspicious that the President has decided to give the star wars plan his blessing. To the Soviet Union, for example, last week's first test by the United States of an antisatellite missile (see page 306) and the President's announcement (expected this week in the State of the Union speech) that the government has approved plans for a space station, are bound to look threatening.

A slightly more charitable explanation of the administration's behaviour is that its report on Soviet treaty violations is simply another unsuccessful attempt to paper over its own political troubles. It is an open secret that the administration is divided between those who favour new arms control measures and those who want to break out of the existing regime so that the United States can recover its dominant position in strategic weapons. Complaints in public on the basis of a still-secret document may have been a convenient compromise between the two factions. The effect, however, will be to make the Soviet Union even more reluctant than it already is to answer the serious charges against it. □

West German reform

The Bonn government seems bent on carrying reform to the universities, urgently in need of it.

LEGEND has it that the student prince was almost always penniless but invariably free — free to move from one university to another, to let his inclinations guide his pursuit of knowledge or even, if that seemed more appropriate, the pursuit of other goals. In the hard decades after the Second World War, the species has become almost extinct. Now, only vestiges of the old behaviour-pattern are to be seen. Italian universities have no effective mechanism except discomfort for turning away students whom they cannot hope to teach. In France, in spite of the troubles of 1968 which filled the streets of Paris with would-be student princes, the government seems cleverly to have managed to keep the worst excesses of the old system in check, helped no doubt by economic reality of the kind that touches students. Only in West Germany have the old ways been substantially unchanged, with the result that some universities (the University of Munich, for example) are unmanagably large while others, some of the newer creations in *Nordrhein-Westfallen,* are crying out for students. And the capacity of universities to manage their affairs efficiently has been further undermined by the basic law that distributes decision-making to statutory committees able, if so inclined, to exercise the rights but not the responsibilities of power.

Against this background, the new proposals for reform now in the mill at Bonn are much more radical (and welcome) than they may seem at first sight. The idea that students should be guided to the places where they can be taught is not illiberal (as some will make out), it does make sense that students should not remain students indefinitely, it is high time that some power of decision should be returned to those who are blamed if things go wrong, and it is especially welcome that there should be a scheme for keeping people in the academic system for long enough to tell whether they will be able to find permanent places for themselves. The dangers now lie not so much in Bonn but with the backwoodsmen, the politicians in the *Länder* governments who do not see the need for reform and with the would-be student-princes. It will be interesting to see how many are left.□

0028-0836/84/040304-01$01.00

© 1984 Macmillan Journals Ltd

US nuclear power

Cancellations promise bleak year ahead

Washington

THE new year has brought a new wave of trouble for the US nuclear power industry. Five nuclear power plants now under construction have either been cancelled or have encountered licensing problems in just the last few weeks, chiefly as a result of rising costs and newly discovered safety violations. And a report from the Energy Information Administration (EIA)* has found that average construction time for a new reactor has reached 12 years — more than twice the time it took to build a reactor in the early 1970s. Three-quarters of the plants completed in the past decade or nearing completion now have cost more than twice the original estimate. One in ten of them have cost more than seven times the original estimate.

These escalating costs, together with the failure of electricity demand to keep up with the utilities' projections, have played a large part in the recent cancellations. Last week, the Public Service Company of Indiana announced that it was abandoning the Marble Hill plant on which it has already spent $2,500 million. The River Bend plant No.2 in Louisiana was recently cancelled as well, and the two-reactor Midland plant in Michigan is facing an uncertain future, with its current estimated cost at more than ten times the original figure of $300 million.

Safety violations have aggravated economic difficulties in some other recent cases. On 13 January, the Nuclear Regulatory Commission's safety and licensing board unconditionally denied an operating permit to the Byron plant in Illinois, saying it had "no confidence" in the quality assurance programme of key contractors. Although the board did not point to specific safety deficiencies, it said that the quality assurance was so poor that there was no way to know for sure. According to the Nuclear Regulatory Commission press office, the board, in its 400-page report, charged that Systems Control Corporation, which supplied safety-related equipment, had actually run a "fraudulent and ineffective" quality assurance programme and is being investigated by the Department of Justice.

Spokesmen for the utility said the problems were only in the documentation of the quality assurance efforts, not in the work itself. They also said that the utility was losing $1 million a day as a result of the delay.

Safety troubles with Cincinnati Gas and Electric's Zimmer plant led to an unusual decision over the weekend at that troubled plant, which has stood half-finished since November 1982 when mounting quality assurance failings prompted the Nuclear Regulatory Commission to order a halt in construction. The utility decided that rather than spend an additional $1,000 to $2,000 million to complete the nuclear plant, they would convert the station to a coal-burning facility. The utility had already spent $1,700 million.

A spokesman for the Atomic Industrial Forum said the cancellations were all primarily due to the low growth in electricity demand. He said that "nuclear is a natural target" because of the large capacity of nuclear plants and their high initial capital costs.

The cancellations of half-completed nuclear plants are already sparking heated confrontations over who is to bear the construction costs — stockholders or ratepayers. The decisions in each case will ultimately be made by the state regulatory commissions which have different rules and have come down on different sides of the issue.

Stephen Budiansky

*"1983 Survey of Nuclear Power Plant Construction Costs", DOE/EIA-0439(83).

West German universities

End of old order in sight?

A RADICAL reform of West German education is in the air. Professorial powers will be increased and advance beyond the basic degree will be made more difficult if recommendations made by a commission appointed by the West German Minister of Education are accepted.

The commission's mandate was to examine the working of the 1976 University Law (*Hochschulrahmengesetz*) framed in reaction to the student unrest of the late 1960s. This shifted the balance of power from the professors (the *Ordinarienherrschaft*) to a wider group. Decision-making committees of the universities now comprise representatives including students, administrators, scientists and non-scientists, as well as professors.

The commission finds, in agreement with the *Wissenschaftsrat* and the Conference of West German Rectors, that over-diligent application of the principle of group responsibility has led to the hardening of conflicting interests, inefficiency and a mushrooming bureaucracy.

The commission wants to see the professorial and faculty role strengthened at all levels. However, the number of faculties should be limited to fifteen, and the heads of faculties (*Decane*) represented in the senate. It does not, however, agree with the rectors that the professorial complement in the senate should be increased. At present, university rectors are elected by the professors in universities of six *Länder*. The commission favours this system as against appointment by the president.

Subject to restrictions on admissions to popular faculties (*numerus clausus*) such as medicine, the school-leaving *Abitur* remains a general passport to university education and student careers have traditionally been long even with rising student numbers. The report now recommends that length of study should be controlled and academic criteria strictly applied to courses beyond the basic level.

Many bureaucratic barriers have arisen in the application of private sources of finance in university research. All such support now requires special permission and must be channelled through the university administration. The present government favours closer links between industry and the universities and will therefore agree with the recommendation that restrictions on this source of money be lifted and also that researchers be given freedom to appoint their own staffs.

The report also recommends the redesigning of the academic echelons. The present two highest levels of C3 and C4 will be amalgamated, perhaps to provide a larger pool of professional expertise. The lower C2 grade and general all-purpose university assistant would vanish, to be replaced by a class of scientific assistants who would be in the process of qualifying.

Those obtaining the *Habilitation*, the formal German professorial qualification, would be guaranteed a four-year post as an assistant, giving them time to receive the necessary invitation to a full professorship and at the same time maintaining a reserve pool of available talent.

The report has been a long time in gestation. It reflects the opinion now widely held among West German academics that the reforms of the 1970s, which diffused power widely among statutory university committees and which further strengthened the legal rights of students and junior members of teaching staffs, failed to tackle the outstanding problems of excessive demand for places in some universities and the difficulty of placing young people in any but the most demeaning academically related posts.

The problems encountered in some *Länder* of making full use of newly built universities have helped to win approval for the new proposals in the *Wissenschaftsrat*, the constitutionally unique body representing the federal and *Länder* governments which has been for the past thirty years the most powerful arbiter of policy on higher education.

The report will be the basis for discussion from which a new university law will emerge.

Sarah Tooze

0028-0836/84/040305-01$01.00

© 1984 Macmillan Journals Ltd

Antisatellite weapons

First US test of airborne system

Washington

MORE than a year of lobbying by prominent scientists failed to prevent the US Air Force from conducting its first test-firing last Saturday of a new antisatellite weapon (ASAT) that can be launched from underneath the wing of an ordinary F15 fighter aircraft. The test, in which the missile was fired into space but not at a target, was immediately denounced as the prelude to a dangerous new phase in the arms race with the Soviet Union.

Development of the new weapon has been justified by the Department of Defense on the grounds that the Soviet Union is already believed to possess an operational antisatellite system. But critics complain that the American air-launched system is far more sophisticated than the Soviet system and, because of its small size, will make verification of a treaty banning such weapons virtually impossible.

The Soviet Union is believed to have conducted some 20 tests of its own main ASAT system, in which an explosive interceptor is launched from the ground by a variant of the SS-9 Scarp rocket. Last March, the Pentagon claimed that the Soviet Union would be able to launch the prototype of a space-based laser weapon some time after the mid-1980s and that an operational system capable of destroying satellites within a few thousand kilometres could be completed by the end of the century.

Unlike the existing Soviet system, the American weapon tested last week is launched in flight by an F15 aircraft and consists of a miniature non-explosive homing vehicle carried into space by a two-stage rocket. The vehicle would locate its target with infrared sensors and destroy it by collision. Congress has told the Air Force not to test the new weapon against target satellites until President Reagan provides a report on the administration's efforts to curb an arms race in space.

The administration is not expected to face any substantial difficulty in persuading Congress that the tests should go ahead, however. In recent months the Pentagon has been stressing the rapid strides the Soviet Union is believed to be making in space weapons, and a report last week in *Aviation Week and Space Technology* says the Central Intelligence Agency (CIA) believes the Soviet Union is developing a space-based defence system that could destroy American ballistic missiles launched against the Soviet Union.

At present there are no international treaties forbidding the deployment of ASAT weapons. The 1963 Limited Test Ban Treaty and the 1967 Outer Space Treaty prohibit the placing of nuclear weapons or other weapons of mass destruction in space, and the provisions of the 1982 Salt I treaty prohibit interference with reconnaissance satellites. Negotiations on a treaty to ban ASATs were, however, broken off by the Carter Administration in 1979 after the despatch of Soviet troops to Afghanistan.

Many prominent American scientists have urged the Reagan Administration to take up recent Soviet offers to resume the stalled negotiations. Condemning last week's ASAT tests, the group argues that the deployment of small and sophisticated ASATs would make the negotiation of a verifiable treaty almost impossible. Signatories of a statement criticizing the test include Jerome Wiesner, a former presidential science adviser; Hans Bethe, a physicist from Cornell University; Franklin Long, former associate director of the Arms Control and Disarmament Agency; and Herbert Scoville, former deputy director of CIA. **Peter David**

Human rights

Philippines under scrutiny

Washington

ALLEGATIONS of gross violations of human rights in the Philippines have been made public by a delegation sent there by the American Association for the Advancement of Science (AAAS), the Institute of Medicine and four other scientific societies and human rights groups. Among the delegation's complaints are allegations of torture, "disappearances" of political detainees on the recent Argentinian model and the intimidation of health workers in rural areas.

The delegation was led by Dr Jonathan Vine, a private physician from Boston, and included Dr Robert Lawrence, director of primary care at the Harvard Medical School, and Eric Stover, staff officer of the AAAS committee on scientific freedom and responsibility. During their three-week stay in the Philippines, members of the delegation met government officials, Catholic clergy, health workers, US embassy officials, some of those in detention and also former detainees. The group visited seven detention centres and a variety of rural health facilities.

The rural health workers especially at risk are predominantly those employed on projects set up by the Catholic church to bring health care to remote areas not served by government facilities. According to the delegation, these workers are under constant scrutiny by the military, chiefly because of the suspicion that they are providing assistance for the guerilla force called the New People's Army. Many lay workers for these rural health projects have allegedly been detained and several private physicians have been arrested and held under "Presidential Commitment Orders" authorized by a decree suspending habeus corpus.

One of the incidents described in the delegation's report is that of the assassination in 1982 of a private physician, Dr Bobby de la Paz, who had operated a clinic on the remote Sumar Island. The local army command at first accused the guerillas but, in response to demands for a more thorough investigation, charged an enlisted man with the crime. The family of Dr de la Paz now claims, however, that the man thus identified does not match witnesses' descriptions of the assassin.

The delegation seems also to have been able to document several apparent cases of the torture of prisoners held for subversive activities. Its report includes a gruesome X-ray of the skull of a 37-year-old farmer who had a 4-inch nail driven into his skull while in detention. The police are said to have told the hospital to which the man was eventually taken that he had attempted suicide.

Clergy and others interviewed are reported to have said that there had been no substantial improvement of human rights in the Philippines since the lifting of martial law in 1981. The delegation notes, however, as an encouraging sign, the recent release of three scientists who had been in detention. **Stephen Budiansky**

NIH curbs drive out researcher

Washington

A FEDERAL rule that forbids researchers at the National Institutes of Health (NIH) from conducting experiments on *in vitro* fertilization with human beings has driven out the chief of pregnancy research there. Dr Gary Hodgen, a leading researcher on human reproduction who has been at NIH for 15 years, announced last week that he will be leaving at the end of June to join a research group at the Institute for Reproductive Medicine at Norfolk, Virginia. The institute was among the pioneers of *in vitro* fertilization in the United States.

Under an administrative rule adopted during President Gerald Ford's administration, federal researchers are not allowed to use the procedures of *in vitro* fertilization or to carry out experiments in which human embryos are used. NIH regulations also forbid research with human fetuses except when the objectives include assuring the survival of the fetus or the avoidance of congenital defects.

The Institute for Reproductive Medicine at Norfolk is run by Drs Howard and Georgeanna Jones, a husband and wife team who have carried out some 50 successful *in vitro* fertilizations since 1982. Hodgen is to become the institute's scientific director. Stephen Budiansky

0028-0836/84/040306-01$01.00

© 1984 Macmillan Journals Ltd

UK nuclear power

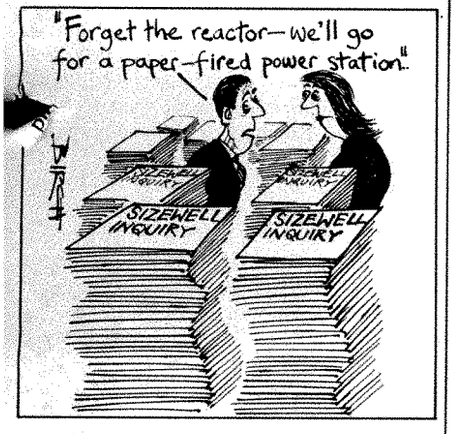
Next reactor still not decided

THE public inquiry into the application from the Central Electricity Generating Board (CEGB) to build Britain's first pressurized water reactor (PWR) in Suffolk has now entered its second year, with no prospect of finishing before the autumn. It might easily take a further six to nine months for the Inspector, Sir Frank Layfield, to complete his report and submit it with recommendations to the Secretary of State for the Environment, who will take the final decision on whether the board should be allowed to go ahead.

The inquiry is being held in a former malting-house now converted into a fine concert hall, in the tiny village of Snape in Suffolk. The planned site for the PWR is just a few miles away at Sizewell, near an existing gas-cooled nuclear power station. The inquiry hall could hold 800 spectators, but since early last year it has rarely held more than a handful of people. All the activity takes place on a raised platform where Sir Frank sits, surrounded by crates of documents and under the continual glow of spotlights, attended by two assessors and an assortment of lawyers.

CEGB has a similar posse of lawyers on the platform ready to jump up and introduce procedural points, while the objectors, running their case on a limited budget, have a smaller contingent but manage to keep the words flowing.

A great many words are involved. Every one is taken down in shorthand, and the inquiry's secretariat now estimates that, including supporting documents, it distributes 200,000 sheets of paper a week. The



proportion of these that are ever read is a different matter.

Local residents have mixed feelings about the effects of the inquiry on Snape itself, but there can be no doubt that money from CEGB has largely financed the conversion of the concert hall complex into a potentially profitable leisure centre, and that jobs will be created if the new reactor is built. Under the terms of the inquiry, the board pays for everything, from the inspector's salary down to the bus that every day runs to and from the nearest town, Ipswich. More often than not, however, the bus is empty. The one pub in the vicinity appears to be enjoying a good trade from board employees and objectors alike, although one person who is not to be seen in *The Plough and Sail* is Sir Frank Layfield: protocol demands that he is not seen dining, and presumably drinking, with either party.

The first three months of the inquiry were taken up by the electricity utility reading out its evidence, and were followed by nine months of detailed examination of the economic justification for the board's case. The objectors claim to have significantly weakened it: the board itself admits to having refined some of its assumptions but insists the case holds. Now, attention has turned to safety aspects.

Friends of the Earth, the British environmental group, has hired a barrister to make its case that the board's design is not acceptably safe, and reckons to have spent £100,000 on the inquiry so far. The group will retire from the inquiry in a few weeks' time, to be replaced by the Stop Sizewell B Association, which will concentrate on attacking the board's operational safety claims. In particular, the association will be pressing that the design should include electromagnetic filters in the reactor's coolant circuit at a cost £5 million. The filters will, it is claimed, reduce radiation doses to operators by 50 per cent.

This week, Friends of the Earth intends to raise the temperature of the proceedings by introducing a document produced by the UK Atomic Energy Authority but not publicly available in Britain. The document provisionally endorses a controversial study by the United States Oak Ridge National Laboratories which found actual rates of mishaps in reactors significantly in excess of predicted rates. Friends of the Earth says the document became available only because of an application in the United States under the Freedom of Information Act, and that the study of the frequency of mishaps at British reactors had been carried out under contract for a US customer.

If CEGB's application is granted, the result will be the introduction of a new type of reactor technology into Britain. The board's previous experience with building power stations is not encouraging, but it says that the PWR could be built in a record 19 months once it gets clearance. However, plans are already two years behind schedule and costs are rising. **Tim Beardsley**

Indian universities

Can academic politics be banned?

New Delhi

INDIA'S University Grants Commission (UCG) has called on the ruling political parties in the central and state governments to "set an example" by refraining from interfering in the internal affairs of universities. At the same time, the commission urged academics and students to avoid bringing politics into university matters. Universities cannot be run effectively, Mrs Madhuri R. Shah, head of UGC, observed, while the present "free for all" is considered to be an expression of "democracy" in the universities.

Among those spheres of academic life which UGC considers should be free from "politics" are appointments, recruitment, admissions and some administrative and financial matters.

All these spheres have, of late, become imbued with politics. Thus a recent dispute over the appointment of a new vice-chancellor at the University of Calcutta developed into a major confrontation (complete with students' strike) between the governor of West Bengal, Mr A.P. Sharma (who is *ex officio* chancellor of the university) and the Left Front state government. And a student campaign in Bangalore ostensibly demanding universal access to "scientific and democratic" higher education is closely linked with the conflict between the Janata state government and the Central Congress (I) government, and the opposition in southern India to Mrs Gandhi's hints that Hindi should replace English as India's "link language". In Assam, the All-Assam Students Union is allegedly involved in plans to bring down the state legislature.

UGC therefore suggests a formal separation of political and university life. Central and state ministers, it urges, should not seek appointment to university bodies, and university lecturers and employees who are elected to central or state legislative assemblies should be granted unpaid leave during their term of office.

University vice-chancellors should be given the "full trust" of government officials instead of, as often happens at present, the latter giving their overt or covert support to groups opposed to the university authorities. In the seven central universities (which are supported by the Delhi government), vice-chancellors should have direct access to the "highest quarters" of government, in recognition of their status as national institutions.

UGC does not spell out how these objectives might be attained, but simply appeals to the "maturity" of the political parties, calling on them to unite to save the universities "which have a key role in building the country's future". In the present atmosphere of tension between the central and state governments and the current militancy of Indian students, however, such exhortations seem somewhat utopian. **Vera Rich**

0028-0836/84/040307-01$01.00

© 1984 Macmillan Journals Ltd

Halley's comet

Europe's hopes for second probe

GIOTTO, the European Space Agency's first interplanetary satellite, designed to intercept Halley's comet, should be backed up by a second version which could be built at relatively little extra cost, some Europeans claim. Spare parts and a spare framework are already available, and could be assembled to make a Giotto II.

British Aerospace, prime contractors for Giotto, was not prepared last week to quote a price for such a project but, according to some estimates, a second Giotto, including launch, would cost only half as much as the current mission: another £50 million on top of the present £100 million.

The advantages of a second Giotto would be as a safeguard against the failure of the Ariane launcher, two of whose last six launches have failed. But if that launch were successful, a second Giotto could provide a matching experiment to send to another comet. The other missions to Halley, the Soviet Union's Vega craft and Japan's Planet-A and MST-5, are already double missions.

"This is a hot potato in ESA (European Space Agency) at the moment", said a Giotto specialist last week. It would put the £80-million-a-year ESA scientific programme back only a few months, he claimed, saying that it is "up to the scientific community" to decide whether it wanted to do it.

One planetologist in favour of a second spacecraft is Tony McDonnell of the University of Canterbury. He has already proposed a mission called Comex, which would be a modified Giotto II designed to intercept a number of comets by using direct orbital injection from Ariane and by carrying a liquid fuel motor aboard. Comets differ greatly from one another, McDonnell points out, some bursting, others splitting with a variety of tails and emission characteristics, and there is a danger that the Halley data will give too selective a view of cometary structure and chemistry.

ESA planners, however, take the view that any modification of the tightly-designed Giotto mission would entail its total redesign and thus destroy the advantages of using Giotto spare parts. Giotto uses a solid fuel booster which can only be fired once to reach its interception orbit with Halley, and so a Giotto II could be used effectively only for another single comet.

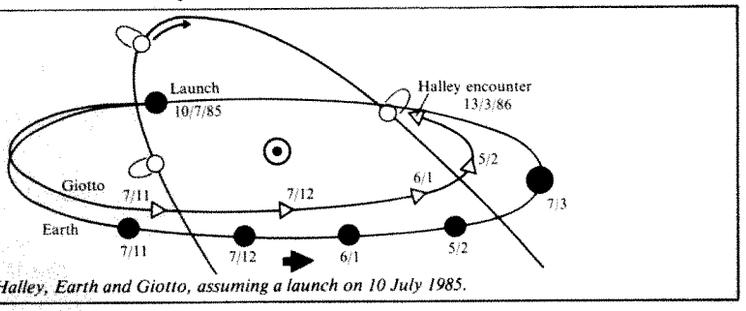
There may, however, be other arguments for a Giotto II. Giotto will go far closer to the comet nucleus than any other mission. (The closest approach will be less than 1,000 km, compared with upwards of 10,000 km for the Soviet and Japanese probes and for the American ISEE satellite recently diverted to comet Jacobini-Zimmer.) So, says Giotto's project scientist Ruediger Reinhard, Giotto has only a small chance of surviving the encounter.

Moreover, it is now clearly admitted, impacts with cometary dust in at least the last "tens of seconds" of the 30-minute passage of Giotto through the cometary coma to the nucleus are likely to throw out the close (1°) alignment of telemetry with the Earth, so that the last portion of data is in danger of being lost. And this assumes the dust cloud to be uniform; if Giotto were unfortunate enough to meet a jet of emission (such as are observed), misalignment would occur earlier.

There will be attempts to steer Giotto between any jets observed from Earth by taking the best straight-line path. But it is agreed that the whole exercise is risky. "We will know the risk sometime between 1830 hrs on 13 March 1986 and 0330 hrs on the next day", when the encounter takes place, said a British Aerospace spokesman.

● A rumour is circulating at the British Aerospace Dynamics Group near Bristol, where Giotto is being assembled, that Mrs Margaret Thatcher the British Prime Minister has enquired of an official whether Halley would be destroyed if Giotto targetting were so good that the satellite hit the nucleus (which is believed to be about 3 km in radius). The political consequences of such a conflagration would clearly be incalculable. But whether or not the question was actually put, British Aerospace did tackle the calculation. Little damage would be done, it seems, even though the impact would take place at 68 km per second. This velocity gives Giotto kinetic energy equivalent to about 250 kilotons of TNT, but the latent heat of melting of the cometary snowball would be some 100 million times greater.

Robert Walgate



The orbits of Halley, Earth and Giotto, assuming a launch on 10 July 1985.

European pollution

West Germany takes the lead

WEST Germany is well on its way to leading the European Economic Community (EEC) in environmental legislation. The impetus is the growing anxiety about the condition of West German forests (see figure opposite, and *Nature* **307**, 97; 1983). A government committee, *Der Rat von Sachverständigen für Umweltfragen*, has now stressed that the phenomenon "can no longer be explained on the basis of traditional forest experience", but that acid air pollution must be an important contributory cause. But there are now also fears that air pollution is increasing mortality among the vulnerable trees — the old and the very young. Mortality among the young may be especially severe in West Germany, which now has the lowest success rate for new trees in the world.

The response to the anxiety has been impressive. In a debate in the Bundestag, the Federal Minister of the Interior, Dr Friedrich Zimmermann outlined a series of measures being taken in West Germany. The federal government is spending DM56 million (£14 million) a year on research related to forest death (compared with an estimated £0.5 million in the United Kingdom. Individual *Länder* are also reacting — Baden-Württemberg, which sent a delegation to Japan to investigate power-plant desulphurizing techniques, now has three pilot plants built at a total cost of DM 60 million (£15 million) without federal or EEC support.

West Germany emits 3.5 million tonnes of sulphur dioxide a year into the atmosphere, 43 per cent of it from power stations. Regulations introduced last year set the emission levels for new power plants at 1,800 mg m^{-3} and twice that for old plant. The utilities claim the new regulations will cost them DM10,000–15,000 million (£2,500–3,740 million); 20–30 per cent of the cost of new plant is already attributed to measures designed to protect the environment.

The West German environment council is nevertheless pressing for the emission levels to be further reduced, perhaps closer to the Japanese limit of 100–500 mg m^{-3}. Even so, new regulations would reduce total annual emission of sulphur dioxide to 0.55 million tonnes a year within 10 years. (The opening by *Rheinischen Braunkohlenwerke* AG of what will be the largest brown coal mine in the world, with reserves of 2,500 million tonnes to be exploited over the next 50 years, may make that harder to achieve.)

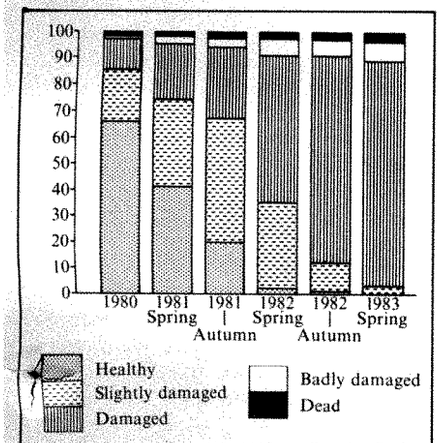
Nitrogen oxides from vehicle exhausts are another target for current concern. One controversial estimate is that the introduction of a speed limit, of 80 kilometres per hour in general and 100 kilometres per hour for autobahns, would reduce emis-

0028-0836/84/0308-01$01.00

© 1984 Macmillan Journals Ltd

sions of nitrogen oxides by 13 per cent. But the German speed lobby has more than a little in common with the gun lobby in the United States.

In the European Communities as a whole, forest covers one-fifth of the total land area (some 35 million hectares) and provides employment for 1.4 million people. Nevertheless, the EEC is an importer of wood, bringing in 60 per cent of its needs in 1980.



The health of silver fir Abies alba *in Baden-Wurttemberg. Adapted from a report of the federal technology ministry. Observations were made on 1,675 trees.*

Damage to forests such as has been found in West Germany is widespread throughout the Community and two proposals for directives are now being considered in Brussels. One would limit emissions from large installations to 250 mg m^{-3} by 1995 and reduce total emissions by 60 per cent for sulphur dioxide, 40 per cent for nitrogen oxides and 40 per cent for dust. The height of flue stacks would not be allowed to exceed 200 metres except under exceptional circumstances, limiting long range transport of pollutants. A second proposal on nitrogen oxides would forbid air concentrations above 200 mg m^{-3} for more than 2 per cent of the time. Both proposals include requirements for strengthening the monitoring system and supplying environmental information to Brussels.

Not all the economic considerations are negative. A study commissioned by the EEC estimates that air pollution from sulphur dioxide and nitrogen oxides costs the Community US $1,400–4,200 million a year without taking account of damage to historic monuments and setting the cost of damage to public health at only $20 million. The cost of control measures for large installations is estimated at $370 million per annum for 25 years.

Sarah Tooze

● THE environment committee of the European parliament has now entered the fray on acid rain. The committee last week tabled a heavily critical report based on public hearings and written evidence, in which it claims that damage caused by acid dust and rain goes beyond even the 3–5 per cent of European community gross national product estimated by the Organization for Economic Cooperation and Development. The report calls for heavy investment in smoke-scrubbing equipment, rapid legislation to limit emissions and for more research.

On Monday, the chairman of the committee, Scottish Labour MEP Kenneth Collins, said he hoped to put heavy pressure on the European Commission, and the decision-making council of ministers, to take action. There is "widespread agreement" in the council, said Collins, but one member state — the United Kingdom — was resisting all measures in the absence of incontrovertible evidence both of acid rain damage and of the origin of the acidity.

But evidence of this kind will never be watertight, says Collins. Moreover, the United Kingdom wished to wait at least until the findings of the Royal Society study, performed in conjunction with CEGB and the National Coal Board, and this might take five years.

Collins is hoping for some pressure on the United Kingdom from the French, during the present six months' French presidency of the European Council. France is only just beginning to feel the effects of acid rain, largely in the Vosges in the east of the country, but it is more willing to act than the United Kingdom, Collins believes.

Meanwhile further pressure may come from the environment committee of the UK House of Commons, which will begin an investigation of acid rain within the next few weeks. Members of the committee will be visiting European countries including West Germany and Scadanavia.

And acid rain is now beginning to cause fears outside Europe and the United States, according to the International Union for Conservation of Nature and Natural Resources (IUCN). In response to a worldwide request for information, IUCN has learned of fears in South Africa for the Eastern Transvaal Highveld, which lie downwind of coal-oil conversion plants and coal-fired power stations centred on Witwatersrand and Johannesburg, and acidification down to a *p*H of 3.7–4.7 in San Paolo State, Brazil. IUCN has established a task force to study the worldwide effects of acidification. **Robert Walgate**

Afforestation

UK woodland policy disputed

A REVIEW of forestry policy in the United Kingdom by the British Association of Nature Conservationists has now concluded that the decline of ancient semi-natural woodland in Britain is being encouraged by a tax loophole. The review, entitled *The Future of Forestry*, endorses a Treasury study of 1971 which found that new afforestation is not justified in the national interest either strategically or economically. That report was overtaken by political events and by a government decision in favour of afforestation.

Now, according to the conservationists' report, private foresters are able, by changing ownership when a plantation becomes profitable, to switch between tax schedules so that initial costs are set against other income while productive stands are taxed only at their land value. The Inland Revenue accepts that these provisions are being exploited by syndicates of high-rate taxpayers but appears unable to remedy the situation. The annual loss through this "unscrutinized subsidy" may be up to £25 million.

Plantation with conifers, which give better commercial returns, often has damaging effects on wildlife, says the association. This is true especially where a high proportion of the land area is covered, as in parts of southern Scotland. The association recommends that the Forestry Commission, which is now disposing of many of its plantations in accordance with government policy, should cease further coniferization and that it should recommend private foresters to do likewise.

The report also bemoans the conservation grant system which allows the Department of the Environment, through the Nature Conservancy Council, to buy woodland thought worthy of conservation from the Forestry Commission, which simply returns the money to the Treasury.

The Forestry Commission has yet to consider its reply to the charges made against it, but officials say that the decline of ancient woodland will not continue under present policies and that the commission already encourages plantation of broad-leaved woodland.

Private foresters are represented by the Royal Society of Foresters in England and Wales. Mr Esmond Harris, director of the society, objects to foresters' taxation arrangements being described as "manipulation". "They are," he says, "an incentive comparable with the government's Small Business Investment Scheme." And he argues that it is "sensible" to expand the home base.

Mr Harris also challenges the view that afforestation with conifers is damaging to wildlife interests. He claims the re-establishment of goshawks in Britain and the increasing numbers of golden eagles as proof of this, citing the support of scientists at the Institute of Terrestrial Ecology for this view.

According to Dr Ian Newton of the Institute of Terrestrial Ecology, the main complaint against coniferization is when it is done in blanket fashion. He also points out that, as the golden eagle needs open ground in which to hunt, its recovery can hardly be claimed as a success for forestry policies. **Tim Beardsley**

0028-0836/84/040309-01$01.00

© 1984 Macmillan Journals Ltd

UK research

Review of research by numbers

THE Prime Minister, Mrs Margaret Thatcher, announced in the House of Commons last week the publication of the first of a series of annual reviews of government-supported research and development. The need for such a review was argued in 1981 by the House of Lords Select Committee on Science and Technology, which doubted the ability of the Treasury adequately to judge individual departments' research programmes against their responsibilities.

The science and technology secretariat at the Cabinet Office has carried out the first review which enables some trends to be discerned. As a public document, the review is ostentatiously laconic, replete with figures showing the sums of money spent by various government departments (which are listed consecutively) but entirely free from value judgements.

Nevertheless, the review touches base with most of the issues now causing anxiety within the British system of research and higher education. It refers to the dual-support system (but does not say whether it has broken down), mentions the problems encountered by the research councils in paying overseas subscriptions (but recommending no specific remedies), and so on.

It is not immediately apparent whether this is the kind of document for which the House of Lords was asking two years ago, although the political problems that would arise if the Cabinet Office were seen publicly to have a view on how government departments conduct their affairs are readily appreciated.

In one respect, however, the review does break new ground in Britain by adopting as the basis for its calculations a set of definitions of basic research, applied research and development which were first promulgated by the Organization for Economic Cooperation and Development (OECD) in its "Frascati Manual".

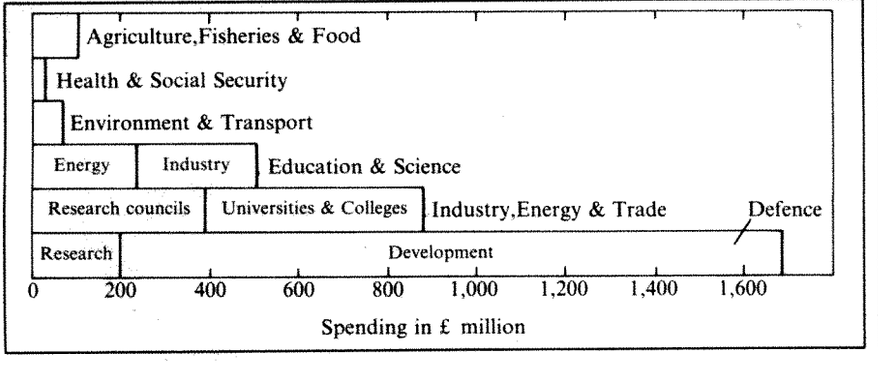
Evidently the Cabinet Office intends to apply these definitions to the classification of British research and development, raising in the process questions such as whether the collection of data as part of some routine operation, or the application of known knowledge to concrete tasks, should be classified as research.

On the figures now published, in which the most recent records are for 1981–82, defence spending shows the biggest increase between 1973/74 and 1981/82 while the proportion of all government-supported research and development by the Trade and Industry and Energy departments fell from 21.5 to 15.4 per cent over the same period. Expenditure by the Department of Education and Science shows a modest increase, from 24.6 to 27.4 per cent of the total.

The decrease in the figures for the Department of Trade and Industry is attributed to the running down of the programmes of support for the RB-211 aeroengine and Concorde civil aircraft. The large figure for defence includes the cost of development of specific items of equipment. □

● The increase in the share of the Department of Education and Science is a surprise. At a meeting in London last week, Dr John Burnett, Principal of the University of Edinburgh, argued that in the university sector, forward commitments on research equipment now exceed the ability of the system to pay for them. But the steep drop of support from the University Grants Committee in 1981 was preceded by a period of declining support for equipment and consumables, according to Sir David Philips, chairman of the Advisory Board for the Research Councils. And Professor John Kingman, chairman of the Science and Engineering Research Council, said that the diminished ability of his council to accede to all first-rate project grant applications was in part a consequence of the increasing sophistication of equipment but that government support for particular initiatives, such as that in information technology, had come out of the science budget. Not every body agrees that academic research is being hurt however. Drs Ben Martin and John Irvine of the Science Policy Research Unit at the University of Sussex argued that the evidence for a decline is largely anecdotal, and advocated a new approach to monitoring research output: the peer-review system, they said, might not be an adequate way of making decisions about closures.

Tim Beardsley



Information technology

Europlan in doldrums

ESPRIT, Europe's £1,000-million three-year plan for joint pre-competitive research in information technology, hung in the balance earlier this week as foreign ministers clashed in Brussels over the future finances of the European Communities. Esprit was to begin on 1 January, but the wrangles over British and German contributions to the EEC budget, and over Europe's enormous agricultural surpluses (bought in by Brussels at supported prices) have brought the project to a halt. On Monday the EEC commissioner for research, Viscount Etienne Davignon, was berating ministers for ignoring Esprit and he claimed, in effect, that the economic future of Europe might depend on it.

Davignon's future certainly does, for Esprit is the *leitmotif* of his efforts to use the Brussels machinery to establish a truly European high-technology industry and infrastructure. But is his moment passing?

Esprit is already formally approved, but will have no money, probably until Britain and West Germany agree to disentangle the issue from the wider problems of the community. British officials have been hopeful, West German officials non-committal, about the prospects of this happening. Meanwhile both countries have been forging ahead with their own national plans. In both countries, companies are clearly divided about allegiance to Esprit or a national programme.

And beyond that, three European companies — Britain's ICL, France's Bull and Germany's Siemens — have already established a joint research centre (in Munich) said to be going so well that other companies are beginning to see the advantages of purely private cooperation.

In Britain, the "Alvey directorate", running a five-year £500 million programme in information technology, is getting wind of a new industrial view: that Brussels should concern itself more with the problem of setting standards (communications and design protocols and so on) than with research itself. This is but a minor part of the Esprit concept as it stands.

What seems to have happened is that the seductive possibility that one nation might win the information battle alone has taken hold. According to Mr Brian Oakley, director of the Alvey programme, Britain, with by far the fastest-growing population of microcomputers in Europe, is faced with "an incredible opportunity". This may be for the wrong reasons — people are playing "Pac-man" and "Space Invaders" rather than writing programs. But Oakley believes there is no smoke without fire.

Oakley is also proud of his team in the directorate, most of them on secondement from industry and still paid by their original employers. This arrangment has

0028-0836/84/040310-01$01.00

© 1984 Macmillan Journals Ltd

caused some companies to fear loss of confidentiality, but those have been overcome, Oakley says. Even so, after Oakley's many years in government, he can say "I have never been in such a position where I can find out so rapidly exactly what companies think". With his £500 million, this gives Oakley a position of power and pulse-taking that Davignon or his staff would clearly like to exercise on the European scale.

There is some evidence that West Germany may follow the British lead. A plan for information technology is said to be in gestation — it will probably go to the German parliament in March, after the 28 February deadline Davignon seeks for agreement on Esprit. The upshot, that the two main complainants over the EEC budget may have made their own arrangements by them, does not augre well for Esprit.

Robert Walgate

East German chemicals

Back to coal

EAST Germany is planning a return to coal and, in particular, to domestically available lignite as the basis of its chemical industry, according to a report from the German Institute for Economic Research in West Berlin. Carbon chemistry has declined in importance in East Germany since 1975, with oil being used as the primary chemical feedstock.

Last November, at a meeting of the Central Committee of the United Socialist Party (SED), party leader Erich Honecker stressed that he attached the "greatest importance" to coal gasification and liquefaction. Current plans call for 80 per cent of the growth in lignite production to be allotted as a feedstock for the chemical industry. Expansion of coal tar and, above all, carbide production is a prime objective.

According to Mr Honecker, processing chemistry — including coal and lignite — ranks with biotechnology and microelectronics among the sciences which must "increasingly permeate" the East German economy. The target date for the large-scale liquefaction of lignite is, at the latest, 1992, with a planned annual processing of 10 million tonnes of lignite into fuel and liquid feedstocks. A team of 150 scientists at the carbo-chemical centre in Boehlen is said to be working on new sophisticated processes for lignite gasification and processing.

At present, however, the return to coal and acetylene seems to be based on a revival of old methods and technologies. This, according to the German Institute for Economic Research, involves a considerable loss of labour productivity and an increase in energy consumption, since in existing plants, nine to ten tonnes of raw lignite and a "very high input" of hydrogen are needed to replace one tonne of oil.

Vera Rich

US–Soviet space research

Moves for joint programme

MOSCOW radio's English-language service for North America has dropped several hints during the past few weeks suggesting a Soviet willingness to resume joint research projects in space. Such cooperation has, in fact, never entirely stopped. There is still some joint work on space medicine — including animal experiments on calcium loss due to weightlessness — and Soviet–American meetings of planetologists involved in Venus studies.

The recent hints suggest, however, that the Soviet side would like to see a resumption of plans for a joint manned mission. One commentator noted that there was at one time a plan for a link-up between the US space shuttle and a Soviet Salyut station. This, he claimed, was "unilaterally suspended" by the American administration, which has "increasingly been expanding military space projects at the expense of scientific research in space".

The same attitude of blaming the Americans for the cessation of these plans was adopted by Vladislav Dobrosel'skii, head of the external relations administration of the Soviet Academy of Sciences. He maintained that, in spite of highly-publicized official embargoes, the Washington government has quietly maintained a policy of preserving exchanges and contacts in areas where the Soviet Union is undoubtedly ahead. In the field of space medicine and biology, in which Soviet achievements are "obvious", the Soviet Union had however been guided by "humanitarian motives" in making their experience and knowhow available to the Americans.

Ostensibly, Dobrosel'skii's call was for a restoration of "broad scientific ties" between the United States and the Soviet Union. His stress on the Soviet lead position in space, and the advantages which, even now, the Americans receive from it, suggests that renewed cooperation in space was the prime purpose of the broadcast.

What political ambience the Soviet Union would demand as a prerequisite for a major joint space experiment has not, so far, been spelled out. (So far the commentators have simply urged that such cooperation would be conducive to peace and detente.) Dobrosel'skii himself urged that cooperation should be "on a footing of equality, without any discrimination" and "free of contingency fluctuations" (presumably of political origin). It is possible, therefore, that the suggestions were intended as a warm-up to the latest round of "confidence building" negotiations which opened in Stockholm last week.

It should be remembered, however, that in 1982, a Moscow radio commentator hinted that the United Kingdom could qualify, like France and India, for participation in the manned "Interkosmos" programme — provided it first rejected the siting of US missiles on its soil.

If the possibility of a joint United States–Soviet major space effort goes further than the recent preliminary kite-flying, it seems highly likely that some concession on arms limitation will be demanded by the Soviet side as a prerequisite to participation.

Vera Rich

UK observatories reprieved

GROUND-based astronomers in Britain and particularly those at the Royal Greenwich Observatory (RGO) should be breathing a sigh of relief this week. The Willmore panel set up to advise the Science and Engineering Research Council (SERC) on the future of the British observatories has recommended that RGO, the Royal Observatory Edinburgh (ROE) and Rutherford Appleton Laboratory (RAL) should all continue to be supported by the SERC. This recommendation has been accepted by the body directly responsible for UK astronomy, SERC's Astronomy Space and Radio (ASR) Board, thus stilling fears that RGO might be abolished in the near future (see *Nature* 301, 102; 1983).

The final decision, morever, rests with the council, due to meet in mid-February, although there is no reason to believe that it will not follow the recommendation. But the observatories will probably not escape totally unscathed, for the plans now laid for instrumentation of the new telescopes at La Palma and Hawaii will probably be cut back, or be streched out. Those working at RGO, who have been expecting to co-ordinate the remote operation by satellite of the La Palma telescopes, will for example be displeased by the news that the initial demonstration of this technique, both for La Palma and for the millimetre-wave telescope being constructed in Hawaii, will be carried out at the Edinburgh Observatory.

While these decisions relate to the long-term future of the ASR Board's activities, immediate problems remain. Deficits arising from the effects of exchange rate fluctuations on international subscriptions have been partly compensated for by the Treasury, to the tune of £7 million. But for the financial year 1984-85, the board still has to cut about £2 million from its original estimate. To achieve this, the board intends to cut funds from research grants by 5 per cent, RGO and RAL manpower by about 10 per cent and 15 per cent respectively and other institutional costs by about 10 per cent.

Philip Campbell

© 1984 Macmillan Journals Ltd

0028-0836/84/040311-01$01.00

India's science defended

SIR — Dr Malviya (*Nature* **306**, 10; 1983) says that scientists in India are "not terribly science-minded" and that there is no competition among them. I imagine that, science being universal, "competition" ought to be among the scientists in the world rather than among those of a given country. And if one wishes to compete for recognition in science, one obviously ought to be science-minded.

I believe that Indian scientists working in India are not all that bad. During the past decade at least seven of them have been elected to the Royal Society of London, others as fellows of academies in the United States, the Soviet Union and in European countries while some hold high offices in international scientific unions and the like.

India is a vast heterogeneous nation with pockets of brilliance where the scientists are very good, and also many places of mediocrity, not strikingly different from the spectrum of the quality of Indian expatriates abroad: some very good and some mediocre.

On the hostility of Indian scientists towards their countrymen abroad, the Indian National Science Academy has about 30 of them as fellows in a total membership of some 500, while the Indian Academy of Sciences, in its total fellowship of about 490, has more than 30 Indians settled abroad. And we have not heard of any such hostility from the more than 100 expatriate Indian scientists (Dr Malviya included) who have visited our laboratory at our invitation in the past 2 years.

The question of improving the quality of Indian research necessarily involves the creation and generous support of centres of excellence and this infusion of new blood in its universities and research centres by the creation of additional research chairs with good salaries and facilities. The last idea has already been taken up in the form of a cadre of "research scientist" positions at existing universities. Such positions are open to both residents and expatriates, as they should be. No special consideration for expatriates seems to be necessary.

In considering how to improve the quality of scientific research in India, an appreciation of the existing efforts and problems is essential. Simplistic solutions or blanket condemnations are of no value. I am thus particularly dismayed at Dr Malviya's claim that the people who control science in India are scientifically most incompetent. Those concerned, many of them FRSs, include those who brought in the green (and now the white) revolution, who have put up Indian satellites and sent Indian research vessels to the Antarctic, set up nuclear plants for power production, trebled the amount of irrigation water in 30 years and controlled communicable diseases.

These scientists advise the central cabinet of ministers on scientific matters. And it is this scientific competence, utilizing the science and technology base within, that has made India self-sufficient and not a market-place for giant multinational manufacturers.

Let us therefore not tie ourselves into knots of self-flagellation and also keep our temper — the scientific kind — so that we can attempt to solve our problems. (Incidentally, the issue of *Nature* that carries Dr Malviya's letter also carries an article on the cloning of rice embryo histone genes by Indian scientists working in India.)

D. BALASUBRAMANIAN
Centre for Cellular Molecular Biology, Hyderabad 500 007, India

Who's playing God?

SIR — In reviewing Paul Davies's *God and the New Physics* (*Nature* **305**, 833; 1983) Professor McCrea eschews "any close critical assessment of the work". This approach is akin to that followed by most theologians and practising religionists; to construct elaborate hypotheses based on conjecture and wishful thinking and not squarely to examine the evidence.

While not discounting Dr Davies's scholasticism, it seems a bit over-stated for McCrea to experience "humility" in the light of Davies's "courageous thinking". How courageous must one be, in these times, to espouse ideas dating back to Epicurus? Davies, like many who have preceded him, is merely attempting to intellectualize and dilute the god-concept in order to make it more palatable to those emotionally-weak individuals whose educations would normally preclude such irrational ideas, but who are nonetheless attracted to Universe-schemes which give them a sense of value and purpose other than that which they are able to create for themselves.

This type of accommodation of science by religion has been repeatedly used by the Church throughout the ages in order for it to remain afloat in a sea of scepticism. Any definition of "God" which is interchangeable with "Universe" (another vague term) or "all in all" is completely useless both as a separate concept and as a source of meaning in human existence. Is it not better respectfully to retire such antiquated ideas?

In a decade when science and education are continually being undermined and assaulted by creationists and other pseudo-scientists, it is high time that the scientific community should cease patronizing religion, which we have seen can be quite a formidable opponent to progress and the betterment of the human condition.

SCOTT A. KERNS
(Director, American Atheists, Houston Chapter)
Department of Microbiology, Baylor College of Medicine, Houston, Texas 77030, USA

Inviable usage

SIR — I share the concern indicated by Vimala Sarma, regarding the obfuscation in the recent report of the NH & MRC of Australia, which has adopted a recommendation on rules for fetal research (*Nature* **306**, 308; 1983).

Viable, which means capable of maintaining life, is an adjective which may be applied to the embryo, fetus or neonate; for, in the appropriate environment, each is indeed capable of maintaining its life, given adequate oxygen and nutrition, at least. "Previable" is a term which has no scientific basis, either in embryology or neonatology, to explain a given status of an embryo, fetus or neonate. The report itself indicates as much when it states — "dissection of the fetus should not be carried out while a *heartbeat* is still apparent or there are other *obvious* signs of life".

This seems a clear case of scientific jargon obscuring meaning.

PATRICK W. GILL
Neath General Hospital, Neath, Glamorgan SA11 2LQ, UK

New words for old

SIR — Your offer (*Nature* **306**, 134; 1983) to make space available for comment on the abuse of language is welcome. I submit a plea that when new words are coined, they should be unambiguous. It is a pity that the pedantic rule against mixing languages is so often violated. *Prebiotic* may be cited as an example: *probiotic or prevital* are preferable.

Some words, for example, *dyke* and *watershed*, have antithetical meanings. In Britain, *watershed* means the line separating catchment areas, and that is the metaphorical meaning in the United States. When used non-metaphorically in the United States, it means a catchment area. So *watershed* is a meaningless word in an international journal. Chemical logic has introduced a few possibilities of ambiguity in written English, although context usually distinguishes the two meanings of such words as *periodic* and *retinal*.

Ambiguity could be avoided if editors took more care to correct the carelessness or ignorance of authors. As T.S. Eliot remarked: ". . . (words) slip, slide, perish, decay with imprecision . . .". We are in danger of losing the distinction between *enormity* and *enormousness*, between *militate* and *mitigate*, and between *suck* and *suckle* (the baby sucks, the mother suckles). The original clearly defined meaning of *visualize* will be lost if the word is stretched to mean to stain or otherwise make visible. Perhaps another word is needed to carry that meaning — I suggest *iconize* or *phanerize*.

N. W. PIRIE
Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, UK

Dispute over scale of Universe

Controversy continues about the scale of the expanding Universe, with the two camps separated by a factor of two. Resolution of the conflict is more urgent than the disputants acknowledge.

OF all the natural constants, Hubble's constant must be the least deserving of the name. Those not directly concerned with the measurement of the distance-scale of the Universe may be forgiven if they harbour the impression that, for the past quarter of a century, the extant value of H_0 has stubbornly refused to settle down within some bounded range of all possible values, eventually to approximate ever more closely to what might be called a true value. Instead, successive estimates of H_0 seem to have fluctuated between two extremes differing numerically by a factor of nearly two.

Behind each value, there is a different school of opinion. Sandage and Tammann, whose latest estimate of Hubble's constant appears in this issue on page 326, are among those who hold that H_0, the measure of the rate at which the expansion velocity of the Universe increases with distance, is unlikely to be very different from 50 km s^{-1} per megaparsec (abbreviated to Mpc). The opposing school, represented for example by Dr G. de Vaucouleurs of the University of Texas at Austin, considers that the value of H_0 is more likely to be twice as large, approximately 100 km s^{-1} Mpc^{-1}. In some ways, the latest contribution from Sandage and Tammann appears to be a direct response to a challenge issued by de Vaucouleurs two years ago (*Nature* **299**, 303; 1982), when he argued that a careful examination of the hydrogen-line radio-emission from receding galaxies should provide one of five crucial tests of the conflict between the two distance scales.

This long-standing issue is unlikely, however, to be settled by the appearance of just one more contribution, and neither Sandage and Tammann nor de Vaucouleurs should be blamed for that. For one thing, the problem is uncommonly difficult, as Hubble himself recognized more than half a century ago. Essentially it is that of attempting to construct a distance scale applicable to objects in the Universe so distant that yardsticks usable on smaller scales (for example within our galaxy) are not directly applicable. Instead, they must be translated into secondary yardsticks by processes never free from assumptions about the physical nature of extragalactic objects (and even the space between them).

Then, the problem seems always to become more complicated. While the uniform (in space) and constant (in time) expansion implied by the definition of the Hubble constant may be a good first approximation to the Universe in the large, only in the past fifteen years has it become plain that we perforce look out at the Universe from an exceptional place, one near enough to the Virgo cluster for the motions of most nearby galaxies (on which previous estimates of distance have largely been based) to be streaming towards its centre of gravity, and even for the motion of our own galaxy (as measured by the anisotropy of the microwave background radiation) to be influenced as well.

Inivitably, these tenuous chains of inference run into serious trouble in the most distant reaches of the Universe, where the galaxies may be physically different (witness the quasars) from those in our immediate neighbourhood. One practical consequence is that people seem for the time being to have abandoned hope (still strong less than a decade ago) of being able to determine accurately from direct observation any but the first linear approximation to the expansion of the Universe. The Hubble constant may be uncertain, but the the range of values admissible for the deceleration of the expansion — the next approximation — is so great that the argument whether the Universe is open or closed has reverted entirely to the theorists.

The article by Sandage and Tammann will find a wide hearing simply because it offers a novel test of a sensible prediction of the relationship that should exist between the intrinsic luminosity (or mass) of a galaxy and the maximum velocity of its interstellar gas, predominantly hydrogen. Crudely, the greater the mass within some distance of the centre of a galaxy, the greater will be the gravitational potential at that distance so that, other things being equal, the greater will be the velocity of freely moving material at that point.

So, according to what is known as the Tulley–Fisher relationship, it should be possible to infer, from the broadening of say the 21-centimetre hydrogen line, the spread of velocities of hydrogen clouds within the galaxy and that, by comparison with other galaxies of the same type, should yield an estimate of the intrinsic magnitude of the distant galaxy. The use of the Tulley–Fisher relationship is not of course new; two years ago, de Vaucouleurs used exactly such an argument to support his view that the Hubble constant is roughly twice that now quoted by Sandage and Tammann.

How can such discrepancies arise in the analysis of the same data? And how will they eventually be resolved? The obvious difficulty is that the process of extending the distance scale beyond nearby galaxies is far from being objective. Whatever physical principle is used to help determine the intrinsic brightness of a distant galaxy, people will eventually be driven to select, from among all galaxies for which measurements are available, a sample which can be held to be comparable with each other.

The snag, as the experience of past decades has shown, is that this process of selection can also be a source of bias, involving as it does explicit or even hidden assumptions about the physical reasons why one type of galaxy differs from another. Indeed, these chains of inference are so complicated that many of the minor controversies that arise within the ambit of the larger controversy are discovered, in the end, to have arisen because some set of apparently independent measurements is found to hang on a few or even a single one of them.

The only remedy is the old remedy — more data. In the long run, the construction of a reliable distance scale will require such a detailed study of the properties of the galaxies involved that the propriety of the comparisons made can be verified directly. The search for novel ways of comparing the intrinsic brightness of near and far galaxies should of course continue (as it will), but there should by now be enough experience, most of it disappointing, for the practitioners to know that there is no magic solution on the cards.

What, in the meantime, is to happen to the controversy between the opposing camps, each of which is convinced of the correctness of its own estimates of the distance scale? Sandage and Tammann point out that their own estimate of Hubble's constant has the virtue of suggesting an age for the Universe as a whole which is more nearly in agreement with Astrophysical estimates of the age of the objects which it contains than the shorter time-scale implied by the longer distance scale of de Vaucouleurs and others (which does not, of course, prove that it is correct). This circumstance seems to infuriate the other camp, with the consequence that the controversy is no longer seemly. The ideal would be that those concerned should acknowledge that the crying need is for a reliable distance-scale, not for a proof that one or other of the two candidates now in the field is the better.

John Maddox

0028-0836/84/040313-01$01.00

© 1984 Macmillan Journals Ltd

Archaeology

Clues to recent human evolution from specialized technologies?

from Marek Zvelebil

A WEALTH of new studies of the technology used by hunters and gatherers to procure their food are making it possible to guess what part advances in technology played in human evolution. Particularly interesting are two recent analyses by R. Torrence[1] and R. Dennell[2], building on earlier work by Binford[3–6], of the way in which the development of specialized stone tool technologies allowed foraging societies dependent on a narrow range of resources to live successfullly in cold northern climates. The analyses show that devoting time to the production of special tools may have made possible the replacement of the neanderthal by modern man during the last glaciation, and the colonization of sub-Arctic areas some 30,000 years ago.

In their efforts to survive, prehistoric societies employed various technologies. The most ancient of them — that of stone — has been in use for more than two million years. But the making of tools takes time — time which could be spent on other activities, including the search for food. Benefits derived from the use of any range of tools must be carefully weighed against the time and effort needed to make them, and their manufacturer and the search for raw materials must be organized so that they complement other activities required for subsistence. In favourable environments, where resources are evenly distributed in space and over seasons, people can usually obtain their food with the aid of only relatively simple technology. Using data collected by Oswalt[7], Torrence[1] has shown that the use of simple general-purpose instruments and weapons is associated with hunter-gatherer societies that live in tropical and sub-tropical climates, where most of the food comes from stationary reliable plant resources. In contrast, where resources are seasonal and mobile, as in the Arctic and sub-Arctic, highly specialized complex tools are found that require considerable skill and planning to produce.

To exploit seasonal and mobile resources (the characteristics often occur together, as in migratory herbivores, water-fowl, seals or anadromous fish) requires a long time: both to locate prey (search time) and to capture it (pursuit time); but the time available is limited. Foragers can increase the reliability and productivity of their subsistence strategy by using time-saving devices: by budgeting their time and by preparing in advance more specialized but also more complicated tools, designed for each of the number of tasks involved (Fig. 1). In societies dependent on stationary ubiquitous resources, time is not so important and the use of a complex and specialized technology would not bring benefits that would justify the time and effort spent in its development.

In a recent publication, Dennell[2] linked the technological developments of the Upper Palaeolithic with the conceptual advances which marked the difference between modern man and neanderthals. Compared with the Mousterian — the technology which spanned the middle Palaeolithic — upper Palaeolithic industries were more complex: the number of techniques used for working raw materials increased, as did the range of raw materials; and the number of components making up an artefact increased, as did the diversity of tool types. The number of stages involved in making artefacts increased significantly; according to Dennell[2] it is this feature that marks the crucial difference between middle and Upper Palaeolithic technologies. The middle Palaeolithic Mousterian was essentially an expedient technology: tools were made for immediate needs in a short sequence of single-stage operations. It is perhaps for this reason that raw materials which required a large amount of time and labour to make into artefacts, such as bone, ivory or antler, were not used extensively in tool making until after 30,000 BP. In contrast, upper Palaeolithic artefacts were made in anticipation of future needs in a series of temporally and conceptually separate stages, employing a variety of different techniques and separate manufacturing processes. This required advances in planning and, possibly, advances in manual dexterity that might have been beyond neanderthals' mental and physical capabilities.

The similarity between the new upper Palaeolithic technology and the technology used by modern foragers in cold environments seems remarkable until we realize that both populations were trying to cope with the same type of time-stressed seasonal environments: the cold temperate and Arctic habitats, where resources tend to be not only seasonal and migratory but also to contain fewer spec[illegible] These characteristics mutually reinforce each other in encouraging the development of specialized technology: seasonality and

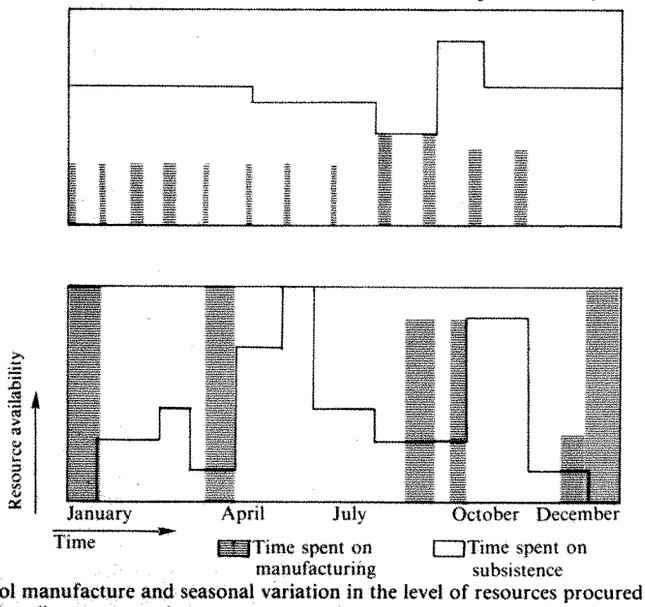


Fig. 1 Tool manufacture and seasonal variation in the level of resources procured. The upper diagram describes a system where permanent stationary resources are exploited using generalized technology (as, for example, in tropical gatherer communities). Little time is spent on manufacturing, which is evenly spread throughout the subsistence activities. The lower diagram shows how seasonal and migratory resources are exploited using specialized technology (for example by boreal hunter-fishers). Here, food procurement time is at a premium. Manufacturing time is greater and is located in periods of minimum subsistence activity.

1. Torrence, R. in *Hunter-Gatherer Economy in Prehistory* (ed. Bailey, G.) 11 (Cambridge University Press, 1983).
2. Dennell, R. *European Economic Prehistory* (Academic, London, 1983).
3. Binford, L. in *Stone Tools as Cultural Markers* (ed. Wright, V.S.) 24 (Australian Institute for Aboriginal Studies, Canberra, (1977).
4. Binford, L. *J. anthrop. Res.* **35**, 255 (1979).
5. Binford, L. *Am. Antiq.* **45**, 4 (1980).
6. Binford, L. *Nunamiut Ethnoarchaeology* (Academic, London, 1978).
7. Oswalt, W.H. *An Anthropological Analysis of Food Getting Technology* (Wiley, New York, 1976).
8. Dennell, R. *Nature, News & Views* **301**, 199 (1983).
9. Trinkaus, E. in *Mousterian Legacy* (ed. Trinkaus, E.) (British Archaeological Reports, Int. Ser. 164, Oxford, 1983).
10. Trinkaus, E. & Howells, W.W. *Scient. Am.* **241**, no.1, 94 (1979).
11. Brose, D.S. & Wolpoff, M.H. *Am. Anthrop.* **73**, 1156 (1971).
12. Klein, R.G. *Quat. Res.* 1 (1971).
13. Mochanov, Yu. A. in *Early Man in America from a Circum-Pacific Perspective* (ed. Bryan, A.L.) (Edmonton, 1978).
14. Morlan, R.G. in *Early Man in America from a Circum-Pacific Perspective* (ed. Bryan, A.L.) (Edmonton, 1978).

0028-0836/84/040314-01$01.00

© 1984 Macmillan Journals Ltd

mobility because specialized technology saves search and pursuit time; low diversity because specialized technology acts as an insurance against failure.

Organizational changes brought about by the use of specialized technology[1,5] must have aided upper palaeolithic man considerably to cope with the cold and seasonal environments of the temperate zone during the later part of the last glaciation[8]. In competition with these more flexible cultural adaptations, the physiological adaptations of neanderthal man to cold climate[9,10] proved inadequate. Assuming that in western Europe we can equate neanderthal man with the old Mousterian technology and modern man with the new — an assumption not universally accepted[11] — then the disappearance of neanderthals around 35,000–30,000 BP can probably be attributed to their failure to compete successfully with the better equipped *Homo sapiens sapiens* for the mobile and seasonal resources of the last ice age.

The observations made by Dennell and Torrence have some further implications. The failure of the neanderthals may illustrate the inability of human populations to survive and prosper in cold and seasonal climates without the aid of complex and specialized technology. There is no evidence of human occupation of Siberia before 35,000 BP, while the earliest reliable evidence for the presence of humans in North America dates to 27,000 BP (refs 12–14). It may be that successful colonization of these sub-Arctic regions (as they then were) could not have been accomplished without the technological and organizational advances attained by modern man. □

Marek Zvelebil is in the Department of Prehistory and Archaeology, University of Sheffield, Sheffield S10 2TN.

Optical bistability

Towards the optical computer

from S. D. Smith

NEXT month (21 and 22 March) the Royal Society will hold the first European conference devoted exclusively to optical bistability and photonic logic. This is a mark of the growing appreciation of the potential of these devices in the design of new kinds of computers that will not merely be faster than, but different from, the machines now being used.

Sequential binary logic has had success enough in the fields of signal processing, transmission and computing using one-dimensional electronic circuits as to be now accepted as one of the vital elements of our new industrial economies. But this familiar method, because it is sequential and because the signals are confined to a conducting wire, is clumsy and primitive. The disadvantages are overcome, say for a computer calculation or in building up a television picture, only by means of a large number of high-speed switching operations.

The possibility that optical devices might be superior goes back to 1969, when Abraham Szöke and colleagues at MIT put forward a first statement of a principle of optical bistability[1] based on the combination of nonlinearity, represented by saturable absorption, with positive feedback provided by an optical resonator. This led to the simple idea of an optical device consisting of a Fabry-Perot interferometer with an absorbing spacer layer illuminated by a laser and having two stable states — one at low power in which there is no interference and low transmission and one in which, at high power, the spacer absorption is saturated, constructive interference can take place and both transmission and internal intensities in the resonator are large. Once reached, the second state could in principle be maintained with a lower power than required to achieve it. The output and input of the device are thus related by a bistable loop (Fig. 1).

In reality, the effect was not observed until 1976, when a bistable optical effect was found in sodium vapour at the Bell Laboratories[2]. But analysis showed that the effect was due to refraction, not absorption, almost coincidentally with the publication of a theory of refractive optical bistability[3] based on the idea that refractive index can vary with light intensity. In retrospect, it turns out that absorptive bistability is much less easily observed than refractive bistability, basically because of the greater sensitivity of the interferometric effects when a resonator length is tunable through the intensity dependence of refractive index.

The device operates as follows (see Fig. 2). An interferometer is illuminated by a laser at a wavelength slightly detuned from resonance at some intensity (called the 'holding power'). Increase of intensity changes the optical thickness, moving the device towards the resonance. But, as resonance is approached, the ratio of the internal field to the incident field increases as interference becomes more constructive. Because it is the internal intensity that causes the nonlinearity and hence the optical thickness change and the rate of tuning, positive feedback is provided. The rate of tuning increases dramatically and the resonator can switch in a bistable manner.

The intrinsic interest of optically bistable effects caused an outburst of theoretical work, mainly concerned with two-level atomic systems, but the most significant steps towards a potential new technology were perhaps our discovery in 1976 at the

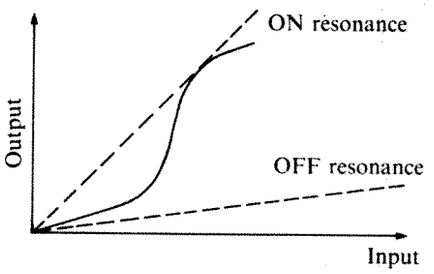


Fig. 1 Schematic relationship between input and output light intensities for a bistable device (full line). The sharp increase of output with input in the middle of the diagram explains how such a device may be used as a fast switching device.

Heriot-Watt University in Edinburgh of giant band-gap resonant nonlinear refraction in a narrow-gap semiconductor[4] and of similar effects, albeit a thousand times smaller, near the exciton frequency in GaAs by a Bell Laboratories group[6] in 1979.

The first discovery, giving a nonlinear refractive index n defined by $n = n_o + n_2 I$ of the order $n_2 = 1\ \mathrm{cm}_2\ \mathrm{kW}^{-1}$, meant that nonlinear optics was transformed from megawatt to milliwatt powers. The origin of this previously unnoticed effect lies in the 'blocking' or saturation effect of promoting electrons into states near the band-gap, with consequent large changes in the real part of the dielectric constant. The development also meant that devices a few micrometres in dimensions could be constructed. Thus in 1979 we[5] and the group at the Bell Laboratories[6] simultaneously reported the observation of optical bistability in small semiconductor resonators. Our group at the same time described 'transphasor' (that is, optical transistor) action with a two-beam signal gain of about 10 (see text below and Fig. 6).

These experiments have now set the scene for a complete family of optical circuit elements[7] (see Figs 3–5). Simply by adjusting the initial detuning, either single-valued characteristics with steps of slope greater than 1 (gain) or bistable loops of variable width can be obtained. Moreover, a system such as InSb at 77K can be held by

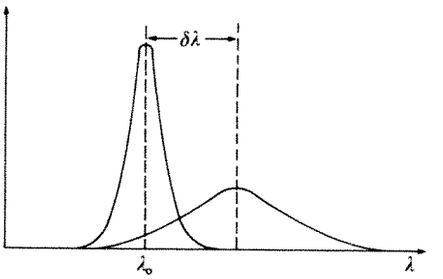


Fig. 2 Change of state of nonlinear refractive optical resonator with increasing light intensity. The refractive index n is $n_0 + n_2 I$, where n_0 and n_2 are constants, and I is light intensity. Increasing intensity increases n and thus reduces the wavelength λ to coincide with a value λ_0, an integral sub-multiple of the length of the resonator.

0028-0836/84/040315-01$01.00

© 1984 Macmillan Journals Ltd

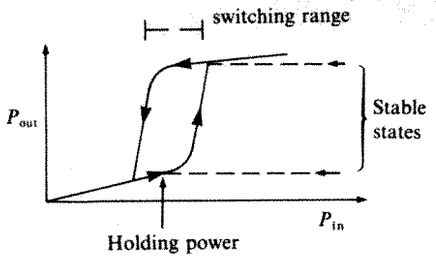


Fig. 3 Bistable optical element as a binary memory device.

a continuous bias beam at a given point on the characteristic and then addressed by a light pulse, and such a system was used in 1982 to demonstrate optical AND/OR and NAND/NOR logic gates using both reflection and transmission[8].

In the past year, devices that function at room temperature have been made from a variety of systems such as GaAlAs multiple-quantum-well systems (Bell and Arizona, at 0.8 μm), Te (Jena, at 10.6 μm), InSb (Heriot-Watt, at 10.6 μm) and liquid crystals (Berkeley, visible). At the same time, other narrow-gap semiconducting materials have been introduced such as CuCl (Strasbourg, visible), CdS (GTE and Frankfurt), CdHgTe (RSRE) and InAs (University of Southern California) at cryogenic temperatures. The speeds of these devices and the light intensity required vary considerably, and with the present rapid progress, it is too soon to know which materials will prove most suitable for which purpose. But many systems suggest switch-up times of the order of picoseconds. Fast switch-down has yet to be confirmed, but seems possible.

Novel approaches to either the geometry of the feedback process (such as focusing) or the nonlinearity have also recently been pursued. One intriguing development has been the use of radiation pressure[9] on a freely mounted mirror forming part of a Fabry-Perot device (Max Planck Institute for Quantum Optics, and the University of Munich). In this experiment, the length of the resonating cavity is changed simply by using the radiation pressure impinging on the mirror *in vacuo*. The system is clearly sensitive to vibration and, indeed, has already been recognized as constituting a sensitive seismometer. Direct conversion of acoustic waves to optical modulation (a microphone for optical fibres?) is another possibility.

With the development of this growing family of optical devices based on optical bistability, all the elements required to make an optical computer have now been demonstrated. Where will this lead? If devices based on optical bistability are to compete with those of other switching technologies solely in terms of speed and power, their application would be limited by the continuing advance of silicon technology. But optical devices have qualitative advantages. A lens or mirror can easily communicate thousands of channels of information in parallel, while one optical beam can be passed through another without interference.

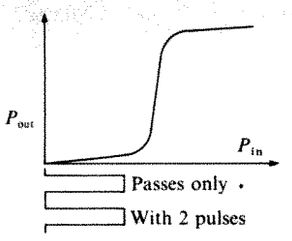


Fig. 4 Optical logic element as an AND gate, giving significant output only when two pulses separately not intense enough to switch states coincide in time and exceed threshold.

By contrast, electronic signals must be individually guided and protected from interacting, and in the design of future electronic computers, switching speeds will certainly increase as very large-scale integration (VLSI) proceeds, but so will the band-width needed for the interaction of components. But communication usually entails some circuit capacitance, and as sizes are reduced, time-constants increase.

In designing a computer architecture to take advantage of the new optical circuit elements, it may well be that interconnection time and the use of parallel channels will be the key points. The 'van Neuman bottle-neck', perhaps the fundamental limitation of digital computers, is the trade-off time for interconnections needed to make up for the inability of electronics to support multiple communications channels in parallel. These are the processes that limit the time taken for a computer to perform a given task.

At this stage, only general statements can be made about the form which an optical computer may take. One of the concepts being investigated, for example, is a two-dimensional array of optical NOR gates with optics, including prisms and lenses, capable of offsetting an output image of the NOR gate array by one pixel and then feeding the output image into the input. More prosaically, if the performance of optically bistable devices at low power

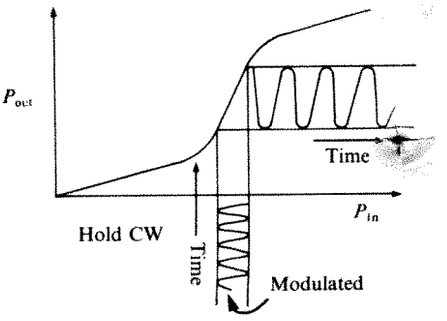


Fig. 5 Transphasor (signal amplifier) in which optically bistable element is held at linear part of input–output relationship. The gain of this device is given by the slope of the curve and may be as great as 10,000 (see text).

levels presently obtained in the infrared can be reproduced with suitable materials in the visible spectrum, technically simple optically-addressed display technology would become available. Power limiters for laser pulses are certainly a practical proposition and, since the most recent work at Edinburgh has demonstrated a transphasor gain of 10,000, all-optical repeaters for fibre communication lines may be possible. Further, incoherent light, which could be in the form of an image, has been shown to cause bistable switching and amplification strongly suggesting that the technique will be useful in image processing. □

S. D. Smith is Professor of Physics, Heriot-Watt University, Edinburgh EH14 4AS.

1. Szöke, A., Daneu, V., Goldhar, J., & Kurnit, N.A. *Appl. Phys. Lett.* **15**, 376 (1969).
2. Gibbs, H.M., McCall, S.L. & Venkatesan, T.N.C. *Phys. Rev. Lett.* **36**, 1135 (1976).
3. Felber, F.S. & Marburger, J.H. *Appl. Phys. Lett.* **28**, 731 (1976).
4. Miller, D.A.B., Mozolowski, M.H., Miller, A. & Smith, S.D. *Opt. Commun.* **27**, 133 (1978); for earlier reference to the experimental discovery, see Ironside, C.N. thesis, Heriot-Watt Univ. (1978).
5. Miller, D.A.B., Smith, S.D. & Johnston, A. *Appl. Phys. Lett.* **35**, 658 (1979).
6. Gibbs, H.M. *et al. Appl. Phys. Lett.* **35**, 451 (1979).
7. Abraham, E. & Smith, S.D. *Rep. Prog. Phys.* **45**, 815 (1982).
8. Seaton, C.T., Smith S.D., Tooley, F.A.P., Prise, M.E. & Taghizadeh, M.R. *Appl. Phys. Lett.* **42**, 131 (1983).



100 years ago

GEOGRAPHICAL NOTES

THE first report of Prof. Hull, dated from Gaza, January 1, has been received. It is necessarily brief, the details being reserved for the full report to follow, but it announces the success of the expedition so far. The professor has made a complete geological survey of the Wady Arabah and the Dead Sea, with a traverse across Southern Palestine. Capt. Kitchener, R.E., who accompanied him, has made a trigonometrical survey. Akabah he found to be laid down too far south; the south part of the Dead Sea as shown on the maps, is quite out of its true shape and position, and the Lisan has to be shifted three miles. From Gaza, when the rest of the party were in quarantine, Capt. Kitchener rode back to Egypt, accompanied by four Arabs only. He took a previously unknown route, particulars as to which will follow, and arrived at Ismailia after a ride of 200 miles. He was everywhere well received by the Arabs, who took him for a cousin of Sheikh Abdullah (the late Prof. Palmer), whose memory is still revered among them, and whose murder they still deplore. They are also reported to be deeply impressed with the energy and pertinacity of Sir Charles Warren's pursuit of the murderers.

From *Nature* **29**, 318; January 31, 1884.

0028-0836/84/040316-01$01.00

© 1984 Macmillan Journals Ltd

Immunology

Mechanism of T-cell tolerance

from Roger B. Taylor

IN spite of the burgeoning literature on suppressor cells and immunological networks, there has been persistent support for the notion that responsive lymphoid cells may be specifically deleted. This term is now broadly interpreted to mean some internal change occurring in the cell to render it either temporarily or permanently inactive.

We already know that B cells, especially in their immature stages, can enter a condition of lasting unresponsiveness — sometimes accompanied by a defect in the expression of receptors. So far, however, the evidence for deletion of helper T cells has been restricted to experiments *in vivo*, almost entirely in mice, using mammalian serum proteins in high dosage as tolerogens.

To be certain that no suppressor influences are at work one should ideally study tolerance in monotypic cell populations, and preferably clones, *in vitro*. It was therefore a breakthrough, important in more than one sense, when a group working at University College, London, recently achieved the induction of tolerance in human helper T-cell clones *in vitro*[1].

These clones were specific for peptides derived from the influenza haemagglutinin molecule, and were propagated by stimulation with the specific peptide in the presence of unseparated human mononuclear cells which served as antigen-presenting cells. After such stimulation the cells would both exhibit a proliferative response and help B cells to produce antibody. Lamb and his co-workers then tried incubating the clones with high concentrations of the peptides, in the absence of the human mononuclear cells. On subsequently testing the response to the usual low stimulatory concentrations in the presence of the human mononuclear cells they found that the T cells had become specifically unresponsive. The tolerized cells would continue to divide in the presence of interleukin-2 but only for a period of 1–2 weeks, after which their viability declined. A critical finding was that the 'tolerized' cells had lost the surface marker T3 which is thought to be closely associated with the antigen receptor[2]. It confirmed that some demonstrable change had occurred in the cells, and thus argued that the tolerance was not due merely to blocking of the receptors by the high concentration of peptide. A parallel finding was made by Schlossman's group in Boston, who induced a similar state of deletion in human T cells by means not of antigen but of anti-receptor antibodies[3].

Against this background, interest now focuses on the mechanism by which such deletion comes about. A pertinent report has recently appeared from Schwartz's group at the National Institutes of Health, on cloned mouse T cells[4]. These clones, specific for pigeon cytochrome *c*, were propagated by stimulation with this antigen on irradiated mouse spleen cells or a B-lymphoma cell line as antigen-presenting cells. The proliferative response was measured in the presence of varying concentrations of both antigen and the relevant Ia. (The latter was varied by changing the numbers of presenting cells, blocking with anti-Ia antibody, or by the choice of cells homozygous or heterozygous for the relevant Ia.) Bell-shaped curves were obtained, in which the response was inhibited at high concentrations of either antigen or Ia or of both. A clear relationship emerged whereby the response depended on the product of the concentrations of antigen and of Ia. One implication is that a physical product of these two molecules must be the critical driving force for T-cell activation. For the high-dose inhibition to be classed as tolerance, it would need to render the cells refractory to subsequent stimulation with optimal doses. This has not yet been reported. Nevertheless the similarity to the human T-cell work is obvious.

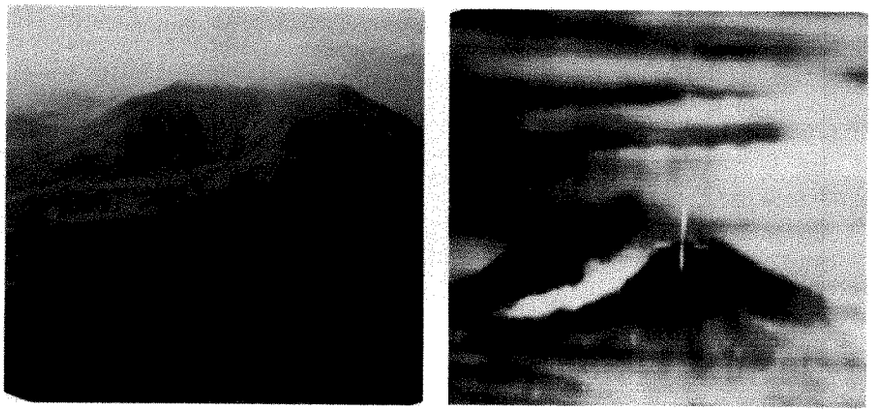
The question now arising is how the T cell is inhibited. Does the occupation of a high proportion of receptors release a negative signal to the cell interior? Or does intense stimulation cause the cell to release critical amounts of an external feedback signal? The authors suggest that immune interferon could serve as such a feedback control — particularly as its production is not subject to the same degree of inhibition at high doses of antigen as the proliferative response[5]. There is no evidence so far, however, that the inhibitory effects of interferon on immune responses can persist long enough to be classed as tolerance.

Interferon can also enhance immune responses, as exemplified by a paper in this issue of *Nature*[6] (see p.381). It is shown that interferon both increases antibody responses *in vivo*, and also increases the expression of Ia on an antigen-presenting macrophage cell line. The authors suggest that the effect of interferon on the antibody response is achieved by virtue of its effect on Ia expression. If this is the case, then the bell-shaped curve derived by Schwartz and colleagues leads us to expect that, at high concentrations of antigen, interferon should aid the induction of tolerance rather than immunity. Its activity might then be seen as to shift the curve to the left, and so influence the quantity of signal delivered via the receptors rather than acting itself as a negative signal. This might also be the role of a variety of other 'adjuvant' influences, many of which can have either positive or negative effects in different circumstances. □

Roger B. Taylor is in the Department of Pathology, University of Bristol, The Medical School, University Walk, Bristol BS8 1TD.

1. Lamb, J.R., Skidmore, B.J., Chiller, J.M. & Feldmann, M. *J. exp. Med.* **157**, 143 (1983).
2. Lamb, J.R. & Feldmann, M. *Nature* **300**, 456 (1982).
3. Meuer, S.C. *et al. J. exp. Med.* **157**, 705 (1983).
4. Matis, L.A., Glimcher, L.H., Paul, W.E. & Schwartz, R.H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6019 (1983).
5. Hecht, T.T., Longo, D.L. & Matis, L.A. *J. Immun.* (in the press).
6. Nakamura, M., Manser, T., Pearson, G.D.N., Daley, M.J. & Gefter, M.L. *Nature* **306**, 381 (1984).

Latest view of Mount St Helens



MOUNT St Helens photographed during its eruption on 18 May 1980 in normal light (top), and in a recently-released US Navy infrared image. White areas on the infrared image represent hotter areas of the volcano and allow the lava flow near the crater and thermal events not usually seen by the naked eye to be detected. The infrared image was taken from a Hughes A-6E Intruder aircraft using its 'Detecting and Ranging Set', a thermal imaging sensor which, when not photographing volcanoes, helps the Intruder's aircrew see and attack surface targets at night or through smoke and haze. □

0028-0836/84/040317-01$01.00

© 1984 Macmillan Journals Ltd

Molecular biology

Genetic noise in evolution?

from Darryl Reanney

IN 1949 Shannon[1] published an important theorem which states, in essence, that information cannot be transmitted over long periods of time without experiencing some deterioration in quality due to 'noise' in the copier process. Nucleic acids obey this general law as the information they encode is degradable by errors in the copier machinery or by agents such as heat, radiation and mutagenic chemicals[2].

How important has noise been in evolution? The error frequency in DNA replication in modern eukaryotes is about one incorrect nucleotide incorporated for every 10^9–10^{11} bases polymerized during any one round of replication[3]. But studies on non-enzymatic synthesis in simulated prebiotic environments suggest that the error rate in the 'first' genes was of the order of 10^{-1}–10^{-2} substitutions per base per generation[4,5]. Thus as we go back in evolution the amount of noise in the genetic copier mechanism rises by a staggering factor of one hundred million or more.

The amount of information a self-reproducing system can stably maintain is inversely proportional to the error rate of the copier system[5]. Since the accepted mutation rate of enzymatically catalysed polynucleotide synthesis in the absence of repair is about 10^{-3}–10^{-4} (refs 5,6) one can predict that the maximum amount of information encodable in a single genetic molecule at this stage of evolution was of the order of 10^5 nucleotides[5] (in point of fact it was probably somewhat less because such a system could not correct errors introduced by external factors such as heat, radiation or *p*H). In this kind of situation selection would almost certainly favour systems with multiple copies of their genomes since redundancy makes it possible to mask the deleterious effects of harmful mutations by complementation[7]. Thus I believe we can be reasonably confident that early genetic systems were 'polyploid' in the sense that their genetic information was represented several times over. This contrasts sharply with the traditional view that early cells were haploid.

Self-replicating error-prone genomes are today found only in the RNA rivuses and of these only the retroviruses possess the genome redundancy postulated to have been a key feature of early genetic systems. Retroviruses are important however because they offer a significant contemporary insight into the influence of noise in evolution. Viruses of this group contain a reverse transcriptase enzyme which makes DNA on a RNA template. Reverse transcription is the only surviving mode of DNA synthesis which is about as error-prone as RNA synthesis[8]. On passing from single-stranded RNA (the free virus) to double-stranded DNA (the integrated nuclear copy) retrovirus information experiences a millionfold drop in noise levels (see the figure). When this information is transcribed back into RNA the error rate rises by a comparable factor because DNA-dependent RNA synthesis lacks corrective functions. It is difficult to believe that these passages between low- and high-fidelity copier systems have not affected the evolutionary success of retroviruses. When integrated into cellular DNA the viral genes can exploit the repair systems of their host chromosomes and thus maintain strict homogeneity of nucleotide sequence during copying. On re-entering an independent life, the virus can offer a spread of variant sequences (that is survival options) to the test of selection. Retroviruses have the best of both worlds.

The integrated genomes of retroviruses have some tantalizing similarities to certain families of repeated sequences in the mammalian genome such as the *Alu* family[9] and the genes encoding some small nuclear RNAs[9,10]. Thus it is not surprising that these families show evidence of having passed through a RNA phase followed by reverse transcription and re-integration into chromosomal DNA[9,10]. The same seems to be true of 'pseudogenes' — partial duplicates of structural genes, many of which lack introns, have a 3′ poly(A) 'tail' and generally resemble mRNAs that have been reverse-transcribed back into DNA. The discovery of pseudogenes for mouse α-globin, human κ immunoglobulin, human β-tubulin, human metallothionein and rat α-tubulin[9] suggests that potentially any sequence in a higher-cell genome can pass through a RNA → DNA pathway. Reverse transcription thus closes the flow of information in higher cells into a loop which causes genes to alternate between low- and high-error copying systems.

Could such a cycling of information make a significant contribution to evolution? In assessing this question it is important to recognize the amount of RNA variation that is available. Since transcriptional copying in modern cells retains the high error rate (10^{-3}–10^{-4}) characteristic of uncorrected nucleic acid synthesis (Fig. 1) most transcripts of nuclear genes may contain at least one error. The amount of sequence polymorphism present may be magnified by the ability of the RNA processing mechanism to direct appropriately miscopied RNAs into a variety of alternative splicing pathways[11]. Given that a single gene in a differentiated cell may generate 50,000 copies of its cognate mRNA[12] it follows that the population of RNA molecules in a clone of cells can experience in a matter of months a degree of sequence variation which their DNA genome in the absence of selection might take hundreds of thousands of years to achieve. The key question is whether such variations can find their way back into the genome?

Two known mechanisms are available. A processed gene may 'donate' its RNA-based mutations to its parent gene by gene conversion, a process which seems to take place rather frequently in higher cell genomes[13]. Indeed, if a cDNA reverse-transcribed from mRNA were matched against its original gene, then conversion in one direction could imprint the restructured intron-deficient or intron-lacking sequence into its genomic homologue while leaving the promotor signals intact. This would allow processed information to feed back into the genome in functional form. It is also possible that RNA molecules transcribed from transposon-like elements may be reverse-transcribed into a heterogeneous pool of 'nomadic' plasmid-like DNA rings as has been observed in the case of the *Alu* family of sequences[14]. These DNA rings would be potentially genetically transmissible through the germline in a non-mendelian fashion without necessarily integrating into chromosomal DNA. Thus cells clonally derived from a common ancestor may become polymorphic in those molecules which have passed through an RNA phase while retaining rigorous sequence identity in the original DNA genes. In this way new adaptive possibilities could be canvassed without erasing the existing phenotype.

Given that effective mechanisms for promoting the RNA-to-DNA pathway exist it is more difficult to assess how often this route of information transfer has been used in evolution. It has been suggested that perhaps 20 per cent of the mammalian genome originated as RNA that was written back into DNA[10]. In many systems the figure may be higher. If one assumes that pseudogenes with 15–30 per cent divergence have been seen or inferred for 10 per cent of genes one can say there has been a reintegration event in the germline for about 10 per cent of genes within the last 10–20 million years. This corresponds to about 0.5 per cent to 1 per cent of genes per million years or once for each gene every 100–200 million years. On the other hand it must be admitted that there is no hard evidence that any functional eukaryotic genes are derived from reintegrated mRNAs since known higher-cell genes (with the exception of the α and β interferons and the histones) contain introns. To date the most likely candidate for a one-way gene conversion event is the rat insulin gene which has two alleles, one of which contains two introns while the other contains only one[10].

While there is no evidence for active 're-integrated' genes in eukaryotes it seems likely that the passage of information through the DNA → RNA → DNA loop has been a factor in the modular evolution

0028-0836/84/040318-01$01.00

© 1984 Macmillan Journals Ltd

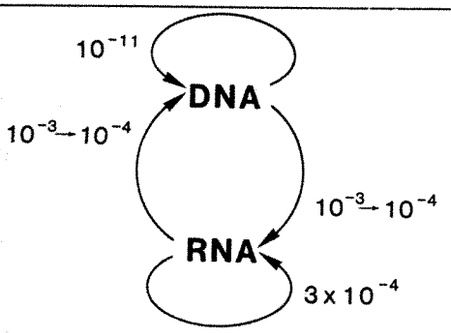


Fig. 1 Information cycles in genetic systems. The error-prone DNA → RNA step occurs at high levels in active cells during the normal course of protein synthesis. The error-prone RNA → DNA step occurs as an essential part of the life cycle of retroviruses. There is now good evidence that cellular sequences may also follow this pathway on occasions. If one assumes that pseudogenes with 15–30 per cent divergence exist for about 10 per cent of genes one can estimate that reverse transcription of a cellular sequence followed by re-integration occurs in the germ line once for each gene every 100–200 million years. This is slow but significant on the evolutionary time scale. The actual rate may of course be much higher.

of split genes. Blake and others have suggested that exons correspond to discrete peptide substructures which often have specific functions[15,16]. Each time a mosaic gene (in whole or part) cycles through an RNA intermediate back into the genome, intron removal knits the exons together into more complicated structures. Such multi-exon aggregates would normally be inactive but they could regain activity by conversion, or by incorporation into other transcriptional units followed by mutation. Such processes would, over evolutionary time, increase the complexity of protein structure by building more and more functional capacities into the one polypeptide. On this basis older genes would consist of very short exons embedded in an excess of 'junk' while modern genes would contain fewer introns and more complex exons.

Many contemporary genes bear the stamp of this kind of process. According to Gō, the globin gene, which has two introns, is made up of four peptide modules, not three (see ref. 17). Gō believes the now contiguous central coding region of the gene was once split by an intron[17] which has since been precisely removed, presumably by passing through an RNA processing stage. Similar processes seem to have occurred in the genes for egg white lysozyme and cytochrome *c*[17]. The end products of this bit-by-bit process of protein evolution are the uninterrupted genes of prokaryotes, where, according to Doolittle[18] and others, all introns have been removed in the interests of efficiency and economy.

Apart from its possible relevance to protein evolution the ability of nuclear information to occasionally pass through a noisy RNA copier has implications for students of evolution who seek to estimate times of (recent) evolutionary divergence by comparing the base sequence of an intron-lacking pseudogene with that of its intron-plus parent (see for example ref. 19). Such calculations should be tempered by the knowledge that a molecular clock based on RNA runs at a much faster rate than one based on DNA.

While DNA is a high-fidelity copier system this very stability may deny it a preponderant role in short-term adaptation. By contrast the noisiness which makes RNA a genetic liability (where 'large' genomes are concerned) also makes it an ideal channel for the controlled introduction of novelty. In a limited but important sense then DNA may be regarded as the guardian and RNA the modifier of genetic identity. By switching back and forth between these extreme levels of noise evolution may have struck an effective balance between conservatism and change. □

Darryl Reanney is Senior Lecturer in Microbiology at La Trobe University, Bundoora, Victoria, Australia 3083.

1. Shannon, C.E. in *The Mathematical Theory of Communication* (eds Shannon, C.E. & Weaver, W.) (University of Illinois Press, Urbana, 1949).
2. Drake, J.W. *Symp. Soc. gen. Microbiol.* **24**, 41 (1974).
3. Kornberg, A. *DNA Replication* (Freeman, San Francisco, 1980).
4. Inoue, T. & Orgel, L.E. *Science* **219**, 859 (1983).
5. Eigen, M. & Schuster, P. *Naturwissenschaften* **64**, 541 (1977).
6. Domingo, E., Sabo, D., Taniguchi, T. & Weissman, C. *Cell* **13**, 735 (1978).
7. Reanney, D.C., MacPhee, D.G. & Pressing, J. *Aust. J. biol. Sci.* **36**, 77 (1983).
8. Battula, N. & Loeb, L. *J. biol. Chem.* **250**, 4405 (1975).
9. Sharp, P.A. *Nature* **301**, 471 (1983).
10. Lewin, R. *Science* **219**, 1052 (1983).
11. Mount, S. & Steitz, J. *Nature* **303**, 380 (1983).
12. Long, E. & Dawid, I. *A. Rev. Biochem.* **49**, 727 (1980).
13. Dover, G. *Nature* **299**, 111 (1982).
14. Shmookler Reis, R. *et al. Nature* **301**, 394 (1983).
15. Blake, C.C.F. *Nature* **277**, 598 (1979).
16. Inana, G. *et al. Nature* **302**, 310 (1983).
17. Lewin, R. *Science* **217**, 921 (1982).
18. Doolittle, W. *Nature* **272**, 581 (1978).
19. Lemischka, I. & Sharp, P.A. *Nature* **300**, 330 (1983).

Cell patterning

Plants under strain

from Clive Lloyd

BEING nasty to your houseplant might put it under strain but, for a plant developmental biologist, the word strain takes on a different meaning: it denotes those tensions, propagated across cells and tissues, that have been hypothesized to orientate the insertion of new cross-walls, and so play a part in cell patterning. Scientists have been trying for some time to come to terms with the forces that influence the partitioning of cells in tissues. Observation of groups of cells can persuade that purely physical rules are involved and nineteenth century scientists (and, later D'Arcy Thompson) compared the behaviour of young cross-walls, of which no more than three walls are seen to meet at one point, to liquid films. But bricks, rather than bubbles, provided the analogy for Sinnott and Bloch's pioneering work in the 1940s.

They noted that when a new cross-wall formed in one file of cells, it avoided lining up with a cross-wall in the neighbouring file — much as bricks are staggered between courses in a wall. However, they also recorded that there was no such avoidance in cells induced to divide by wounding; thereby illustrating that previously formulated 'rules' are frequently broken[1,2] because the quality of the response seems to be affected by local conditions. Other examples of 'rule breaking' appeared. New cell walls may initially be formed parallel to the sides of the mother cell but, upon further centrifugal development, the growing cell plate can swerve towards one side, producing a smaller wedge-shaped cell — from a purely physical analogy it could be argued that the new cross-wall should be of the minimum surface area required to divide the cell.

The lasting contribution that Sinnott and Bloch[3] made to plant histogenesis was to emphasize that in vacuolate cells, a plate of cytoplasmic strands becomes positioned across the vacuole, with the ends of the strand attached to the mother cell walls along the line where the new cell plate will join them, before even mitosis has taken place. This structure, the phragmosome, can lay claim to the title of being the organelle least understood yet most likely to help our understanding of pattern formation — the task now being to find out more about its structure and about the forces that orientate it.

Forces that orientate the new cell plate are the subject of a letter in today's *Nature* by Lintilhac and Vesecky (see p.363). The point they make is that extracellular mechanical cues are sensed by cells in tissues and the new cell plate is orientated so that it is maximally protected from shear. Lintilhac and Vesecky studied tobacco pith, which, when taken into culture, grows into a disorganized callus. They attempted to re-establish ordered patterns of division by compressing callus in a frame, on the grounds that the *in vivo* mechanical

0028-0836/84/040319-01$01.00

© 1984 Macmillan Journals Ltd

environment for growth has been lost and cannot be restored by hormonal manipulation alone. After five days under compression, co-planar divisions were found in sections of calli. Allowing for the difficulty of obtaining uniform results from such variable material as callus it is intriguing that compression can yield co-planar divisions up to a depth of ten ranks of cells.

The observation will add fuel to an idea expressed at different times in different ways (see, for example, refs 4–7) that the development of plant form involves epigenetic control over the alignment of cross-walls through a programme of division and expansion which imparts each cell with a different positional value that is 'read' in terms of strain alignment of the cell plate.

At the cellular level, however, it is still not understood how the cell plate is aligned. Vacuolate cells, stimulated to divide by wounding the tissue, possess a circumferential band of microtubules[8] whose orientation forecasts the plane of division in much the same way that the pre-prophase band of microtubules does in meristematic cells (for a review, see ref. 9). However, the question of whether the pre-prophase band is the result or the cause of cytoplasmic polarization remains unresolved, although recently published observations by Galatis *et al.*[10] throw interesting light upon it.

In the stomatal complex of *Triticum* leaves, two pre-prophase bands, rather than one, are sometimes formed simultaneously within a single long cell. Following mitosis, a cell plate is deposited which curves from one of the cortical zones, previously marked by a pre-prophase band, to the other. Again, this behaviour is contrary to the minimal surface area hypothesis for cytokinesis. It is not known whether such curved cell plates are orientated by chemical or mechanical signals, but the observation that pre-prophase and telophase nuclei form acute local extensions (which these investigators liken to the pulling of the nucleus) hints at mechanical involvement. Unfortunately, all we have at the moment is hints; physical connections between nucleus and cortical cytoplasm are not yet resolved in higher plants but, if real, may provide a fuller understanding of how the growth of plant cells is patterned. □

Clive Lloyd is in the Department of Biology, John Innes Institute, Colney Lane, Norwich NR4 7UH.

1. Sinnott, E.W. & Bloch, R. *Am. J. Bot.* **28**, 607 (1941).
2. Sinnott, E.W. *Torreya* **43**, 29 (1943).
3. Sinnott, E.W. & Bloch, R. *Proc. natn. Acad. Sci. U.S.A.* **26**, 223 (1940).
4. Sobota, A.E. & Partanen, C.R. *Can. J. Bot.* **44**, 497 (1966).
5. Cooke, T.J. & Paolillo, D.J. *Am. J. Bot.* **67**, 1320 (1980).
6. Miller, J.H. *Am. J. Bot.* **67**, 534 (1980).
7. Lintilhac, P.M. & Vesecky, T.B. *Am. J. Bot.* **68**, 1222 (1981).
8. Venverloo, C.J., Hovenkamp, P.H., Weeda, A.J. & Libbenga, K.R. *Z. Pflanzenphysiol.* **100**, 161 (1980).
9. Gunning, B.E.S. in *The Cytoskeleton in Plant Growth and Development.* (ed. Lloyd, C.W.) 228 (Academic, New York, 1983).
10. Galatis, B., Apostolamos, P. & Katsaros, C. *Protoplasma* **117**, 24 (1983).

Astronomy

IRAS circular 6

The source name consists of four parts: (1) the letters 'IRAS' to indicate the origin; (2) the right ascension (RA) in hours and minutes, seconds omitted; (3) declination (Dec) in decimal degrees, multiplied by 10 and then truncated (thus +32° 42.3 becomes +327); (4) an appendix starting with 'P' and followed by the number of the circular; this appendix stresses that the data are preliminary. Position is given at Equinox 1950.0. The measurements were made between epochs 1983.1 and 1983.6. Sources labelled PO3 were published in IRAS circular no. 3 and are included for completeness.

Source IRAS	RA (1950) h min s	Dec (1950) deg arc min arc s	Identification	Flux density (Jy) 12 μm	25 μm	60 μm	100 μm
0125+848P03	01 25 27.9	+84 45 11	U1039	<0.2	<0.2	0.50	2.3
0353+261P06	03 53 19.8	+26 05 54		<0.4	<0.4	0.52	<1.5
0355+184P06	03 55 19.3	+18 26 32		<0.2	<0.2	0.62	<1.2
0356+202P06	03 56 05.1	+20 11 56		<0.7	<0.3	0.57	<1.7
0356+217P03	03 56 33.0	+21 39 16	ZW487.004	<0.2	<0.2	0.76	1.9
0357+209P06	03 57 46.7	+20 54 40	M+03−11−004	<0.4	<0.3	0.65	<1.2
0359+140P06	03 59 50.6	+14 01 32		<0.4	<0.2	0.71	<1.6
0400+248P06	04 00 53.5	+24 46 35		<0.3	<0.3	0.80	<1.4
0402+212P03	04 02 19.2	+21 14 20		<0.2	<0.2	1.22	2.6
0402+112P06	04 02 52.1	+11 10 03		<0.2	<0.3	0.77	<2.3
0406+085P03	04 06 29.9	+08 31 05	U2970	<0.2	<0.2	0.95	4.8
0409+054P03	04 09 42.2	+05 25 08	U2982	0.55	0.79	9.3	20.8
0412+064P06	04 12 04.3	+06 22 10		<0.3	<0.2	0.70	<1.4
0413+081P03	04 13 24.3	+08 03 29	U2997	<0.2	0.68	5.37	7.0
0413+026P06	04 13 57.3	+02 38 02	U2998	<0.2	<0.2	0.74	3.2
0414+011P03	04 14 07.3	+01 03 35		<0.2	<0.2	0.77	2.3
0414+001P03	04 14 11.0	+00 09 01		<0.2	<0.39	1.99	4.0
0414+103P03	04 14 28.9	+10 20 04		<0.2	<0.3	3.14	8.5
0414+047P06	04 14 36.8	+04 39 38	U3003	<0.2	<0.2	0.65	1.8
0414+023P06	04 14 43.2	+02 18 47	U3004	<0.2	<0.2	1.51	5.1
0415+014P06	04 15 05.3	+01 26 08	2ZW007	<0.2	<0.4	3.05	6.6
0416+031P03	04 16 12.9	+03 06 33		<0.2	<0.2	0.75	2.9
0417+020P06	04 17 16.9	+01 58 27	U3014	<0.2	<0.2	0.92	2.5
0417−012P06	04 17 17.0	−01 11 25		<0.2	<0.2	0.58	1.3
0417−011P06	04 17 30.4	−01 06 51		<0.2	<0.2	0.82	2.7
0417+001P06	04 17 31.3	+00 05 55	M+00−12−005	<0.2	<0.2	1.43	5.1
0418−021P06	04 18 02.1	−0.2 08 57	M+00−12−010	<0.2	<0.2	0.56	1.8
0418+007P06	04 18 45.7	+00 42 36		<0.2	<0.2	0.68	1.7
0418+058P06	04 18 48.4	+05 48 32		<0.2	<0.2	0.49	2.1
0419+039P06	04 19 18.0	+03 55 49	ZW419.002	<0.2	0.3	2.12	5.5
0419−009P06	04 19 26.6	−00 55 31	U3022	<0.2	<0.2	0.53	1.9
0420+044P06	04 20 24.2	+04 25 48		<0.2	<0.2	0.51	2.1
0421+040P06	04 21 01.4	+04 01 00		<0.2	0.35	0.68	<1.8
1648−024P06	16 48 47.0	−02 22 15	U10578	<0.2	<0.3	2.67	6.2
1650−022P06	16 50 08.1	−02 10 11		<0.2	<0.2	0.50	<1.5
1653−012P06	16 53 23.7	−01 10 18		<0.2	<0.3	4.08	8.2
1654+030P06	16 54 42.6	+02 57 35		<0.2	<0.2	1.76	2.2
1657+026P06	16 57 15.0	+02 35 02	U10640	<0.2	<0.2	0.58	<1.5
1658−018P06	16 58 22.6	−01 46 29		<0.2	<0.4	0.52	1.6
1658+069P06	16 58 42.7	+06 55 49	ZW053.023	<0.1	<0.3	1.41	1.8
1658+054P06	16 58 54.2	+05 21 19	ZW053.024	<0.2	<0.2	2.22	4.6
1700+770P06	17 00 19.9	+77 02 26		<0.2	<0.2	0.56	1.8
1701+043P06	17 01 15.8	+04 18 53	ZW053.027	<0.2	<0.2	0.55	1.3
1701+030P06	17 01 31.8	+03 00 23		<0.3	<0.2	0.73	<1.2
1702+772P06	17 02 00.5	+77 14 17		<0.2	<0.2	0.61	1.4
1702+100P06	17 02 36.9	+09 59 47	M+02−43−006	<0.2	<0.2	0.57	2.5
1702+081P06	17 02 44.1	+08 03 26		<0.2	0.45	2.48	3.9
1703+104P06	17 03 56.9	+10 26 28	U10699	<0.2	<0.2	2.25	6.0
1704+066P06	17 04 06.5	+06 36 15		<0.2	<0.2	0.50	2.3
1706+041P06	17 06 14.1	+04 06 45	U10718	<0.2	<0.2	0.86	2.6
1709+081P06	17 09 06.7	+08 03 13	U10743	<0.2	<0.2	3.39	4.3
1710+166P06	17 10 10.0	+16 37 15	ZW111.012	<0.2	<0.2	0.48	2.0
1711+129P06	17 11 45.7	+12 53 33		<0.2	<0.2	0.71	4.9
1711+788P06	17 11 56.0	+78 49 56		<0.3	<0.2	0.45	<1.4
1715+197P06	17 15 15.5	+19 40 17		<0.2	0.44	2.33	4.8
1715+117P06	17 15 25.1	+11 41 26		<0.2	<0.2	1.80	3.8
1715+126P06	17 15 37.5	+12 38 12		<0.2	<0.2	0.93	<1.1
1717+167P06	17 17 40.5	+16 42 43	U10807	<0.2	<0.2	0.72	2.9
1721+212P06	17 21 51.6	+21 10 53	ZW140.018	<0.2	<0.3	2.37	5.6
1722+191P06	17 22 22.5	+19 06 43		<0.3	<0.2	0.66	<1.1
1725+211P06	17 25 20.3	+21 08 34		<0.2	<0.2	0.91	2.3
1730+202P06	17 30 00.6	+20 09 39		<0.2	<0.2	0.50	1.7
1733+803P06	17 33 00.9	+80 16 34		<0.2	<0.2	0.77	1.8
1735+263P06	17 35 18.4	+26 16 25	ZW140.055	<0.4	<0.2	0.52	<1.5
1736+250P06	17 36 23.9	+24 58 54	U10926	<0.2	<0.2	1.20	2.5
1737+287P06	17 37 46.6	+28 44 59		<0.2	<0.2	0.62	1.7
1738+291P06	17 38 41.4	+29 08 45		<0.3	<0.2	0.67	<1.0
1740+256P06	17 40 01.3	+25 38 27	M+04−42−002	<0.2	<0.2	1.16	2.8
1744+307P06	17 44 33.9	+30 43 17	U10976/7	<0.2	<0.2	1.92	6.1
1751+319P06	17 51 21.1	+31 53 00		<0.4	<0.2	0.61	<1.4
1751+339P06	17 51 55.8	+33 51 20		<0.2	0.39	1.40	2.6
1752+329P06	17 52 39.2	+32 53 34	U11035	<0.3	<0.42	3.59	9.2
1753+348P06	17 53 04.3	+34 47 02	U11041	0.34	0.74	6.38	16.8
1755+326P06	17 55 00.7	+32 38 46	U11058	<0.2	<0.2	0.55	2.2
1755+313P06	17 55 46.9	+31 17 06		<0.3	<0.2	0.61	1.4
1803+338P06	18 03 55.8	+33 49 28		<0.2	<0.2	0.67	1.9
1803+347P06	18 03 57.5	+34 44 48	M+06−40−003	<0.2	<0.2	0.65	2.1
1804+340P06	18 04 03.6	+34 00 37	U11118	<0.2	<0.2	0.45	2.0
1805+356P06	18 05 40.9	+35 33 27	U11124	<0.2	<0.2	0.60	1.9
1806+359P06	18 06 04.9	+35 52 10	M+06−40−006	<0.2	<0.2	0.68	2.2
1806+397P06	18 06 28.8	+39 42 39		<0.2	<0.2	0.74	<1.3
1813+373P06	18 13 01.5	+37 20 44		<0.2	<0.2	0.83	1.9
1816+398P06	18 16 28.3	+39 48 00	M+07−37−036	<0.2	<0.2	0.89	3.1
1820+416P06	18 20 17.1	+41 33 14		<0.2	<0.2	0.49	6.1
2126+871P06	21 26 16.8	+87 05 13		<0.2	<0.2	0.63	<1.7
2327+853P06	23 27 02.0	+85 18 34	7ZW941	<0.2	0.2	1.81	6.1

0028-0836/84/040320-01$01.00

© 1984 Macmillan Journals Ltd

The complexity and stability of ecosystems

Stuart L. Pimm

Department of Zoology and Graduate Program in Ecology, University of Tennessee, Knoxville, Tennessee 37996, USA

Early studies suggested that simple ecosystems were less stable than complex ones, but later studies came to the opposite conclusion. Confusion arose because of the many different meanings of 'complexity' and 'stability'. Most of the possible questions about the relationship between stability–complexity have not been asked. Those that have yield a variety of answers.

ELTON[1] noted the dangers of human simplification of the natural environment if ecosystems become less stable as they become more simple. The consequence may be increasingly unstable populations leading to extinctions, further simplification and even more instability. That there might be a single relationship between such gross variables as stability and complexity is intriguing theoretically, in a field where generalizations are scarce. The evidence on this topic is controversial. Early theoretical studies were logically simplistic, if appealing, in suggesting that complex system were more stable. Later theoretical studies were more sophisticated and suggested exactly the opposite. Field studies are, superficially, ambiguous and contradictory. I shall try here to resolve some of this controversy by pointing to several different definitions of stability, of complexity and of various ecological variables of interest. Several score permutations of these definitions are possible and different ones will yield different results. The earlier theoretical treatments and field studies typically address different combinations from those of later studies; in the few cases where they treat the same combination, there is often good agreement. Most combinations, however, have not been explored and very few have been studied extensively by both theoreticians and field workers.

Early studies[1–4] argued that increased complexity enhanced ecosystem stability. This seemed so certain that the idea became a central feature of ecology texts. Watt[5] makes one of his core principles of ecology '... the accumulation of biological diversity ... promotes population stability'. Later studies[6–21] typically came to the opposite conclusion. To see why early confidence was misplaced is easy with hindsight. The first of Elton's six arguments was a theoretical one: simple population models are characterized by oscillations; more complex models were expected to fluctuate less. Another theoretical argument, that of MacArthur[2], was that the more pathways for energy to reach a consumer, the less severe would be the failure of any one pathway. Perhaps because it represents conventional wisdom ('don't put all your eggs in one basket'), the argument was not given a formal mathematical treatment. The conflict between this argument and the diametrically opposite one from later studies is one I shall try to resolve.

One of Elton's lines of evidence involved the increased chance of pest outbreaks in agricultural systems, another the absence of pest outbreaks in tropical (but not temperate) forests and the prevalence of population cycles in the Arctic and, finally, the ease with which species can invade small, remote (and hence species-poor) oceanic islands. Elton's data were few: the absence of pest outbreaks in the tropics was based on a casual conversation with three tropical foresters[1]. Furthermore, man's impacts on agricultural systems are many and varied: simplification may not be the only, or even the main cause of their instability.

Simply, the early theoretical arguments and field studies were heterogeneous and incomplete[22]. Yet this heterogeneity only points to the fact that there are many questions to be answered in the discussion of stability–complexity relationships. No single question has logical supremacy.

Definitions

Table 1 lists definitions I shall use to build an array of complexity–stability questions. This set of definitions is not exhaustive[23–26]; I have included those definitions that have been most studied or which promise to be amenable to both theoretical and field studies. One can create 'complexity–stability' questions by taking the possible combinations of measures of complexity, stability and variables of interest. Looking at all the possible combinations suggests some features that should be discussed before answers are sought.

(1) The definitions of stability imply different kinds of comparisons. If a system is not stable (in the strict sense of Table 1), we will probably not observe it except in transition to a new equilibrium[27]. Rather special dynamics are required to permit

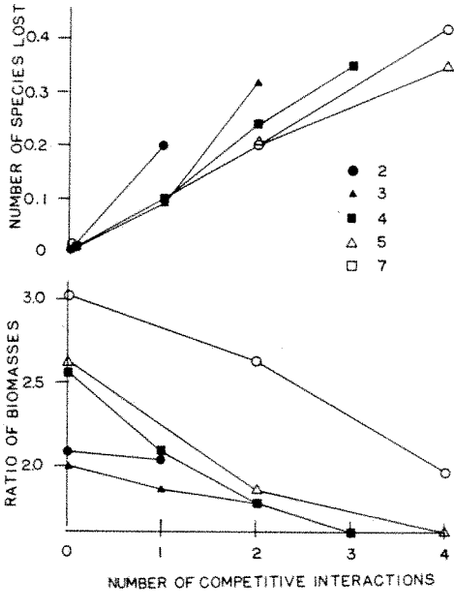


Fig. 1 An illustration of the diametrically opposite effects of increasing complexity on two different measures of stability. Results are from model systems where the single herbivore is removed from a system of possibly competing plants. As the number of competitive interactions between plants increases (abscissa), the system is more likely to lose plant species (upper graph) but change biomass less (lower graph) when the herbivore is removed. In the lower graph, the ratio of biomass plotted is the total biomass of plants without herbivore/total biomass of plants with herbivore. Numbers indicate numbers of plant species in the system.

0028-0836/84/040321—06$01.00

© 1984 Macmillan Journals Ltd

Table 1 Definitions of variables

Complexity	
Species richness	The no. of species in a system.
Connectance	The no. of actual interspecific interactions divided by the number of possible interspecific interactions.
Interaction strength	The mean magnitude of interspecific interaction: the size of the effect of one species' density on the growth rate of another species.
Evenness	The second moment (variance) of the species abundance distribution measures how uneven are the abundances. (Diversity indices are measures that combine the evenness of the species' abundances (equitability) and species richness. The relative weighting of these two components varies from index to index. One member of this family is the information theoretic index, *H*.)
Stability	
Stable (units nondimensional and binary: 0 unstable, 1 stable)	A system is deemed stable if and only if the variables all return to the initial equilibrium following their being perturbed from it. A system is locally stable if this return is known to apply only certainly for small perturbations and globally stable if the system returns from all possible perturbations. The set of all values of the variables from which the system returns to a particular equilibrium is known as the domain of attraction.
Resilience (units of time; characteristic return time is time taken for a perturbation to return to 1/e (~37%) of initial value.)	How fast the variables return towards their equilibrium following a perturbation. Resilience is not, therefore, defined for unstable systems.
Persistence (units of time)	The time a variable lasts before it is changed to a new value. Turnover is the reciprocal of persistence.
Resistance (units nondimensional and continuous)	The degree to which a variable is changed, following a perturbation.
Variability (the units are those of animals squared per unit area (variance), animals per unit area (s.d.) dimensionless (c.v.)	The variance of population densities over time, or allied measures such as the standard deviation of population densities, or the coefficient of variation (standard deviation divided by the mean density c.v.).
Variables of interest	
Individual species abundances	The densities of all the species in the system.
Species composition	The list of all the species in the system.
Trophic level abundance	The total density (or biomass) of all the species in a particular trophic level (or for comparable definitions, for some other interesting set of species).

species to persist with population densities which do not return to an equilibrium, but instead, cycle indefinitely. Such dynamics are theoretically fascinating but seem to characterize only a few populations[28], such as lemmings and lynx. Thus, when we investigate the chances of stability of systems with different complexities we are implicitly comparing existing with hypothetical systems. For the other measures of stability, however, we could ask whether existing systems are more or less resilient, resistant, persistent or variable. (Although if they are not resilient, resistant or persistent enough or too variable, then, indeed, we may not observe them for long.)

(2) There are many kinds of perturbations to natural systems. Detailed answers to specific complexity–stability questions may depend on what is perturbed, when, and by how much. Some perturbations may involve changes in species abundances (like the English winter of 1962–63 which temporarily depressed bird populations[29]); others may involve the removal of some or all of the species (as in secondary plant succession) and thus involve recoveries of a much longer duration. Consequently, the definitions of resilience, persistence and resistance pose problems of scale when we attempt to measure them in the field[30]. It is obvious that large perturbations will disappear more slowly than small ones. The time taken for a perturbation to diminish to a given percentage of its initial value, however, may be relatively independent of the size of the perturbation. Such a statistic thus avoids the complication of the initial size of the perturbation, which will depend not only on the size of the disturbance but also on the system's resistance. The notion of persistence is more difficult to deal with. How long a system persists will not only depend on the system, but also on the properties of the disturbances. Defining comparable disturbances for different systems may often be difficult or impossible. But for some cases to be discussed, the differences in persistence clearly can be seen to depend on the intrinsic differences between systems and not on the disturbances.

It is also obvious that long-lived organisms (say, trees) will be less resilient and more persistent in numbers than short-lived organisms (say, annual plants) on a time scale measured in years[30]. Why some systems are dominated by organisms with short or long life histories may be the answer to some questions about resilience and resistence. For others, we may wish to scale resilience or persistence further by correcting for the generation times of the species involved.

Perturbations also need to be defined spatially. Disturbing 1 m^2 is not merely a smaller perturbation than disturbing 1 km^2: in the latter, the boundary is smaller relative to the total area, so immigration and emigration are likely to be relatively less important than birth and death processes.

In short, because of the problems of spatial and temporal scales, it may be impossible to compare widely different ecosystems. Yet for similar systems, contrasts of resilience, resistance, persistence and variability should be possible. Indeed, the field studies I shall discuss confirm this.

(3) The definitions of stability are interrelated in complicated ways. Under some circumstances, resistant systems, because they change less under a given disturbance, will appear more persistent and less variable. For some populations, increasing resilience implies decreasing variability[29] and, as discussed above, resilience and resistance may or may not be confounded depending on how the former is scaled. I shall not discuss these relationships, in part because Harrison[26] has already done so. However, the principal reason is that despite the potential for the reverse, theoretical and even field results fall cleanly into the various definitions of stability; and even if the correlation between any two definitions is absolute, we will retain both when, under different circumstances, each variable becomes an

Table 2 Theoretical and field studies on various combinations of measures of complexity, variables of interest and measures of stability

		THEORETICAL STUDIES		FIELD STUDIES	
		SPECIES RICHNESS	CONNECTANCE AND INTERACATION STRENGTH	SPECIES RICHNESS	CONNECTANCE AND INTERACTION STRENGTH
Individual species abundances	STABLE	Local stability: with more species there is less chance of stability[6-21] Modifications: (1) feasibility[32] (2) structure[10,11,31] (3) parameters[10,11] (4) donor-control[10] (5) succession, deletion and evolution[33-36] (6) predator foraging patterns[42,43]	Local stability: with more connectance and and greater interaction there is less chance of stability[6-9]	1) Cn ≤ constant[44-46]	2) Food webs lack links that are particularly destabilizing[27]. 3) Insect systems are more connected than vertebrate dominated ones[50]
	RESILIENT	With more trophic levels there is less resilience[53,54]	With more connectance there is greater resilience[11,27]	With more species there is less resilience[55]	
	PERSISTENT	1) Systems with more species are harder to invade[36,56] 2) With more species, it is less likely an invader will be a new species	With more interaction systems are harder to invade[56,57]	1) Successions: older, systems with more species are more persistent[58] 2) Islands: those with fewer species are easier to invade[1,59,60]	
	RESISTANT				
Species composition	STABLE	Species deletion: with more species there is less chance of stability (except for donor-control where the reverse is true[38,61])	Species deletion: with more connectance there is less chance of stability (except for donor-control where the reverse is true[38,61])	Indirect evidence: most systems are changed considerably by predator removals and are thus not donor-controlled[38]	
	RESILIENT				
	PERSISTENT			Geologically, systems with more species persist less[63]	
	RESISTANT				
Total Density	RESILIENT		With more connectance there is possibly more resilience[64,65]		
	RESISTANT	With more species, there is usually less resistance[66]	With more connectance there is more resistance[66]		

easier or more appropriate measurement of stability.

(4) The variables of interest are hierarchial in nature. If individual species abundances are maintained, then so are the number of species in the system, species composition and the total density or biomass of any subset of species. Yet species composition may remain the same while the densities of the species change, or vice versa.

(5) Connectance and interaction strength are closely related: their product measures how strongly all the species in the system interact. Theoretically, it is convenient to distinguish species pairs that interact from those that do not. Practically, we might expect a continuous gradation of interaction strengths from the strong to the weak and for these two measures to affect stability in comparable ways.

Table 2 compiles theoretical and field studies on some of the combinations of measures of complexity, variables of interest and measures of stability. Later, I shall discuss evenness as a measure of complexity and variability as a measure of stability. I shall first summarize what results are available, then conclude by pointing to some questions which appear experimentally and theoretically tractable and yet which do not appear to have been answered.

Individual species abundances

Stability. The majority of theoretical studies have examined local stability of species abundances. Initial results[6-9] found that increasing species richness, connectance or interaction strength decrease the chance that randomly assembled communities would be stable. Random assembly is an unreasonable assumption and other studies relaxed the assumptions on the structure and limits of the interaction parameters[10,11,31,32]. In addition,

models were allowed to develop through the successive additions or deletions of species[33–36].

The many subtleties to this work are reviewed by May[37], McDonald[13] and Pimm[27] and one feature seems capable of completely reversing the original result. In most models, increasing the density of the predator, decreases the population growth rate of the prey. Alternatively, predators may consume prey that are likely to die or have died from other causes—disease or starvation, for example. For these donor-controlled models, increasing connectance and species richness increases the chances of finding a locally stable model[10]. This result matches MacArthur's[2] idea that complexity buffers the consequences of density variations in one prey species. His argument is, however, incomplete: it fails to consider the variations in densities of species at higher trophic levels. For these, the opposite is true: variations propagate more widely the more connected the system. It is this latter effect that predominates in many models but, by definition, is absent in donor-controlled models. Donor-control dynamics predict that predator removal should have no effect on prey species densities and certainly not on the resultant community's species composition. Usually this is not the case[38]. With the rejection of donor-control dynamics goes one plausible model where increased stability comes from increased complexity.

In Lotka–Volterra models, resource limitation of species at the base of the food web helps to stabilize the system, and predator effects on prey density (absent in donor-controlled models) are destabilizing. However, the predator interactions, themselves, can be stabilizing. Typical examples of this involve some mechanisms for the prey to escape predation at low densities. Here, predators might not be able physically to attack the prey because the latter are hiding or, perhaps, because low densities cannot be profitably exploited. In these models there can be stable interactions with prey depressed to levels far below those set by resource limitation. Detailed case histories[39], general arguments about the structure of herbivore communities[40], or about herbivores in general[41] all suggest that many species may be limited primarily by predators rather than resources. For such cases Nunney[42] showed that increasing complexity and species richness could lead to an increased chance of local stability. The generality of Nunney's arguments have been questioned[43] but they also require the product of connectance (C) and species richness (n) to be sufficiently large ($Cn > 6$) to reverse the usual decrease in stability. I shall return to this point presently.

Is there any field evidence for these theoretical results? As argued above, the nature of the definition of local stability makes it unlikely (though not impossible) that we will observe anything other than locally stable systems. Many models suggest that, if interaction strength (i) is assumed to be relatively constant, then C should vary with $1/n$ in order to maintain local stability. Data from published food webs[27,44] or from more detailed studies of lizard communities[45] show that C does decrease linearly with $1/n$ or nearly so[46] with $Cn = 3$–4. These values, incidentally, seem too low for Nunney's argument, in the previous paragraph, to hold. Unfortunately, these results say nothing more than that each species interacts with a fixed number of other species as n increases—a result that seems reasonable without recourse to stability explanations[47]. There are, however, certain other patterns of species interactions (particularly those that involve species feeding on more than one trophic level[48], and the grouping of species interactions within a community[49]) that are particularly destabilizing in models. Natural systems show a statistical scarcity of these patterns[49,50]. Although interaction strength is hard to measure, it is likely to be less between an insect and its comparably sized insect parasitoid than between a large vertebrate predator and its small prey. Thus, stability should permit insect–parasitoid systems to be substantially more connected than vertebrate-dominated systems[48]. They are[50]. (With the exception just noted, most multi-species models do not consider parasitoid or parasite systems. Yet their distinctive features[51] and ecological importance[52], plus their greater potential complexity, suggest that other results from local stability analyses may not be simply transferable to them.)

In sum, most theoretical studies of local stability are best interpreted by saying that the systems we observe should not be too complex. Field evidence suggests this is correct; indeed, patterns of species interactions that are particularly destabilizing in models are also rare in nature.

Resilience. In models, systems with more trophic levels (one way of adding species) are less resilient[53,54] and, for a given number of trophic levels, stable systems will often[11,27], but not always[43], be more resilient the more complex they are. Resilience is amenable to field determination[29] and unlike stability, permits comparisons of existing natural systems. However, I know of only one such study[55]. It found that plants in a species-poor system recovered more quickly from an unusual drought than those in a nearby species-rich field.

Persistence. How long should species densities continue to return to a given equilibrium and not to some other equilibrium involving the same or some different set of species? One long-term question is whether constantly simple systems persist longer than complex ones (for example, are the species abundances of species-poor desert communities more persistent than those of species-rich tropical forests?). I shall consider this topic below. A second, relatively short-term view, posits changes in both composition and individual abundances, effected, perhaps, by the addition of a species to the system. This presumes that the community has fewer species than the set of those capable of reaching the community (this set is called the species pool). This topic is particularly relevant to islands (either typically oceanic ones or abstract islands of isolated habitat which may lack a full complement of species) or communities which, following a major disturbance, may also lack species.

How hard are communities to invade? Those that are hard to invade will be persistent and vice versa. Models that examine species competing for resources arranged along some gradient[56] and more general models of food web assembly[36,57] suggest that communities with more species are harder to invade. In addition, the smaller the fraction of the species pool present in a community, the more likely an invading individual will be of a new species. The more new species that invade per unit time, the more likely it is that one of them will be able to increase and so change the existing system's equilibrium. Simply, both effects mean that the fewer the species in the system, the less persistent that system will be. The effect of increased interaction between species is also to increase persistence[56,57].

There are two kinds of field evidence for these effects. First, during succession, species that enter the community later (when there are usually more species) persist longer than those that enter earlier[58]. In part, this must be due to longer-lived organisms arriving later. The question of whether later-arriving organisms persist longer relative to their generation times has not been asked. Of course, that longer-lived organisms arrive later may simply be a reflection of the greater likely persistence of later successional stages. The second evidence avoids these difficulties, for even among species with similar generation times, species are more likely to colonize remote, species-poor islands successfully than species-rich continents[1,59,60].

Species composition

Stability. If individual species abundances remain fixed, then so does species composition; thus, much of the previous section applies to this one. It remains to ask, under what conditions does species composition remain unchanged, even though individual abundances change considerably? There are two kinds of studies. The first kind considers the effect of removing species on the composition of the remaining species ('species deletion stability'). Other studies consider whether, following changes in density, species abundances will return to their original equilibrium or move to some new equilbrium involving different abundances but the same composition.

Theoretically, species deletion stability decreases with increasing numbers of species and connectance[61], but it also depends

critically on which species are selected for removal[38]. If model plants are removed, more species and greater connectance will lead to a lessened chance of losing more species: these results confirm MacArthur's[2] intuition. For model predators, however, the result is reversed. Of course, donor-controlled systems are not affected by predator removals and, for these, more complex systems will be more species deletion stable[38]. As noted above, donor-controlled dynamics do not predominate in nature[38].

A second problem involves populations which appear to continue at one level for years, then shift to another one and remain there. Lotka–Volterra models do not have this property—more complex dynamics with more parameters are required[62]. In this special sense, systems with greater (dynamic) complexity may permit species composition to remain unchanged when comparable simple models would predict species losses.

Resilience. How fast species composition returns to equilibrium following, say, a major reduction in species, is a question central to the topic of ecological succession. Data are certainly available on how fast succession proceeds. However, I know of no synthesis that relates the rate at which disturbed systems approach their former composition to the various definitions of complexity.

Persistence. I have already noted that species composition should and does last longer when more species are present—at least when viewing the process of succession. But what about the non-successional differences among systems, the species richness of forests versus deserts, for example? In the long term, we might expect development of better prey or predators and changing environmental conditions to cause species losses. From the results on species deletions, we might expect systems with more species to last for shorter periods. The relationship of the persistence of species composition to species richness, therefore, may be dependent on the timescale considered. Boucot[63] finds that for marine fossil assemblages 'trophically complex systems... are far more fragile and subject to extinctions' than simpler systems and, hence, tend to last for shorter periods. As E. R. Pianka has observed (personal communication), excepting succession, species compositions of many systems are persistent over at least human life spans.

Total density or biomass

The total density (or biomass) of all or some subset of the species in an ecosystem may correlate far more closely with our notions of what defines an ecosystem than the abundances or identities of particular species. For example, we readily recognize a deciduous forest. But consider, say, 1 km^2 plots of deciduous forest in eastern North America or western Europe. Within these plots there will be a wide range of tree species, an even greater variation in the abundance of individual tree species, but not, I suspect, a large variance in the total biomass of all tree species.

Resilience. Theoretically, densities of certain sets of species can be more resilient than those of their constituent species and, moreover, this resilience can increase with increasing connectance[64,65]. I know of no field study that relates biomass resilience during succession to complexity.

Resistance. There are both theoretical[66,67] and experimental[55,68,69] studies of the degree of change of biomass and density with the addition or removal of a species or resource. Two of these studies[66,69] focus on the effects of large mammalian grazers on plant biomass. Models with few plant species but more connectance (interactions between plants) have greater resistance[66]. The former result also holds for very complicated ecosystem models[67]. Greater evenness of prey abundances (a measure of complexity not yet discussed) correlates with greater resistance both theoretically[66] and in field studies of mammalian grazing[69].

Other variables

I have not discussed variability as a measure of stability because I believe there are difficulties in testing the various theoretical ideas. How much populations vary will depend not only on intrinsic factors involving ecosystem complexity[70–72], but on the extrinsic nature and frequency of the perturbations. In the species-rich tropics, populations do not vary less than in temperate systems[22,72,73]. Yet populations do vary more in climatically unpredictable systems than in predictable ones[73], suggesting that extrinsic factors may govern variability more than intrinsic ones.

Nor have I discussed pest outbreaks. While it may be that pest species are the most variable[74], it is more likely that they are simply those species whose densities are high relative to what humans require them to be. Pimental's[75] finding of lower densities of herbivorous insects with greater species richness might be explained by considerations of equilibrium densities rather than by the definitions of stability used here. The determinants of equilibrium densities[76] or human requirements[77] fall outside the scope of this review, but within its scope is the question of what determines how fast an insect population returns to an economically unacceptable equilibrium after being severely reduced by insecticides. I have already considered resilience a measure of stability. In contrast, for pest outbreaks, a resilient population might be considered unstable, while one that recovered slowly might be considered stable. Resilience might indicate stability or instability depending on one's viewpoint[78]. As noted above, with fewer species, resilience tends to increase: whether this is a general explanation of outbreaks is an intriguing possibility, but one clearly based on too few theoretical and field studies.

Conclusions

Theoretically, the more species that are present in a community: (1) the less connected it should be, if it is to be stable, (2) the less resilient will be its populations, (3) the greater will be the change in composition and in biomass when a species is removed, (4) the longer the persistence of species composition in the absence of a species removal. The more connected a community: (1) the fewer species it must have if it is to be stable, (2) the more likely it is to lose other species if one is removed, but (3) the more resilient will be its populations, (4) the more persistent will be its composition and (5) the more resistant will be its biomass if a species is removed. Clearly, different complexity–stability questions yield different answers (Fig. 1). The fact that they do, often explains some of the earlier controversy generated, it is now obvious, by different authors addressing different questions.

Which questions have not been examined? Local stability is a clearly defined concept mathematically and the majority of theoretical studies examine it, alone[26]. Field studies have not usually addressed local stability because, I suspect, it involves a comparison of existing and hypothetical systems. What field studies have examined are questions that permit comparisons of existing systems and which involve more general views of a system than individual species' abundances, How fast does species composition or total biomass return to equilibrium, how long does composition remain unchanged and how much is composition or total biomass changed following, say, the addition of a species? These questions are as theoretically tractable as those of local stability. Similarly, some accessible theoretical ideas—like resilience—are rarely measured in the field, despite the potential to do so. Put simply, progress in our understanding of the complexity and stability of ecosystems will come from more theoretical and field studies, but is likely to be fastest when these studies consider which questions are both theoretically tractable and experimentally testable: I suggest the many gaps in Table 2 provide a guide to what these questions are.

I thank R. A. Armstrong, J. H. Lawton, R. M. May, S. J. McNaughton and E. R. Pianka for comments on earlier versions of this paper. This research was supported by the NSF's Ecosystems Studies Program under Interagency Agreement DEB 77-25781 with the US Department of Energy under contract W-7405-eng-26 with Union Carbide Corporation: Environmental Sciences, Oak Ridge National Laboratory.

1. Elton, C. S. *The Ecology of Invasions by Animals and Plants* (Chapman & Hall, London, 1958).
2. MacArthur, R. H. *Ecology* **36,** 533–536 (1955).
3. Hutchinson, G. E. *Am. Nat.* **93,** 145–159 (1959).
4. Margalef, R. *Perspectives in Ecological Theory* (University of Chicago Press, 1968).
5. Watt, K. E. F. *Principles of Environmental Science* (McGraw-Hill, New York, 1973).
6. Gardner, M. R. & Ashby, W. R. *Nature* **228,** 784 (1970).
7. May, R. M. *Stability and Complexity in Model Ecosystems* (Princeton University Press, 1973).
8. McMurtie, A. J. *J. theor. Biol.* **50,** 1–11 (1975).
9. Gilpin, M. E. *Nature* **254,** 137–139 (1975).
10. DeAngelis, D. L. *Ecology* **56,** 238–243 (1975).
11. Pimm, S. L. *Theor. Popul. Biol.* **16,** 144–158 (1979).
12. Siljak, D. *Nature* **249,** 280 (1974).
13. MacDonald, N. *Nature* **275,** 117–118 (1978).
14. Tuljapurkar, S. D. & Semura, J. S. *Nature* **275,** 388–389 (1975).
15. Jeffries, C. *Theor. Popul. Biol.* **7,** 149–166 (1975).
16. May, R. M. *Nature* **238,** 413–414 (1972).
17. Tansky, M. *Mem. Coll. Sci. Kyoto Univ.* **B7,** 87–94 (1978).
18. Webber, M. I. in *Ecological Stability* (eds Usher, M. B. & Williamson, M. W.) 165–178 (Chapman & Hall, London, 1974).
19. Kirkwood, R. S. M. & Lawton, J. H. *J. theor. Biol.* **93,** 225–237 (1981).
20. Goh, B. S. *Math. Biosci.* **40,** 157–166 (1978).
21. Kindlemann, P. & Rejmanek, M. *J. theor. Biol.* **94,** 989–993 (1982).
22. Goodman, D. *Q. Rev. Biol.* **50,** 237–266 (1975).
23. Botkin, D. B. & Sobel, M. J. in *Ecosystem Analysis and Prediction* (ed. Levin, S. A.) 144–150 (Society of Industrial and Applied Mathematics, Philadelphia, 1975).
24. Innis, G. in *Ecosystem Analysis and Prediction* (ed. Levin, S. A.) 131–139 (Society of Industrial and Applied Mathematics, Philadelphia, 1975).
25. Orians, G. H. in *Unifying Concepts in Ecology* (eds van Dobben, W. H. & Lowe-McConnell, R. H.) 139–150 (Junk, The Hague, 1975).
26. Harrison, G. W. *Am. Nat.* **113,** 659–669 (1979).
27. Pimm, S. L. *Food Webs* (Chapman & Hall, London, 1982).
28. Tanner, J. T. *Ecology* **47,** 733–745 (1966).
29. Pimm, S. L. in *Ecological Communities: Conceptual Issues and the Evidence* (eds Strong, D. R. Jr, Simberloff, D. S. & Abele, L. G.) (Princeton University Press, in press).
30. Connell, J. H. & Sousa, W. P. *Am. Nat.* **121,** 787–824 (1983).
31. Lawlor, L. R. *Am. Nat.* **112,** 445–447 (1978).
32. Roberts, A. *Nature* **251,** 607–608 (1974).
33. Tregonning, K. & Roberts, A. *Bull. math. Biol.* **40,** 513–524 (1978).
34. Tregonning, K. & Roberts, A. *Nature* **281,** 563–564 (1979).
35. Roberts, A. & Tregonning, K. *Nature* **288,** 265–266 (1981).
36. Post, W. M. & Pimm, S. L. *Math. Biosci.* **64,** 169–192 (1983).
37. May, R. M. in *Population Dynamics* (eds Anderson, R. M., Turner, B. R. & Taylor, L. R.) 385–407 (Blackwell, Oxford, 1979).
38. Pimm, S. L. *Oikos* **35,** 139–149 (1980).
39. Hassell, M. P. *J. Anim. Ecol.* **49,** 603–628 (1980).
40. Lawton, J. H. & Strong, D. R. Jr *Am. Nat.* **118,** 317–338 (1981).
41. Hairston, N. G., Smith, F. E. & Slobodkin, L. B. *Am. Nat.* **84,** 421–425 (1960).
42. Nunney, L. *Am. Nat.* **115,** 639–649 (1980).
43. Abrams, P. A. & Taber, D. A. *Am. Nat.* **119,** 240–249 (1982).
44. Rejmanek, M. & Stary, P. *Nature* **280,** 311–313 (1979).
45. Pianka, E. R. in *Ecology and Evolution of Communities* (eds Cody, M. L. & Diamond, J. M.) 291–314 (Harvard University Press, 1975).
46. Yodzis, P. *Nature* **284,** 544–545 (1980).
47. Pimm, S. L. *Nature* **285,** 591 (1980).
48. Pimm, S. L. & Lawton, J. H. *Nature* **268,** 329–332 (1978).
49. Pimm, S. L. & Lawton, J. H. *J. Anim. Ecol.* **49,** 879–898 (1980).
50. Pimm, S. L. *Ecology* **61,** 219–225 (1980).
51. Dobson, A. P. in *Population Biology of Infectious Diseases* (eds Anderson, R. M. & May, R. M.) 1–25 (Springer, Berlin, 1982).
52. Anderson, R. M. *Population Dynamics of Infectious Diseases* (Chapman & Hall, London, 1982).
53. Pimm, S. L. & Lawton, J. H. *Nature* **268,** 329–331 (1977).
54. Vincent, T. L. & Anderson, L. R. *Theor. Popul. Biol.* **15,** 217–231 (1979).
55. Leps, J., Osbornova-Kosinova, J. & Rejmanek, M. *Vegetatio* **50,** 53–63 (1982).
56. May, R. M. & MacArthur, R. H. *Proc. natn. Acad. Sci. U.S.A.* **69,** 1109–1113 (1972).
57. Robinson, J. V. & Valentine, W. D. *J. theor. Biol.* **81,** 91–104 (1979).
58. Shugart, H. H. & Hett, J. M. *Science* 1279–1281 (1973).
59. Long, J. L. *Introduced Birds of the World* (David & Charles, Newton Abbott, 1981).
60. Moulton, M. P. & Pimm, S. L. *Am. Nat.* **121,** 669–690 (1983).
61. Pimm, S. L. *Oikos* **33,** 351–357 (1979).
62. May, R. M. *Nature* **269,** 471–477 (1977).
63. Boucot, A. J. *J. Paleont.* **57,** 1–30 (1983).
64. Armstrong, R. A. *Am. Nat.* **120,** 391–402 (1982).
65. Armstrong, R. A. *Oak Ridge natn. Lab. Tech. Memo.* (in the press).
66. King, A. W. & Pimm, S. L. *Am. Nat.* **122,** 145–149 (1983).
67. Austin, M. P. & Cook, B. F. *J. theor. Biol.* **45,** 435–458 (1974).
68. Hurd, L. E., Mellinger, M. V., Wolf, L. L. & McNaughton, S. J. *Science* **173,** 1134–1136 (1971).
69. McNaughton, S. J. *Am. Nat.* **111,** 515–525 (1977).
70. DeAngelis, D. L. *Bull. math. Biol.* **37,** 291–299. (1975).
71. Saunders, P. T. & Bazin, M. J. *Nature* **256,** 120–121 (1975).
72. Leigh, E. F. in *Ecology and Evolution of Communities* (eds Cody, M. L. & Diamond, J. M.) 74–80 (Harvard University Press, 1975).
73. Wolda, H. *Am. Nat.* **112,** 1017–1045 (1978).
74. Watt, K. E. F. *Can. Ent.* **97,** 887–895 (1965).
75. Pimentel, D. *Ann. ent. Soc. Am.* **54,** 76–86 (1964).
76. Beddington, J. R., Free, C. A. & Lawton, J. H. *Nature* **273,** 513–519 (1978).
77. Conway, G. in *Theoretical Ecology* (ed. May, R. M.) 356–386 (Blackwell, Oxford, 1981).
78. Turelli, M. *Theor. Popul. Biol.* **13,** 244–267 (1978).

ARTICLES

The Hubble constant as derived from 21 cm linewidths

Allan Sandage & G. A. Tammann*

Mount Wilson and Las Campanas Observatories, Carnegie Institution of Washington, 813 Santa Barbara Street, Pasadena, California 91101, USA

The IR 21 cm linewidth–absolute magnitude relation is calibrated by means of local galaxies with known distances, including new distance determinations of M33 and M81. The calibrated relation is applied to 17 Virgo cluster galaxies of type Sab to Sd and yields a cluster modulus of $(m-M)^0_{Virgo} = 31.47 \pm 0.34$. *Reliable relative distance indicators are used to extend the distance scale to the Coma cluster, for which a distance modulus is found of* $(m-M)^0_{Coma} = 35.39 \pm 0.35$. *The blue 21 cm linewidth–absolute magnitude relation gives slightly larger distances. A combination of the two methods leads to a Hubble constant at the distance of the Coma cluster of* $H_0 = 55 \pm 9$ km s^{-1} Mpc^{-1}. *This value agrees with the global value of* $H_0 = 50 \pm 7$ km s^{-1} Mpc^{-1}, *giving a Hubble time of* $H_0^{-1} = 19.5 \times 10^3$ Myr, *in aggreement with the age of the chemical elements and with the age of our Galaxy, determined from globular clusters.*

In a recent discussion of the Tully–Fisher relation[1] it was stated that the extragalactic distance scale and the value of the Hubble constant depend now only on the infall velocity towards Virgo, and on the distances of the galaxies in the neighbourhood of the Milky Way. Because the former is now well determined from an accurate measurement of the relative distance between the Virgo and Coma clusters[2], and because new fundamental distances to two nearby calibrating galaxies, M33 and M81, have recently become available[3–5], it seems justified to look into the consequences of these new developments.

* Permanent address: Astronomisches Institut der Universität Basel, European Southern Observatory, Venusstrasse 7, CH-4102 Binningen, Switzerland.

The Tully–Fisher relation, that is the dependence of the luminosity of disk galaxies on their 21 cm linewidth, has been widely used to derive extragalactic distances, either by correlating the absorption-corrected blue B magnitude (ref. 6 and refs therein) or the nearly absorption-free IR H magnitude (ref. 1 and refs therein) of a disk galaxy with its inclination-corrected 21 cm linewidth Δv_{21}. So far the IR Tully–Fisher relation has consistently led to a surprisingly low Virgo cluster distance of only 16 Mpc, compared with 22 Mpc which follows from other distance indicators, which are insensitive to selection effects and statistical bias[7,8]. We investigate here this discrepancy, as well as the consequences for the Hubble constant H_0.

0028–0836/84/040326—04$01.00

© 1984 Macmillan Journals Ltd

Data

We restrict the present sample to Sab to Sd spiral galaxies. Very late-type galaxies (Sdm, Sm, Im) are excluded, because knowledge of their intrinsic shape is inadequate to derive reliable inclination angles. Sa galaxies are excluded because there are reasons to doubt that they obey a Tully–Fisher relation; a complete sample of Sa galaxies with $i > 45°$ from the *Revised Shapley–Ames Catalog*[9] does not reveal a significant dependence of M_B on $\log \Delta v_{21}$. This lack of correlation is possibly related to the fact that Sa galaxies have rotation curves which rise steeply in the central regions. The maximum rotation velocity, v_{max}—which essentially determines the 21 cm linewidth—measures only the mass of the rotating bulge, which is a fraction of the total mass (and light) of an Sa galaxy[10].

A Tully–Fisher relation has the form

$$M_H = a - b\ (\log \Delta v_{21} - 2.5) \quad (1)$$

where M_H is the absolute galaxian magnitude in the H band. The constant term a can be determined from nearby calibrator galaxies with known distances (Table 1). The slope b is treated in a first step as a free parameter with values of $b = 10$–13. The best value of b will be discussed later.

Table 1 contains all spiral galaxies for which fundamental distances are available from Cepheids and/or brightest stars and other distance indicators. The Cepheid distances were not increased by a bulk correction of ~0.3 mag, which in the past has seemed to be required by the increased Hyades cluster distance. This open cluster, often used to fix the zero point of the Cepheid period–luminosity function, was originally assigned a distance modulus of $(m-M)^0 = 3.03$, whereas a value of 3.30 seems now much more probable. This change, however, enters with very little weight into the zero point determination of the period–luminosity relation for reasons discussed elsewhere[3]. Briefly, the zero point from Cepheid radius determinations agrees well with our old zero point without the Hyades increase, and an increase of the Magellanic Cloud moduli by 0.3 mag would conflict with their RR Lyrae star moduli. It seems therefore that our original main-sequence fitting was slightly in error, but that error has just been compensated by the increased Hyades modulus, leaving our first Cepheid zero point[11,12] nearly unchanged. This now seems to be established via the open cluster NGC6087 which contains the Cepheid S Nor, on the basis of new observations by J. W. Pel (personal communication).

Table 1 Local calibrators

Galaxy	Type	$(m-M)^0$	M_H	$\log \Delta v_{21}$	Weight
NGC224	Sb	24.19*	−23.28	2.75	1
NGC598	Sc	25.23*	−20.89	2.39	1
NGC300	Sc	26.27†	−19.4	2.35	1/2
NGC55	Sc	26.27‡	−20.3	2.18	1/2
NGC247	Sc	26.8§	−19.1	2.35	1/2
NGC253	Sc	27.3‖	−22.6	2.64	1/2
NGC7793	Sd	27.3‖	−19.4	2.40	1/2
NGC2403	Sc	27.66¶	−21.21	2.48	1
NGC3031	Sb	28.73#	−24.35	2.71	1
NGC5204	Sd	29.20**	−18.90	2.18	1
NGC5585	Sd	29.20**	−19.05	2.31	1

* Ref. 3.
† Ref. 16: the Cepheids in NGC300 are 7.36 mag fainter than those in the Large Magellanic Cloud, for which an apparent modulus of $(m-M)_{AB} = 18.95$ is assumed.
‡ Assumed to be at the same distance as NGC300; compare ref. 15.
§ From the resolution into stars the galaxy is estimated to be 0.5 mag more distant than NGC300; compare with refs 9, 15.
‖ From the resolution into stars the galaxies NGC253 and NGC7793 are estimated to be 1.0 mag more distant than NGC300; compare with refs 9, 15.
¶ Ref. 29; remeasured and slightly adjusted by ref. 5.
Ref. 5.
** Assumed to be at the same distance as M101; compare refs 30, 31.

Two distances in Table 1 need special comment: the distances to M33 and to M81. (1) The distance of M33 (NGC598) has only now been firmly based on Cepheids[4,5]. Its true distance modulus of $(m-M)^0 = 25.33$ (1.1 Mpc) makes it 1.6 times more distant than M31, provided the distance of the latter galaxy is correct. It has been suggested on the basis of IR photometry of the Cepheids in M31, that the true distance modulus of this galaxy is $(m-M)^0 = 24.6$ (ref. 13), which would reduce the distance ratio of M33 and M31 to 1.3. The increased distance to M31 has conservatively not yet been considered here. (2) Lacking a direct distance determination to M81, it has previously been assumed that M81 lies at the same distance as NGC2403. However, recent measurements of the Cepheids, of the colour-magnitude diagram of the brightest blue and red stars, and of the novae in M81 (ref. 5) leads to an apparent blue modulus of $(m-M)^{M81}_{AB} = 28.8$ which is 1.0 mag larger than adopted previously, based as it was then on the modulus of NGC2403. We should also point out that the distance to NGC2403 adopted in Table 1 may again be conservative, because IR photometry of the Cepheids in that galaxy suggests a true distance modulus of $(m-M)^0 = 28.15$ (ref. 14).

As in the case of the M81 group, it appears now as an oversimplification to assume the 'members' of the South Polar Group to lie at a common distance[9,15]. In the latter group a reliable Cepheid distance is only available for NGC300[16]. NGC55 seems to lie at approximately the same distance, judging from its resolution into stars. Unfortunately no H magnitudes are available for these two galaxies; the values in Table 1 were calculated on the assumption that the intrinsic colour of an Sc galaxy is $(B-H) = 1.4$. NGC253 and NGC7793 are judged to be 1 mag more distant on the basis of their resolution into stars, and the same criterion suggests that NGC247 lies between the pairs NGC300/55 and NGC253/7793. These distances are quite tentative, and they are given only half weight in Table 1. NGC300 and NGC55 are also given half weight because of their somewhat uncertain H magnitudes. The IR $H^c_{-0.5}$ magnitudes, corrected for absorption, were taken from refs 17 and 19. The 21 cm linewidths were averaged from refs 6, 17 and 19. The Tully–Fisher relation of the calibrators is plotted in Fig. 1.

Values of the constant term a of equation (1), as determined by the calibrator galaxies, are listed in Table 2 for different values of the slope b. The first set of solutions for a is based on the six calibrator galaxies with full weight in Table 1; the second set of solutions uses all 11 calibrators with the appropriate weights given in Table 1. Comparison of the two sets shows that the value of a is quite insensitive to whether or not the traditional South Polar Group is excluded. For the following discussion the solution adopted is based on only six calibrators.

Table 3 lists all Shapley–Ames galaxies within 6° of the Virgo cluster centre[9] with known IR magnitudes $J^c_{-0.5}$ (refs 17, 18) and 21 cm linewidths[6,18]. Two galaxies, NGC4206 and NGC4498, for which the necessary data are available, were added. The sample is the same as that considered in refs 1 and 18, with the exception that Sa and very late-type galaxies are excluded here. The Tully–Fisher relation of the Virgo galaxies is shown in Fig. 1.

Distance of the Virgo cluster

By means of equation (1) and using the appropriate values of a from Table 2, the individual distance moduli of the Virgo

Table 2 The zero point of the IR Tully–Fisher relation and the distance modulus of the Virgo cluster

b	10.0	11.0	12.0	13.0
a	−21.58	−21.61	−21.64	−21.67
(6 calibrators)	±0.26	±0.29	±0.34	±0.41
a	−21.50	−21.56	−21.61	−21.67
(11 calibrators)	±0.28	±0.30	±0.33	±0.37
$(m-M)^0_{Virgo}$	31.34	31.43	31.52	31.60
(6 calibrators)	±0.12	±0.13	±0.15	±0.17

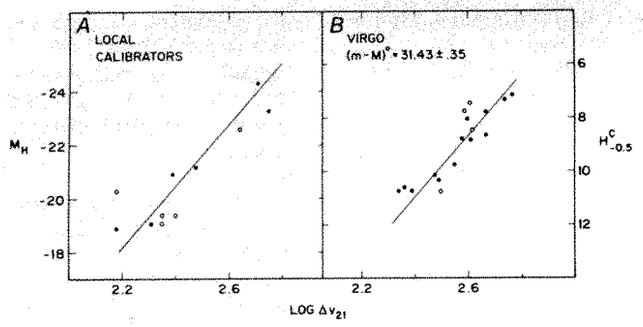


Fig. 1 The IR Tully–Fisher relation. *A*, The local calibrators with absolute IR magnitudes M_H and 21 cm linewidths from Table 1. *B*, Virgo cluster galaxies with apparent IR magnitudes $H^c_{-0.5}$ and 21 cm linewidths from Table 3. The open symbols denote low-weight galaxies. The lines in the two diagrams have the adopted slope $b = 11.5$.

galaxies in Table 3 can be calculated. The resulting mean distance moduli for different adopted values of b are listed in Table 2, as well as the mean standard deviation of that mean. An important conclusion is that the Virgo modulus depends on the value of b, varying from $(m-M)^0_{\text{Virgo}} = 31.34$ for $b = 10$ to $(m-M)^0_{\text{Virgo}} = 31.60$ for $b = 13$. The slope dependence becomes even stronger if the traditional South Polar Group is included among the calibrators. Following ref. 18 the interacting galaxy NGC4438 and the deviating galaxy NGC4569 are excluded here, and NGC4519 and NGC4535 with uncertain inclinations are given half weight. In view of the slope dependence it is important to determine the best value of the slope b. The six calibrating galaxies in Table 1 give very uncertain values of $b = 9.4$ or $b = 10.0$, depending on whether $\log \Delta v_{21}$ or M_H are treated as the independent variable. In the case of the Virgo galaxies the errors should lie mainly in the Δv_{21} values; hence treating M_H as an independent variable yields $b = 10.5$; this value is again quite uncertain and is sensitive to the weight given to individual galaxies. We therefore adopt the value of $b = 11.5$, which was derived for 300 field galaxies of different types, making allowance for a Virgo-centric infall model[1]. With this slope, the r.m.s. scatter of the present Virgo sample becomes $\sigma = 0.53$ mag.

From interpolation in Table 2 one finds for $b = 11.5$ a Virgo modulus of

$$(m-M)^0_{\text{Virgo}} = 31.47 \pm 0.34 \qquad (2)$$

corresponding to a distance of 19.7 ± 3.1 Mpc. Here the mean standard deviation includes the uncertainty of a, but it does not include a remaining systematic error of ± 0.15 mag due to the uncertainty of the slope b.

Table 3 Virgo cluster galaxies

Galaxy	Type	$H^c_{-0.5}$	$\log \Delta v_{21}$
NGC4178	SBc	10.14	2.48
NGC4192	Sb:	7.77	2.67
NGC4206	Sc:	10.34	2.49
NGC4216	Sb	7.33	2.74
NGC4294	SBc	10.77	2.39
NGC4380	Sab	9.77	2.55
NGC4388	Sab	8.86	2.61
NGC4438	Sb (tides)	7.75	2.59
NGC4450	Sab pec	8.05	2.60
NGC4498	SBc	10.76	2.34
NGC4501	Sbc	7.14	2.77
NGC4519	SBc	10.75	2.50
NGC4522	Sc/Sb:	10.60	2.36
NGC4535	SBc	8.45	2.62
NGC4569	Sab	7.45	2.61
NGC4651	Sc	8.65	2.67
NGC4654	SBc	8.79	2.58

Table 4 The relative distance between the Coma and Virgo cluster

Brightest cluster members	3.37 ± 0.40	ref. 32
Ten brightest cluster members	4.22 ± 0.28	ref. 33
$(u-V)$ colours of E galaxies	3.89 ± 0.20	ref. 34
Supernovae of type I	4.22 ± 0.33	ref. 35
Velocity dispersion of E galaxies	3.75 ± 0.18	ref. 2
Mg_2 index of E galaxies	4.00 ± 0.18	ref. 2
$(m-M)^0_{\text{Coma-Virgo}}$	3.92 ± 0.09	

Value of H_o

The distance of the Virgo cluster combined with its mean recession velocity leads to an unreliable value of the Hubble constant, because the observed recession velocity is affected by streaming motions within the Virgo complex and possibly, although to a lesser extent, by the random peculiar motion of the Local Group. Present determinations of the streaming velocities show them to be of the order of 20% of the Virgo cluster velocity (refs 7, 20 and refs therein).

At the distance of the Coma cluster with a mean recession velocity of almost 7,000 km s^{-1} any non-Hubble velocity component can hardly amount to as much as 10%, because it is known from the cosmic microwave background that the largest peculiar motion we partake of is ~600 km s^{-1}.

Fortunately the relative distance between the Virgo cluster and the Coma cluster is now determined with exceptional precision from different (relative) distance indicators. The available determinations are listed in Table 4 and yield a mean modulus difference of $\Delta(m-M)_{\text{Coma-Virgo}} = 3.92 \pm 0.09$, The IR colour–luminosity relation of E galaxies, which give decreasing distance differences with increasing wavelengths[21], have not been considered here, because these colours must either mean that the E galaxies in Virgo and Coma have different IR properties, or they reflect remaining observational difficulties. Indeed, the IR colours are not true simultaneous colour observations, but are the difference of magnitude observations taken by different observers at different telescopes and reduced to a common blue isophote.

The Virgo cluster distance in the previous section combined with the modulus difference from Table 4 gives a Coma modulus of

$$(m-M)_{\text{Coma}} = 35.39 \pm 0.35 \qquad (3)$$

corresponding to a distance of $r = 120 \pm 20$ Mpc. The mean Coma cluster velocity, corrected by $300 \sin l \cos b$ km s^{-1}, is $v_o = 6{,}952 \pm 61$ km s^{-1} (ref. 22) or with our preferred correction to the centroid of the Local Group[23], $6{,}913 \pm 61$ km s^{-1}. From this follows

$$H_0 = \frac{v_0}{r} = 58 \pm 10 \quad \text{km s}^{-1}\,\text{Mpc}^{-1} \qquad (4)$$

Discussion

We have followed the prescriptions given in refs 1 and 18 for the application of the IR magnitude–21 cm linewidth relation, and have shown that the value of the Virgo cluster modulus via the Tully–Fisher route does depend on the adopted slope b. Using the Aaronson–Mould best value of b, we obtain a modulus of 31.47 ± 0.34. Although we believe there are difficulties in the Tully–Fisher method when it is pushed to obtain $\pm 20\%$ accuracies, the purpose of this article is to point out that using the prescriptions advocated in ref. 1, we nearly reproduce the preferred value of $m - M = 31.7$ obtained by other methods[7]. The modulus increase over the Aaronson–Mould value of 31.08 ± 0.11 is due almost entirely to two causes: (1) The new fundamental distances of M33 and M81 have changed the constant term a in equation (1) from $a = -21.39 \pm 0.11$ (ref. 1) to $\sim -21.6 \pm 0.3$ (depending on the adopted slope). The change is not larger, because we have not applied the bulk correction to the calibrating galaxies for the increased Hyades modulus. (2) Aaronson and Mould[1] have adopted a slope of $b = 10$, although

their preferred solution gives a slope of $b = 11.54$. They argue that the choice of the slope has negligible effect on the distance modulus, but as shown in Table 2 their steeper slope makes a difference of $\Delta(m-M) = +0.13$. The sensitivity of the solution to the slope has also been emphasized by Richter and Huchtmeier[6]. (Aaronson and Mould[1] have not included in the quoted error their estimated zero point error of 0.2 mag; with this additional error included our error estimates do not deviate significantly.)

In an investigation of the slope of the blue Tully–Fisher relation, Richter and Huchtmeier[6] have found a slope of $b = 7.17$ and from a large sample of 66 Virgo galaxies a Virgo cluster modulus of $(m-M)_{\text{Virgo}} = 31.82 \pm 0.14$. Adopting their slope, using the local calibrators as shown in Table 1, and including only the 48 Virgo galaxies of type Sab to Sd of their sample, we find $(m-M)_{\text{Virgo}} = 31.66 \pm 0.26$ (the error here includes the error of the constant term $a = -19.82 \pm 0.23$).

It is reassuring for the 21 cm linewidth method, that it yields Virgo cluster distances for IR and blue magnitudes that are almost the same. The mean value of $(m-M)_{\text{Virgo}} = 31.57 \pm 0.25$ agrees well with our independently determined value $(m-M)_{\text{Virgo}} = 31.67 \pm 0.26$ (ref. 7), but we emphasize that the 21 cm method should be viewed with caution until it is better understood why the Hubble-type dependence has not yet been found in the radio data, but is clearly present in the optical results[10]. If one carries the distance scale beyond the Virgo cluster out to the Coma cluster by means of relative distance indicators given in Table 4, one obtains a value of the Hubble constant which must essentially be free of all irregularities of the expansion field and thus must be very close to the global value of H_0. Adopting a Virgo modulus based on the IR Tully–Fisher relation leads then to $H_0 = 58 \pm 10$, or $H_0 = 55 \pm 9$ on the basis of the IR and blue Virgo modulus. Both solutions lie well within the error range of the preferred value of $H_0 = 50 \pm 7$ (ref. 7).

There are remaining problems with the Tully–Fisher relation. In addition to the problem of the dependence on galactic type previously mentioned, there is (1) the possibility that the relation is not the same for field and cluster galaxies[24,25], and (2) the problem that the true intrinsic dispersion of the relation, if large, discriminates against intrinsically faint galaxies of a given linewidth and leads to an underestimate of the distances. It is therefore a matter of personal judgement how much weight one wants to attribute to the method. It is in any case, however, satisfactory to see that the 21 cm linewidth method, as it begins to mature, leads to results which are now consistent with the established distance scale.

This agreement is particularly satisfactory because it reinforces the time-scale agreement between the Hubble time of $H_0^{-1}\,(50) = 19.5 \times 10^3$ Myr the age of the chemical elements at $\sim 20 \times 10^3$ Myr (ref. 26), and the age of the globular clusters in our Galaxy at $\sim 18 \times 10^3$ Myr (refs 27, 28).

G.A.T. is a visiting associate of Mount Wilson and Las Campanas Observatories, Carnegie Institution, and acknowledges support from the Swiss NSI.

Received 24 June; accepted 8 November 1983.

1. Aaronson, M. & Mould, J. *Astrophys. J.* **265,** 1–17 (1983).
2. Dressler, A. Preprint, Mount Wilson and Las Campanas Observatories (1983).
3. Sandage, A. *Astr. J.* **88,** 1108–1125 (1983).
4. Sandage, A. & Carlson, G. *Astrophys. J. Lett.* **267,** L25–28 (1983).
5. Sandage, A. *Astr. J.* (in the press).
6. Richter, O.-G. & Huchtmeier, W. K. *Astr. Astrophys.* (in the press).
7. Sandage, A. & Tammann, G. A. *Astrophysical Cosmology* (eds Brück, H. A., Coyne, G. V. & Longair, M. S.) 23–81 (Pontifica Academia Scientiarum, 1982); *Astrophys. J.* **256,** 339–345 (1982).
8. Tammann, G. A. & Sandage, A. *Highlights Astr.* **6,** 301–313 (1983).
9. Sandage, A. & Tammann, G. A. *A Revised Shapley-Ames Catalog of Bright Galaxies*, 6 (Carnegie Institution, Washington, 1981).
10. Rubin, V. C. *IAU Symp.* No. 100, 3–8 (1983).
11. Sandage, A. & Tammann, G. A. *Astrophys. J.* **151,** 531–545 (1968).
12. Sandage, A. & Tammann, G. A. *Astrophys. J.* **157,** 683–708 (1969).
13. Madore, B. F. Talk at IAU General Assembly, Patras (1982).
14. McAlary, C. W. & Madore, B. F. Preprint, Steward Observatory, Tucson (1983).
15. Graham, J. A. *Astrophys. J.* **252,** 474–480 (1982).
16. Graham, J. A. *Highlights Astr.* **6,** 209–216 (1983).
17. Aaronson, M., *et al. Astrophys J. Suppl.* **50,** 241–262 (1982).
18. Mould, J., Aaronson, M. & Huchra, J. *Astrophys. J.* **238,** 458–470 (1980).
19. Aaronson, M., Mould, J. & Huchra, J. *Astrophys. J.* **237,** 655–665 (1980).
20. Yahil, A., Sandage, A. & Tammann, G. A. *Physical Cosmology* (eds Balian, R., Audouze, J. & Schramm, D. N.) 158 (North Holland, Amsterdam, 1980).
21. Aaronson, M., Persson, S. E. & Frogel, J. A. *Astrophys. J.* **245,** 18–24 (1981).
22. Tifft, W. G. & Gregory, S. A. *Astrophys. J.* **205,** 696–708 (1976).
23. Yahil, A., Sandage, A. & Tammann, G. A. *Astrophys. J.* **217,** 903–915 (1977).
24. Kraan-Korteweg, R. C. *Astr. Astrophys.* **125,** 109–111.
25. Giovannelli, R. C. *IAU Symp.* No. 104, 273–278 (1983).
26. Thielemann, F.-K., Metzinger, J. & Klapdor, H. V. *Z. Phys.* **A309,** 301–317 (1983).
27. Sandage, A. *Astrophys. J.* **252,** 553–573 (1982).
28. VandenBerg, D. A. *Astrophys. J. Suppl.* **51,** 29–65 (1983).
29. Tammann, G. A. & Sandage, A. *Astrophys. J.* **151,** 825–860 (1968).
30. Sandage, A. & Tammann, G. A. *Astrophys. J.* **194,** 223–243 (1974).
31. Sandage, A. *Astr. J.* **88,** 1569–1578 (1983).
32. Sandage, A. & Hardy, E. *Astrophys. J.* **183,** 743–757 (1973).
33. Weedman, D. W. *Astrophys.* **203,** 6–13 (1976).
34. Visvanathan, N. & Sandage, A. *Astrophys. J.* **216,** 214–226 (1977).
35. Tammann, G. A. *Supernovae: A Survey of Current Research* (eds Rees, M. J. & Stoneham, R. J.) 371–403 (1982).

A helix stop signal in the isolated S-peptide of ribonuclease A

Peter S. Kim & Robert L. Baldwin

Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305, USA

The isolated S-peptide (residues 1–20 of ribonuclease A) is known to show partial α-helix formation in aqueous solutions at low temperatures. We show here that the helix is limited to certain residues, including ones that are helical in the intact protein, and that a functional helix termination signal exists in the isolated peptide.

THE α-helix is the most abundant unit of secondary structure in proteins. Most α-helices are quite short: the average length of α-helices in proteins is about 11 residues[1]. In contrast, the average length of α-helices is much longer in synthetic polypeptides (about 100 residues at the transition midpoint)[2], and therefore, α-helix termination signals exist in proteins. The nature of these helix termination signals is of fundamental interest with respect both to the mechanism of protein folding and the prediction of folding from amino acid sequence. An important question concerning the mechanism of protein folding is whether the helix start and stop signals function at early stages in folding when the α-helices and β-sheets are first formed, or whether tertiary interactions are required to fix the ends of helices.

The X-ray structures of RNase A[3] and of RNase S[4] show that RNase contains three short helices. [RNase S is an enzymatically active derivative of RNase A cleaved at the peptide bond between residues 20 and 21; the proteolysis products can be separated and are referred to as the S-peptide (residues 1–20) and the S-protein (residues 21–124)[5].] The structure of interest here is the α-helix formed by residues 3–13 (counting residues containing at least one α-helical H-bond). The crystal structure shows that the amide proton of Asp 14 (the next H-bond donor at the C-terminal end of the 3–13 helix) is H-bonded to the

0028–0836/84/040329—06$01.00

© 1984 Macmillan Journals Ltd

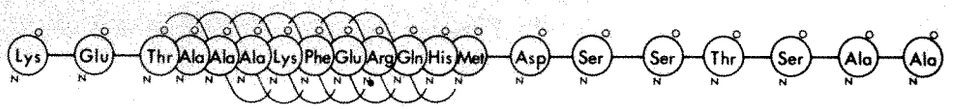


Fig. 1 Amino acid sequence of S-peptide (1–20), showing the α-helix formed by these residues in native RNase A[3] and RNase S[4]. S-peptide (1–20) was prepared and purified for us by V. MacCosham. RNase A (Sigma Type 1-AS, lot no. 81F-8015) was purified[18] and then digested with Subtilisin Carlberg (Sigma Type VIII, lot no. 48C-0164)[19]. S-peptide was purified by ion exchange chromatography (SP-Sephadex C25 in 0.01 M HCl; 0.05–1.0 M NaCl gradient) and by gel filtration (Sephadex G-25 fine in 0.01 M HCl). Two peptides were separated using the purification procedure above, and amino acid analysis (performed at the Department of Biochemistry and Biophysics, University of California, Davis) indicated that the peptides were S-peptide (1–19) and S-peptide (1–20) (see ref. 20). All experiments reported here were made using purified S-peptide (1–20).

carbonyl oxygen of Val 47 in a tertiary interaction (contrast Fig. 4 f of ref. 6). Thus, this tertiary interaction holds residue 14 in a non-helical conformation. One would like to know whether this tertiary interaction is required for the localization of the 3–13 helix, or if a functional helix termination signal exists in the absence of tertiary interactions (for example in an early protein folding intermediate).

One direct approach for investigating the nature of helix termination signals is to study the folding of protein fragments. Until recently, however, this has not been possible because even large fragments of single-domain proteins have been found to be unfolded in aqueous solutions[7,8]. Moreover, 'host-guest' data for α-helix formation in water by synthetic polypeptides[9] indicate that short α-helices (<20 residues) are always unstable in water, regardless of the amino acid composition. These data have led to the conclusion that secondary structure in protein fragments is not stable in the absence of stabilizing tertiary structure interactions.

Nevertheless, both the C-peptide of RNase A (residues 1–13, obtained by cyanogen bromide cleavage at Met-13, yielding HSer-13 lactone as the C-terminal residue), and the S-peptide (residues 1–20) show partial helix formation in aqueous solutions as judged by circular dichroism (CD) spectra and by ^{1}H nuclear magnetic resonance (NMR) studies[10–13]. Some characteristics of the helix formed by the C-peptide are as follows. (1) Up to ~30% helix content is observed in water near 0 °C[11], which is 1,000 times greater than the helix content predicted using data from host–guest studies. (2) Helix formation is strongly dependent on temperature, and C-peptide is essentially unfolded in aqueous solutions at 25 °C[10,11]. (3) Equilibrium sedimentation measurements show that C-peptide is monomeric at concentrations up to 2 mg ml^{-1} in conditions in which helix formation occurs (1 °C, 0.1 M ionic strength)[10]. (4) The *p*H dependence of helix stability follows a bell-shaped curve[11], with maximum helix formation occurring near *p*H 5. A possible explanation for this *p*H dependence has been given[11]: a salt bridge between the ionized sidechains of Glu 9$^-$ and His 12$^+$ could stabilize the helix.

Here we use S-peptide (1–20), which contains the first 20 amino acids of RNase A, to ask whether the α-helix formed by the isolated peptide stops near residue 13 (the last residue of this helix in native RNase A or RNase S). Five residues with low helical propensity occur in a stretch next to the 3–13 helix (residues 14–18, see Fig. 1). Although studies of helix formation in random copolymers allow some amino acid residues to be classified as 'helix formers' and others as 'helix breakers', the reported stability constant (s) values for helix growth span a very narrow range ($s = 0.5$–1.3 for all residues studied)[9]. The 'cooperative length' of an α-helix is about 100 residues[2] = $1/\sigma^{1/2}$, where σ is the helix nucleation constant; $\sigma \sim 10^{-4}$ for all residues studied[9]. Predicted distributions of helix length for short peptides (including end effects) containing a single residue type are discussed by Schellman[14]. For a 20 residue peptide, these results show that a significant fraction of the α-helices formed will include all 20 residues, in the absence of helix termination signals.

To determine whether the α-helix formed by S-peptide (1–20) is localized, we have followed the ^{1}H-NMR chemical shifts of various sidechain groups as the helix is unfolded. The helices formed by S-peptide (1–20) and C-peptide lactone can be unfolded by increasing temperature[11,13], GuHCl[12], or urea, as we report here. Since the stability of the S-peptide (1–20) helix is *p*H dependent[13], we have also monitored the ^{1}H-NMR chemical shifts of sidechain groups as the helix content is changed by varying *p*H. Of particular interest are those amino acid residue types that are present both within and outside the native 3–13 α-helix (Fig. 1). These include the alanine residues (Ala 4, 5, and 6 are in the helix of native RNase A, whereas Ala 19 and 20 are not), and the threonine residues (Thr 3 is in the native helix and Thr 17 is not). The β-CH_3 group of alanine is physically close to the α-helix backbone, and the alanine β-CH_3 resonance is expected to shift with helix formation, as has already been observed for Ala 4, 5, and 6 in the C-peptide lactone helix[12]. The γ-CH_3 resonance of Thr 3 also shifts as the C-peptide lactone helix is formed[11], and here we ask whether the Thr 17 (γ-CH_3) resonance shows a comparable helix shift.

Table 1 Comparison between NMR helix shifts and CD changes in S-peptide (1–20) and C-peptide lactone (1–13).

NMR resonance	Peptide	Helix shift $\Delta\delta$ (p.p.m.)*	Molar peptide CD change $\Delta[\theta]_{222}$ (deg cm^2 dmol^{-1})†	Normalized helix shift $\Delta\delta/\Delta[\theta]_{222}$	Ratio (R) of normalized helix shift S-Peptide (1–20): C-Peptide lactone‡
Thr 3 (γCH_3)	S-peptide (1–20)	0.071	4.30×10^4	1.65×10^{-6}	1.07
Thr 3 (γCH_3)	C-peptide (lactone)	0.103	6.69×10^4	1.54×10^{-6}	
Phe 8 (ϕH)§	S-peptide (1–20)	−0.051	4.30×10^4	-1.19×10^{-6}	1.04
Phe 8 (ϕH)	C-peptide (lactone)	−0.076	6.69×10^4	-1.14×10^{-6}	

* Difference in chemical shift between 4 °C and 47 °C, at the optimum *p*H for helix formation. S-peptide (1–20) data are for *p*H 3.8. Data for C-peptide lactone (*p*H 5.0) are from ref. 11. Temperature dependence of chemical shift, in absence of helix formation, not taken into account.

† Difference in $[\theta]_{222}$ per mole peptide between 3 °C and 40 °C (*p*H 3.8) for S-peptide (1–20)[13] and between 3 °C and 45 °C (*p*H 5.0) for C-peptide lactone[11].

‡ Predicted value for R is 1.0 if the helix is localized to the same residues in both peptides and if the helix shifts are the same for both peptides at 100% helix formation. If the helices propagate to the ends of both peptides, then R is predicted to be ≤ 0.63 ($\frac{12}{19}$, corresponding to the ratio of the number of peptide carbonyl groups). Due to end effects on the apparent molar ellipticity of an α-helix (see ref. 24 and refs therein), R will be <0.63 if the helices include all residues of both peptides.

§ There are six visible resonance lines for Phe 8, and only three of them shift significantly with helix formation[11]. Values are reported for the middle resonance of these three.

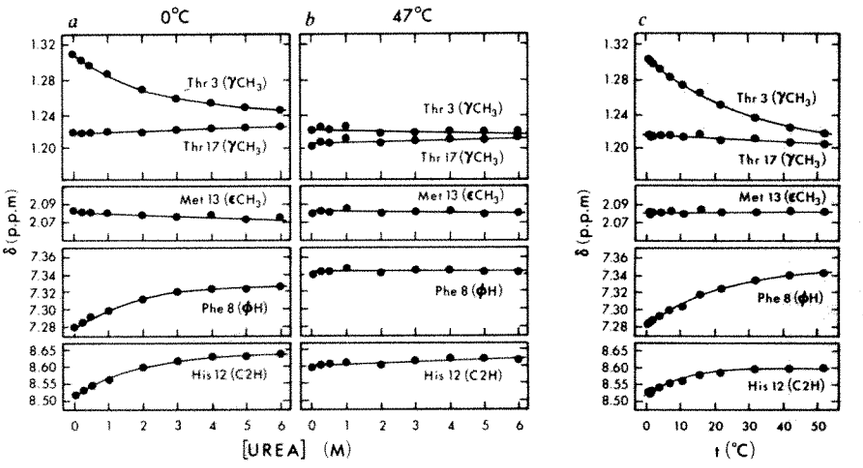


Fig. 2 *a*, Urea dependence of ^{1}H-NMR chemical shift for some side-chain resonances in S-peptide (1–20): 0 °C, *p*H 3.8, 0.1 M NaCl, D_2O. *b*, Urea dependence: 47 °C, *p*H 3.8, 0.1 M NaCl, D_2O. *c*, Temperature dependence of ^{1}H-NMR chemical shift for some side-chain resonances in S-peptide (1–20): *p*H 3.8, 0.1 M NaCl, D_2O. The average chemical shift for the threonine γ-CH_3 doublets are shown. There are six visible resonance lines for Phe 8 (ϕH), and only three of them shift significantly with helix formation. The middle resonance of these three is shown.

Methods: All samples contained 0.1 M NaCl in D_2O. *p*H was adjusted with DCl or NaOD, and *p*H values refer to meter readings without isotope corrections. Urea and GuHCl were Schwarz-Mann ultrapure grade. Urea-d_4 and GuDCl-d_6 were prepared by repeated lyophilization from D_2O (Liquid Carbonic, 99.7% C). All other chemicals were reagent grade. Fourier-transform proton NMR spectra were recorded on a modified Bruker 360 MHz instrument at the Stanford Magnetic Resonance Facility. All chemical shifts are reported relative to the internal standard, TSP. Corrections were made for the *p*H dependence of the TSP signal[21]. Resolution-enhanced NMR spectra were obtained by multiplication of the free-induction decay with either a double exponential[22] or a sine bell function[23]. Damping of the free-induction decay in the presence of urea was observed (contrast ref. 20), but did not affect the spectra of the peptide. Resonance assignments have been or will be given elsewhere[20] (A. Bierzynski *et al.* manuscript in preparation). The Thr 17 (γ-CH_3) resonance was assigned by comparing the spectra of S-peptide (1–15) and S-peptide (1–20) in the unfolded state (5.0 M GuDCl, 0° or 47 °C).

Side-chain resonances

The optimum conditions for α-helix formation in aqueous solutions of S-peptide (1–20), as judged by CD, are *p*H 3.8, 0 °C[13]. As urea is added in these conditions, some side-chain resonances show chemical shift changes while others do not (Fig. 2*a*). The side-chain resonances of Thr 3, Phe 8 and His 12 show changes in chemical shift as the helix is unfolded with urea. However, when the α-helix is already thermally unfolded at 47 °C, urea does not affect the chemical shifts of these resonances (Fig. 2*b*).

In contrast to Thr 3, the side-chain resonance of Thr 17 does not change as the helix is unfolded (Fig. 2). Thus, Thr 17 is probably not in the α-helix formed by the isolated S-peptide (1–20). End effects (helix fraying) are not sufficient to explain the difference observed between Thr 3 and Thr 17, because Thr 3 is the third residue from the amino-terminus and Thr 17 is the fourth residue from the carboxyl-terminus.

The S-CH_3 resonance of Met 13 does not shift as urea is added (Fig. 2). However, the lack of a helix shift for Met 13 is difficult to interpret for two related reasons. (1) The S-CH_3 sidechain group of methionine is four bonds removed from the peptide backbone, and it may be possible for the helix to form without perturbing the magnetic environment of the S-CH_3 protons. (2) We do not have a positive control demonstrating a helix shift for a methionine S-CH_3 resonance, whereas with other resonances (for example threonine γ-CH_3), a positive control exists. It is therefore not possible to tell whether Met 13 is contained in the helix formed by isolated S-peptide (1–20). In native RNase A, Met 13 is the last residue in the α-helix. His 12 (ring C2H resonance) does show a helix shift (Fig. 2). The β-CH_3 resonances of Ala 4, 5, 6, 19, and 20 are not well separated in spectra taken in urea at high temperatures, or at high urea concentrations, 0 °C, and are therefore not depicted in Fig. 2.

The α-helices formed by both C-peptide lactone and S-peptide (1–20) unfold with increasing temperature[11,13]. Figure 2*c* depicts the temperature dependences of the chemical shifts for the resonances shown previously, at *p*H 3.8. The residues that show a helix shift for urea-induced unfolding (Fig. 2*a*) also show a temperature-induced helix shift (Fig. 2*c*). In particular, the Thr 17 resonance, which does not show a urea-induced helix shift (Fig. 2*a*), also does not show a temperature-induced shift (Fig. 2*c*), supporting the suggestion that the S-peptide (1–20) helix is localized.

Guanidine deuterochloride (GuDCl) is known to affect the chemical shifts of many side-chain resonances, and these changes are often not attributable to the unfolding of structure. Moreover, the effects of GuDCl on chemical shifts are dependent on neighbouring groups, suggesting that the effects are related to the binding of GuDCl to the peptide[12]. Our GuDCl titrations of S-peptide (1–20) at 37 °C, where the helix is thermally unfolded, also show GuDCl-induced changes in chemical shift (Fig. 3*b* and 4*b*) and some of these resonances continue to change between 3–5 M GuDCl, where S-peptide (1–20) should be in a 'random coil' conformation.

In addition, GuDCl appears to cause a 'salt effect' on the chemical shifts between 0 and 1 M GuDCl that does not appear to be related to helix structure for the following reasons. (1) The effect is observed even at 37 °C, where the helix is thermally unfolded (Fig. 3*b* and Fig. 4*b*). (2) Essentially all resonances show the salt-effect. These GuDCl-induced chemical shifts may be caused in part by effects of GuDCl on the internal standard (TSP). Both GuDCl and TSP are charged, and we have observed NaCl-dependent shifts of the TSP resonance relative to acetone or dioxane.

When the GuDCl titrations are performed at 0 °C (favouring helix formation), some resonances show changes in chemical shift that evidently are caused by helix unfolding (Fig. 3*a* and Fig. 4*a*). To determine the changes in chemical shift caused by helix unfolding, we have subtracted the GuDCl dependence of the chemical shifts at 37 °C, in the absence of helix formation, from the dependence at 0 °C where the helix is populated. The temperature difference curves for the GuDCl titrations are depicted in Fig. 3*c*. As was found with urea and temperature, the side-chain resonances of Thr 3, Phe 8, and His 12 show helix shifts with GuDCl, whereas those of Met 13 and Thr 17 do not (Fig. 3*c*).

An advantage of using GuDCl is that resonances are spread out which arise in similar regions of the NMR spectrum. In particular, the five alanine β-CH_3 groups (Ala 4, 5, 6, 19, and 20) give rise to ten resonance lines in the NMR spectrum of S-peptide (1–20). There is considerable overlap of these resonances in the spectrum, under conditions in which the helix is unfolded by urea or temperature. The interaction of GuDCl with the peptide results in the separation of these resonances in the spectrum (Fig. 4). The resonances listed as Ala I, II, III correspond to Ala 4, 5, 6 respectively (see Fig. 4 legend).

In S-peptide (1–20), as in C-peptide lactone[12], Ala 4, 5, and 6 show significant helix shifts; the difference between the GuDCl titration curves at 0° and at 37 °C is shown for each resonance in Fig. 4*c*. In marked contrast, Ala 19 and 20 do not show measurable helix shifts (Fig. 4*c*), supporting further the sugges-

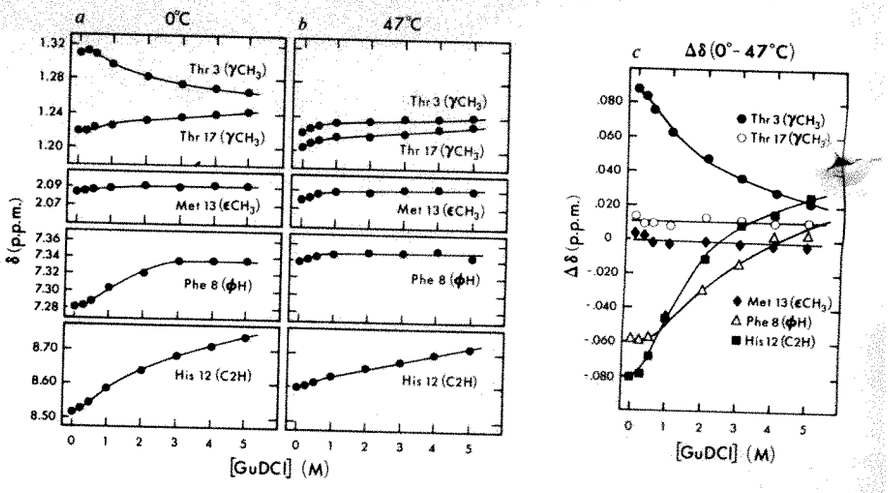


Fig. 3 GuDCl titrations of S-peptide (1–20). *a*, 0 °C, *p*H 3.8, 0.1 M NaCl, D_2O. *b*, 47 °C, *p*H 3.8, 0.1 M NaCl, D_2O. *c*, $\Delta\delta$ (0–47 °C); the difference in chemical shift between 0 °C and 47 °C.

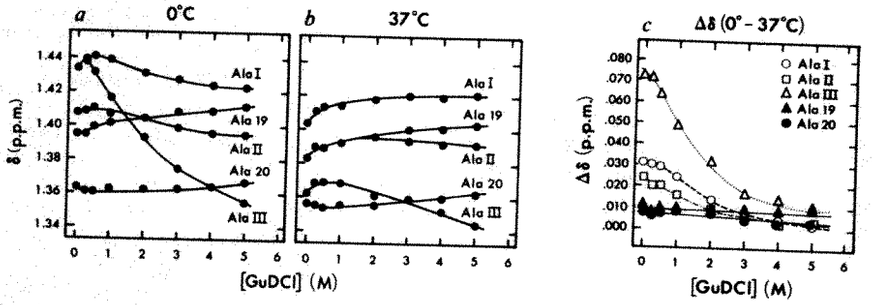


Fig. 4 GuDCl titrations of the alanine β-CH_3 resonances in S-peptide (1–20). *a*, 0 °C, *p*H 3.8, 0.1 M NaCl, D_2O. *b*, 37 °C, *p*H 3.8, 0.1 M NaCl, D_2O. *c*, $\Delta\delta$ (0–37 °C); the difference in chemical shift between 0 °C and 37 °C. The average chemical shift for each alanine β-CH_3 doublet is shown. The resonances numbered Ala I, II, III correspond to Ala 4, 5 and 6 respectively. These assignments were made, after submission of this paper, using a synthesized peptide containing deuterated alanine. The resonance assignments of Ala 19 and 20 were made by comparing the spectra of S-peptide (1–15) and S-peptide (1–20) in the unfolded state (5.0 M GuDCl, 0° or 37 °C) to locate Ala 19 and 20, and by *p*H titration of the α-COOH group of Ala 20, to locate Ala 20.

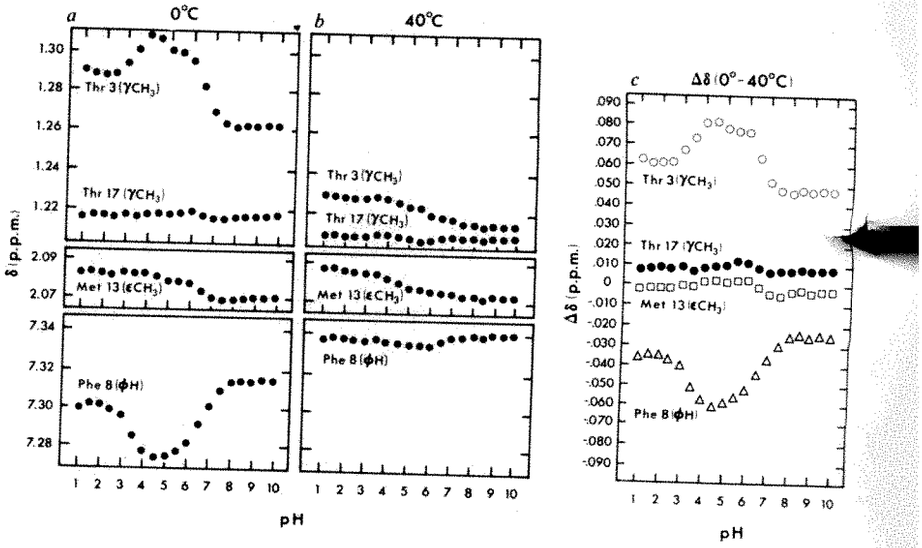


Fig. 5 *p*H titration of S-peptide (1–20). *a*, 0 °C, 0.1 M NaCl, D_2O. *b*, 40 °C, 0.1 M NaCl, D_2O. *c*, $\Delta\delta$ (0–40 °C); the difference in chemical shift between 0 °C and 40 °C. The *p*H titration of His 12 (C2H) is dominated by changes in chemical shift due to the titration of the imidizole ring and is not depicted. The Ala β-CH_3 resonances are not well separated in some conditions and are not shown.

tion that the α-helix formed by isolated S-peptide (1–20) is localized. The alanine β-CH_3 resonances are particularly useful indicators of helix formation. Only one bond separates the alanine β-CH_3 group from the peptide backbone. Moreover, rotation about this bond ($C_\alpha - C_\beta$) is fast on the NMR time scale under all conditions investigated here. A small linear shift is observed for Ala 20 as GuDCl is added, but this shift is clearly distinct from the helix shifts observed for Ala 4, 5, and 6 (Fig. 4*c*). The linear GuDCl shift for Ala 20 is probably related to the fact that Ala 20 is the last residue in the peptide, and contains the α-COO^- group.

Comparison with CD results

A direct comparison between the molar ellipticity change (222 nm) caused by helix formation for C-peptide lactone and for S-peptide (1–20) is not yet possible, for the following reasons. (1) Only partial helix formation has been observed for either peptide, even in the optimal helix-forming conditions. For C-peptide lactone, a helix content of 30% at *p*H 5.0, 0 °C, has been estimated from CD measurements. It is likely that, in these conditions, 30% of the molecules are helical and 70% are in a random coil conformation; almost all resonances of C-peptide lactone show shifts as the helix is formed[11] (A.

Bierzynski *et al.*, manuscript in preparation). Any comparison between the CD changes on helix formation for the two peptides depends critically on the relative stabilities of the helices formed by the two peptides. (2) The *p*H–stability profiles for C-peptide lactone[11] and for S-peptide[13] differ in important respects: the optimal *p*H is 5.0 for C-peptide lactone but 3.8 for S-peptide. Consequently, a comparison between helix stabilities at any given *p*H is arbitrary. A comparison between the molar ellipticity changes on helix formation by the two peptides has been made earlier, at *p*H 5.5, by Brown and Klee[15], who were not aware either of the strong *p*H dependence of helix stability or of the different *p*H–stability profiles of the two helices. Also, they studied helix formation in an unresolved mixture of C-peptide lactone and C-peptide carboxylate, without knowing the striking difference between the helix-forming properties of these two peptides (contrast ref. 13).

We have made an approximate comparison between the molar ellipticity changes on helix formation for C-peptide lactone and S-peptide (1–20) in the following way. We assume that the residues within the helix have the same structure in the helical conformation of both peptides and consequently that the helix shifts for 100% helix formation are the same for both helices, measured for any of several side-chain resonances. From the observed helix shifts, we calculate a ratio for the extent of helix formation for the two peptides. This ratio is then compared to the ratio of ellipticity changes (per mole peptide). The results (Table 1) show that these two ratios are the same, indicating that approximately the same number of residues participate in helix formation in both peptides. This is consistent with the conclusion that the helix in S-peptide (1–20) terminates before Thr 17 and possibly near Met 13.

*p*H dependence of helix stability

As mentioned above, the stabilities of both the S-peptide (1–20) helix and the C-peptide lactone helix are strongly dependent on *p*H. However, the *p*H dependence of $[\theta]_{222}$ is more complex in S-peptide (1–20)[13] than in C-peptide lactone[11]. At low temperatures, where the helix is populated, the *p*H dependence of $[\theta]_{222}$ shows a complex curve[13], in contrast with the almost symmetric bell-shaped curve found with C-peptide lactone[11]. When either peptide is thermally unfolded (40–45 °C), $[\theta]_{222}$ is essentially independent of *p*H[11,13]. These results suggest that charged residue(s) which affect helix stability exist in S-peptide (1–20) that are not present in C-peptide lactone.

We have monitored the *p*H dependence of chemical shift for some resonances of S-peptide (1–20) at 0 °C and 40 °C, and the results are depicted in Fig. 5. At 40 °C, some resonances show *p*H-dependent chemical shifts (Fig. 5*b*) that are unrelated to helix formation. We again take the difference in chemical shift between 0 °C and 40 °C, to obtain the shifts that are due to helix formation. The results (Fig. 5*c*) show that both Thr 3 and Phe 8 give *p*H-dependent helix shifts that are qualitatively similar to the *p*H dependence of $[\theta]_{222}$ for S-peptide (1–20)[13]. In contrast, Thur 17 and Met 13 ($S\text{-}CH_3$) do not show a significant *p*H-dependent shift in the temperature difference plot (Fig. 5*c*). These data agree with the conclusion that S-peptide (1–20) contains a functional helix termination signal, and they suggest that the same residues are contained within the helix at all *p*H values where helix formation is observed.

NMR and helix termination

In the present study, we have monitored side-chain resonances and have measured 'helix shifts' (changes in chemical shift caused by alterations in the helix stability with temperature, urea, GuDCl, or *p*H) to determine whether a given residue participates in helix formation. The fact that self-consistent results are obtained with all four methods of changing the helix content lends support to this procedure. Nevertheless, the results with the $S\text{-}CH_3$ resonance of Met 13 point out a possible danger in using this approach: the group may be too far removed from the peptide backbone to show a significant change in chemical shift on helix formation. In this regard, the results with the $\beta\text{-}CH_3$ resonances of Ala 4, 5, 6, 19, and 20 are particularly valuable in indicating helix termination within the peptide, because the $\beta\text{-}CH_3$ group of Ala is very close to the peptide backbone of the helix. In order to study these five Ala resonances, it was necessary to make use of the ability of GuDCl to spread out the chemical shifts of groups of the same residue type.

Helix termination signal

Measurements of the equilibrium constant for nucleation (σ) in homopolymers and random copolymers[9] indicate that σ is always very small ($\sigma \sim 10^{-4}$). The cooperative length of an α-helix in aqueous solutions is thus predicted to be about 100 residues[2] ($=1/\sigma^{1/2}$). In contrast, our results indicate that the helix formed by S-peptide (1–20) is localized to certain residues. The features of the naturally occurring amino acid sequence of S-peptide (1–20) that are responsible for helix termination are of obvious interest.

In order to understand the mechanism of helix termination in S-peptide (1–20), we will need to have a more detailed description of termination. One wants to know at which residue the helix terminates and whether termination is gradual (spread out over a few residues) or abrupt. In future work it will be useful to use ^{13}C-enriched amino acids to monitor backbone resonances, and to monitor additional side-chain resonances with two-dimensional NMR techniques. One would also like to find conditions (perhaps with new derivatives of the S-peptide and C-peptide) in which helix formation goes to completion for those residues in the helix. Then the molar CD changes for helix formation in different peptides can be compared directly, and the NMR results can be extended by measuring amide proton exchange rates and nuclear Overhauser effects.

We consider here two contrasting models for helix termination in peptides that may also play a role in protein folding. Our present data are largely consistent with a simple helix termination mechanism in which the amino acid sequence increases the helix nucleation constant (σ), and therefore decreases the cooperative length of the helix (for example, increasing σ to 10^{-2} would decrease the cooperative length to 10 residues). Then, a stretch of residues with low helix propensity (for example, residues 14–18 of S-peptide) would prevent propagation of the helix. The observed stabilities of the C-peptide lactone and S-peptide (1–20) helices are 1,000-fold greater than that predicted using the value of $\sigma \sim 10^{-4}$ obtained from polymer studies. If σ were larger in S-peptide, for example due to specific interactions between sidechains, then the increased stability and termination of the S-peptide (1–20) helix could be explained. On the other hand, the mechanism of helix termination may directly involve specific interactions between sidechain groups. For example, model building shows that a H-bonding interaction between the sidechains of His 12^+ and Asp 14^- would prevent propagation of the S-peptide (1–20) helix past residue 13. The *p*H dependence of S-peptide (1–20) is more complex than that of C-peptide lactone[13], and the additional ionizing groups in S-peptide (1–20) involve only Asp 14 and the $\alpha\text{-}COO^-$ group of Ala 20. More work is needed to distinguish between these and other possible explanations of helix termination in S-peptide.

Mechanism of protein folding

Studies of C-peptide lactone[11] and S-peptide[13] have demonstrated that isolated units of secondary structure can have significant stability in water. The data presented here suggest that functional termination signals can exist in isolated units of secondary structure. These results are consistent with the simple framework model for protein folding in which the amino acid sequence directly determines the positions of the α-helices and β-sheets, and these secondary structures then pair to form the tertiary fold. Since C-peptide lactone and S-peptide provide the first examples of α-helix formation and termination in aqueous solutions of a short peptide fragment of a protein, it is important to point out that they provide only isolated examples, and many

more examples will be needed to base conclusions about the folding mechanism.

These data are encouraging with regard to predicting secondary structure from amino acid sequence because they suggest that helix formation in the isolated S-peptide is localized to the same, or nearly the same, residues as in native RNase A. It is interesting that present schemes for predicting secondary structure are more successful in finding the locations of helices than in defining their endpoints[17]. Experiments of the type described here, with peptides of specified amino acid sequence, may provide clues about the nature of helix termination signals.

We thank Drs K. Kuwajima and D. E. Wemmer for discussions, S. G. Boxer for use of his computing facilities and V. MacCosham for technical assistance. This work was supported by NIH grant 2 RO1 GM 19988-22. P.S.K. is a predoctoral fellow of the Medical Scientist Training Program. Use of the Stanford Magnetic Resonance Facility (supported by NSF grant GP 23633 and NIH grant RR 00711) is acknowledged.

Note added in proof: A recent ^{1}H-NMR study[25] (published after submission of this article) of helix formation by S-peptide (1–19) agrees with our conclusion that the helix is limited to certain residues including the helical residues of RNase A.

Received 5 September; accepted 10 October 1983.

1. Schulz, G. E. & Schirmer, R. H. *Principles of Protein Structure* (Springer, New York, 1979).
2. Poland, D. & Scheraga, H. A. *Theory of Helix-Coil Transitions in Biopolymers* (Academic, New York, 1970).
3. Wlodawer, A., Bott, R. & Sjölin, L. *J. biol. Chem.* **257,** 1325–1332 (1982).
4. Richards, F. M. & Wyckoff, H. W. *Atlas of Molecular Structures in Biology 1. Ribonuclease-S* (Clarendon, Oxford, 1973).
5. Richards, F. M. & Vithayathil, P. J. *J. biol. Chem.* **234,** 1459–1465 (1959).
6. Wyckoff, H. W. *et al. J. biol. Chem.* **245,** 305–328 (1970).
7. Epand, R. M. & Scheraga, H. A. *Biochemistry* **7,** 2864–2872 (1968).
8. Taniuchi, H. & Anfinsen, C. B. *J. biol. Chem.* **244,** 3864–3875 (1969).
9. Scheraga, H. A. *Pure appl. Chem.* **50,** 315–324 (1978).
10. Brown, J. E. & Klee, W. A. Biochemistry **10,** 470–476 (1971).
11. Bierzynski, A., Kim, P. S. & Baldwin, R. L. *Proc. natn. Acad. Sci. U.S.A.* **79,** 2470–2474 (1982).
12. Bierzynski, A. & Baldwin, R. L. *J. molec. Biol.* **162,** 173–186 (1982).
13. Kim, P. S., Bierzynski, A. & Baldwin, R. L. *J. molec. Biol.* **162,** 187–199 (1982).
14. Schellman, J. A. *J. chem. Phys.* **62,** 1485–1494 (1958).
15. Brown, J. E. & Klee, W. A. *Biochemistry* **8,** 2876–2879 (1969).
16. Blackburn, P. & Moore, S. *The Enzymes* Vol. 15, 317–433 (Academic, New York, 1983).
17. Schulz, G. E. *et al. Nature* **250,** 140–142 (1974).
18. Garel, J.-R. *Eur. J. Biochem.* **70,** 179–189 (1976).
19. Doscher, M. S. *Meth. Enzym.* **11,** 640–648 (1967).
20. Kuwajima, K. & Baldwin, R. L. *J. molec. Biol.* **169,** 281–297 (1983).
21. DeMarco, A. *J. Mag. Res.* **26,** 527–528 (1977).
22. Ferrige, A. G. & Lindon, J. C. *J. Mag. Res.* **31,** 337–340 (1978).
23. DeMarco, A. & Wüthrich, K. *J. Mag. Res.* **24,** 201–204 (1976).
24. Chen, Y.-H., Yang, J. T. & Chau, K. H. *Biochemistry* **13,** 3350–3359 (1974).
25. Rico, M. *et al. FEBS Lett.* **162,** 314–319 (1983).

Activation of a translocated human c-*myc* gene by an enhancer in the immunoglobulin heavy-chain locus

Adrian C. Hayday*, Stephen D. Gillies*, Haruo Saito*, Charles Wood*, Klas Wiman†, William S. Hayward† & Susumu Tonegawa*

* Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
† Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA

*A tissue-specific transcriptional enhancer element that is associated with the human immunoglobulin heavy-chain locus is defined. In a non-Hodgkin's lymphoma that contains a translocated c-*myc *gene this enhancer is retained on the 14q$^+$ chromosome and occurs within sequences shown to activate previously cryptic promoters of the c-*myc *gene.*

A FUNCTIONAL immunoglobulin gene is generated somatically during the differentiation of B lymphocytes by a set of developmentally regulated gene rearrangements[1]. The major function of these rearrangements is to generate a diverse set of complete immunoglobulin genes from a limited number of inherited gene segments.

Another important role of these rearrangements has recently been identified at least for the mouse immunoglobulin heavy-chain genes[2–4]. In an individual B lymphocyte and its progenies, only one of a few hundred copies of the variable (V) gene segments is expressed. This activation of a specific V gene segment results from its direct rearrangement into the vicinity of a transcriptional enhancer element that is located upstream of the constant (C) region gene segments, between the joining (J) segments and the switch (S) region. Interestingly, the immunoglobulin gene-associated enhancer functions in a tissue-specific manner, suggesting a more general involvement of cellular enhancer elements in tissue-specific expression of eukaryotic genes during cell differentiation.

Another biological process in which gene rearrangements and enhancer elements may play a critical, albeit adventitious, role is tumorigenesis. A model supporting this notion comes from the studies on the chicken leukaemogenesis induced by the non-acute avian leukosis virus (ALV). By integrating close to the cellular oncogene, c-*myc*, the ALV provirus via its transcriptional promoter or enhancer element can lead to increased levels of c-*myc* transcription[5,6]. It has recently been shown that the c-*myc* gene is translocated into an immunoglobulin locus in certain lymphoid neoplasms of both mice and men[7–12]. Thus, murine c-*myc* gene is recombined into the immunoglobulin heavy-chain locus in BALB/c plasmacytomas characterized by t(12:15) translocations[7,9–11]. Likewise, in a majority of the many Burkitt's lymphomas or non-Hodgkin's lymphomas characterized by t(8:14) (q24; q32) translocations, the c-*myc* gene is recombined into the immunoglobulin heavy-chain locus[8,11,12]. It has generally been considered that in these and other neoplasms where nonrandom chromosomal translocations have been observed, c-*onc* genes are activated by the translocations[13–15].

To understand the relationship between the translocation and activation of a c-*myc* gene we have been analysing the structure and expression of normal and translocated human c-*myc* genes. The results presented here demonstrate that the human immunoglobulin heavy-chain locus carries a transcriptional enhancer element analogous to its mouse counterpart, and that this enhancer element may be playing a direct part in the activation of the translocated c-*myc* gene in some lymphoid neoplasms.

c-*myc* translocation in Manca cells

The non-Hodgkin's lymphoma, Manca[16], shows a chromosome translocation (t(8:14)(q24; q32)) that is characteristic of many

0028–0836/84/040334—07$01.00

© 1984 Macmillan Journals Ltd

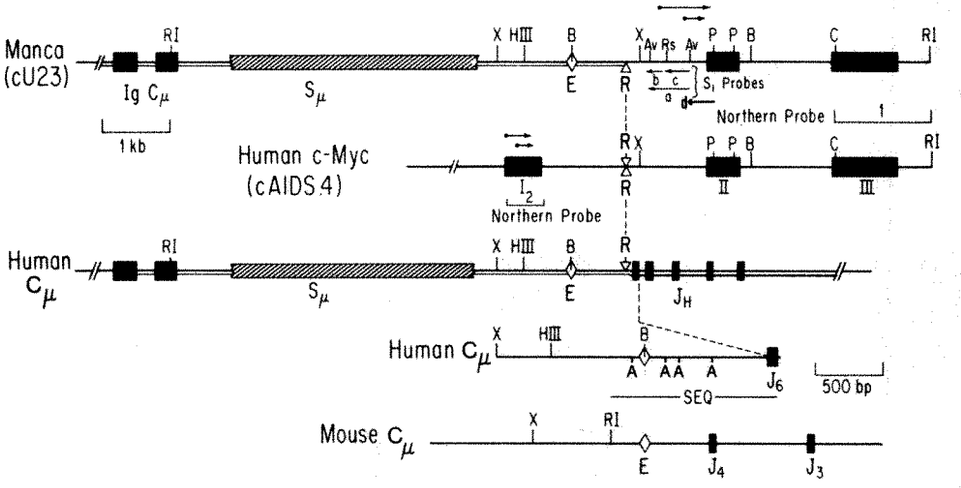


Fig. 1 A comparison of the human c-*myc* gene and the C_μ region of the human immunoglobulin heavy-chain locus to the C_μ–c-*myc* joining region of the 14:8 translocated chromosome from Manca cells. Exons are shown as solid boxes; chromosome 14 sequences as a double line; and chromosome 8 sequences as a single line. R indicates the site of recombination between the two chromosomes. E is a transcriptional enhancer element, which was previously identified for the mouse immunoglobulin heavy-chain locus[2–4] and the identification of the human equivalent is reported here. S_μ, C_μ switch sequences; J_μ, joining segments. The J_μ segments are defined on the basis of data presented in ref. 40, and our own sequencing[41]. The horizontal arrows indicate the initiation sites and directions of transcription. Restriction sites: RI, *Eco*RI; X, *Xba*I; H, *Hin*dIII; Av, *Ava*I; Rs, *Rsa*I; P, *Pst*I; B, *Bgl*II; C, *Cla*I; A, *Alu*I (the complete map is only shown for the first three of these). 1 and 2 delineate the extent of the probes used in the Northern blot experiments. The cloning of the recombination region of the translocated chromosome into cosmid pTCF[19] will be described elsewhere (K.W. *et al.*, manuscript in preparation). The unrearranged c-*myc* gene was also cloned into a cosmid. Colonies were screened with the Northern probe 1. The definition of the c-*myc* gene will be described[22].

Burkitt's lymphomas[14,15]. *In situ* hybridization of a v-*myc* probe[17] to Manca chromosomes indicates that the human c-*myc* locus is on that portion of chromosome 8 [12,18] that is translocated (data not shown).

From a cosmid library[19] of Manca DNA a clone, cU23 was isolated that hybridized both to v-*myc* and to pH18-C1-10, a plasmid containing the immunoglobulin heavy-chain C_μ gene and sequences immediately 5′ to it[20]. Southern blot analysis[21] of Manca DNA indicated that the linkage of c-*myc* and immunoglobulin heavy-chain gene sequences detected in cU23 had occurred in the human cells, and not upon cloning (data not shown). A detailed map of clone cU23 in the region of c-*myc*–C_μ linkage was derived by restriction analysis and DNA sequencing (K. W. *et al.*, manuscript in preparation), and was compared with maps of human germ-line C_μ and c-*myc* genes, contained on plasmid pH18-Cl-10 and cosmid cAIDS4 respectively (Fig. 1). The latter clone was described previously[22]. It can be deduced that in Manca DNA, the human c-*myc* gene is fused head-to-head with the C_μ gene, about 6.5 kilobases (kb) 5′ to the first C_μ exon. The junction on chromosome 14 lies between S_μ and J_H6, at a point that is not normally used for the productive rearrangement of an immunoglobulin heavy-chain gene. The junction on chromosome 8 occurs between exons I and II of the c-*myc* gene. As a result, the Manca DNA cloned in cU23 contains neither exon I nor the normal transcriptional start sites[22] of the human c-*myc* gene. Instead, c-*myc* exons II and III are fused to C_μ sequences that include a stretch of DNA between S_μ and J_H that is in an analogous location to sequences in the mouse genome shown to have tissue-specific transcriptional enhancing activity[2–4] (region 'E' in Fig. 1). The translation of transcripts of the human c-*myc* gene begins within exon II, at an initiation AUG codon that is retained, and presumably used, in transcripts of the translocated c-*myc* gene in Manca cells.

Multiple transcription initiation sites

Northern blot analysis of RNA from Manca cells indicates a high level of c-*myc* transcription (Fig. 2*A*). It is clear that there are multiple transcripts hybridizing to the c-*myc* Northern probe 1 (see Fig. 1). The level of c-*myc* transcription in Manca cells is the highest that we have detected in any cell line (which include other Burkitt's lymphomas, and Epstein–Barr virus-immortalized lymphoblastoid cells) except the promyelocytic leukaemia cell line HL60 (data not shown). The relevance of this comparison is unclear, however, as all these cells are growth transformed. An alternative approach is to compare the level of transcription of the rearranged c-*myc* gene in Manca cells with that of the unrearranged allele; with a probe specific for exon I no transcription of the unrearranged c-*myc* gene can be detected in Manca cells (K.W. *et al.*, manuscript in preparation). Therefore, the high c-*myc* level of transcription in Manca cells all derives from the translocated allele.

The initiation points of c-*myc* transcripts in Manca cells were mapped by S_1 nuclease protection experiments[23,24] using the probes shown at the bottom of Fig. 2. The major initiation site (site 1; see Fig. 2) for c-*myc* transcripts occurs about 636 base pairs (bp) upstream of c-*myc* exon II, within what would ordinarily be intron I of the c-*myc* gene. The nucleotide numbering system used to denote the initiation points is that of Colby *et al.*[25], whose sequence begins within intron I of the human c-*myc* gene such that nucleotide 1,000 defines the start of exon II. Transcripts initiating at site I (nucleotide 364) form S_1-resistant hybrids with probes b and a of 66 base pairs (bp) and 359 bp respectively (Fig. 2*B*, *C*). Downstream of site I, at nucleotide 378, is a weaker initiation point, Ia (Fig. 2*B*, *C*). As has been previously noted[25], upstream of initiation sites I and Ia are sequences related to the consensus TATA box[26,27] commonly found 5′ of genes transcribed by RNA polymerase II (see Fig. 3). Initiating at around nucleotide 873 are further transcripts, which form S_1 nuclease-resistant hybrids of length 192 bp with probe d (data not shown), and 28 bp upstream of this site occurs the sequence TTTATT (Fig. 3). In addition to these initiation sites, an S_1 sensitive site (X) in hybrids between Manca RNA and either probe a or c is detected at around nucleotide 520. However, the assignment of an initiation site to this position is uncertain, an S_1-sensitive site also being apparent at this position in hybrids between probes a or c and yeast RNA on long exposures.

If transcripts initiating at these sites were to have no functional splice sites 5′ of the junction between exon II and the following intron, then the processed RNAs would range in size from 1,660 to 2,450 nucleotides (not including the poly(A) tail[28]). That this is the case is suggested by cDNA cloning, which has revealed a contiguous transcript extending upstream from exon II at least as far as position 660. Beyond this point the S_1 nuclease-sensitive sites have been confirmed as initiation (as opposed to splice) sites by primer extension analyses. For example, reverse transcripts of Manca cell RNA using the primer shown in Fig. 2*B* extend as far as nucleotide 364. Further primer extensions (for example using S_1 probe b) indicate no initiation sites upstream of those characterized here (data not shown).

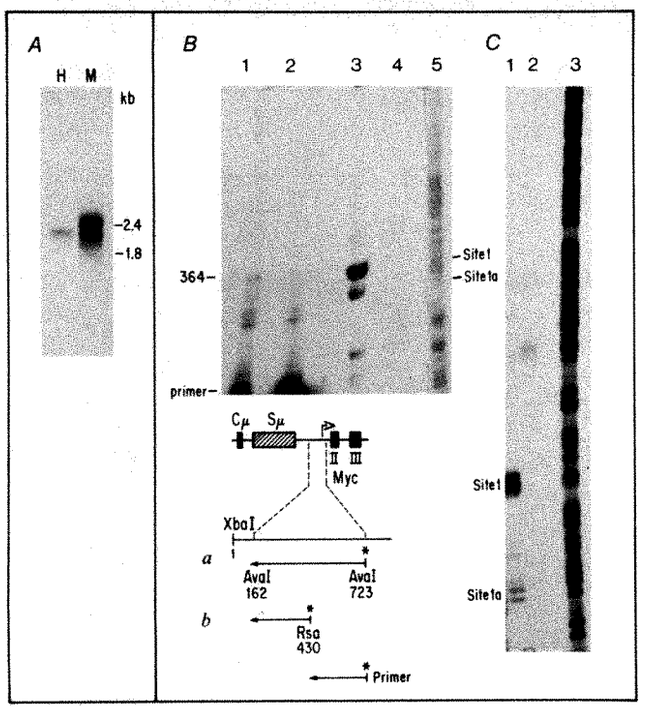


Fig. 2 ***A***, Northern blot analysis[42] using the probe 1 shown in Fig. 1 of mRNA from: lane H, HeLa cells (1 μg); lane M, Manca cells (0.3 μg). ***B***, Lanes 1 and 2 show a primer extension analysis[43], and lanes 3 and 4 an S_1 nuclease mapping analysis[23,24] of: lanes 1 and 3, Manca cell mRNA; lanes 2 and 4, yeast mRNA. The probes used are 'a' and 'primer' shown in the diagram below the photograph of the blot, and were strand separated and end labelled[41] before hybridization. The nucleotide numbering system derives from ref. 25, and starts (nucleotide 1) at the *Xba*I site in intron I of the human c-*myc* gene. Sites 1 and 1a are referred to in the text; site 1 centres on nucleotide 364, and the mark indicating this position on the right of the figure shows the correspondence between the primer extension and S_1 signals in lanes 1 and 3. Lane 5 is a pyrimidine analysis[41] of the sense strand of the probes, from which the nucleotide positions of the DNA–RNA discontinuity could be determined (see Fig. 3). ***C***, S_1 nuclease analysis using the probe 'b' shown in the diagram below *B* of mRNA from: lane 1, Manca cells; lane 2, yeast cells. Lane 3 is the same type of reaction as shown in lane 5 of *B*, but using probe b.
Methods: The S_1 nuclease reactions contained either 100 ng Manca cell mRNA plus 20 μg yeast RNA, or only the yeast RNA. For the S_1 analysis 5×10^4 c.p.m. of probe was used per reaction, and for the primer extension analysis 2×10^4 c.p.m. of probe was used per reaction. The probes were annealed to the RNA at 53 °C for 4 h (except in the case of probe b, which was annealed at 30 °C for 12–16 h). S_1 reactions were performed at 15 °C for 3 h, and primer extension reactions at 41 °C for 45 min. A 6% acrylamide gel was used for *B*, and run for 7 h, and an 8% gel run for 3 h for *C*.

Transcriptional enhancer element

We have previously shown that sequences derived from the J_H–C_μ region of the mouse immunoglobulin heavy-chain locus contain a DNA element that enhances the transcription of the associated gene in a manner largely independent of both position and orientation[2]. The translocation of the c-*myc* gene in Manca cell DNA just 300 bp downstream of the human J_H cluster (Fig. 1) suggested that the high level of c-*myc* transcription in Manca cells (Fig. 2*A*) might be due to a human enhancer element located in an analogous region to that in the mouse ('E' in Fig. 1).

To test this hypothesis, we first attempted to identify the putative human enhancer element. For this purpose, the 2.2 kilobase (kb) *Xba*I fragment (see Fig. 1) containing most of the sequence between the J_H cluster and S_μ, plus about 330 bp derived from the 5′ flanking region of the translocated, truncated c-*myc* gene, was inserted into the *Eco*RI site of plasmid pSER[2]. This plasmid was derived from pSV2.gpt[29] (which contains the mycophenolic acid-resistance conferring gene *Ecogpt*) by deletion from the latter of the SV40 enhancer sequences. As a result, pSER transforms cells to mycophenolic acid resistance (gpt^+ phenotype) at a much lower frequency than does pSV2.gpt (see Table 1). The original level of transformation can be restored by the insertion into the *Eco*RI site of pSER of the mouse immunoglobulin heavy-chain locus enhancer element[2]. As shown in Table 1, the insertion in either orientation of the human *Xba*I fragment into the *Eco*RI site of pSER (2.3 kb upstream of the *Ecogpt* gene) restored the gpt^+ transformation efficiency to a level even exceeding (by about eightfold) that of pSV2 gpt. The transformation efficiency of pSV2 gpt was itself increased 5–10-fold by insertion of the *Xba*I into its *Eco*RI site. Other fragments of mouse and human immunoglobulin heavy-chain loci DNA had no significant effects on transformation frequencies when inserted into pSER of pSV2.gpt. Also, like its murine counterpart the effect of the *Xba*I fragment was tissue-specific: with rodent fibroblasts the transformation efficiencies of pSERXa and pSERXb were over 100 times lower that of pSV2gpt. In a further experiment the 2.2 kb *Xba*I fragment was dissected by cleavage with *Alu*I, and some of the resulting fragments also tested in the pSER assay. Most, if not all the enhancing activity could be attributed to the 279 bp fragment, *Alu*.a (delineated by the two *Alu*I sites flanking the *Bgl*I site shown in the expanded map of the human C_μ gene in Fig. 1).

These transformation experiments strongly suggest that the 2.2 kb human *Xba*I fragment contains a tissue-specific transcriptional enhancer element. To demonstrate this point more directly we examined the effect of this DNA fragment on the transcription of a mouse immunoglobulin γ2b gene. The plasmid pSV.γ2bΔ$X_{2/4}$ contains a functionally rearranged mouse γ2b gene from which most of the sequences (including the enhancer element) between *V–D–J* and *C* exons have been deleted[2]. The plasmid also contains the selectable *Ecogpt* gene. J558L cells transformed to mycophenolic acid resistance by pSV.γ2bΔ$X_{2/4}$ express almost no γ2b heavy-chain RNA[2]. A high level of γ2b expression can be restored by re-insertion (either upstream or downstream of the *V–D–J* promoter) into plasmid pSV·γ2bΔ$X_{2/4}$ of the mouse enhancer[2], and similarly by insertion of the human *Xba*I fragment (Fig. 4). In this experiment, pools of cells transformed to a gpt^+ phenotype by pSV·γ2bΔ$X_{2/4}$ and its derivatives were assayed for γ2b gene expression by S_1 nuclease protection. The mouse enhancer was equally effective whether inserted upstream or downstream of the γ2b *V–D–J* promoter. By contrast the level of the γ2b gene transcription attained by the human enhancer was somewhat variable depending on its position, the highest level of expression being obtained when the enhancer was inserted upstream of the promoter in the opposite orientation to the direction of transcription (Fig. 4). Interestingly, the relative orientations of enhancer and promoter in this case are equivalent to those of the enhancer and the altered c-*myc* promoters in Manca cell DNA (see Fig. 1).

The nucleotide sequence of a portion of the J_H–C_μ region of the human germ-line clone pH18-CL-10 was determined and compared with that of the corresponding region of mouse DNA (Fig. 5), which contains the enhancer core sequences[30] which are common to enhancers so far defined. Core-like sequences are present in the human DNA: there is a 67% matching between the 140 bp of mouse DNA that contains most, if not all, of the murine enhancing activity and the corresponding human sequence. The similarity between the mouse and human sequences throughout the region shown in Fig. 5 is sufficiently strong to allow a unique alignment, with several insertions and deletions.

Activation of c-*myc* transcription

To begin to investigate whether the high level of c-*myc* transcription in Manca cells was due to proximity to the immunoglobulin

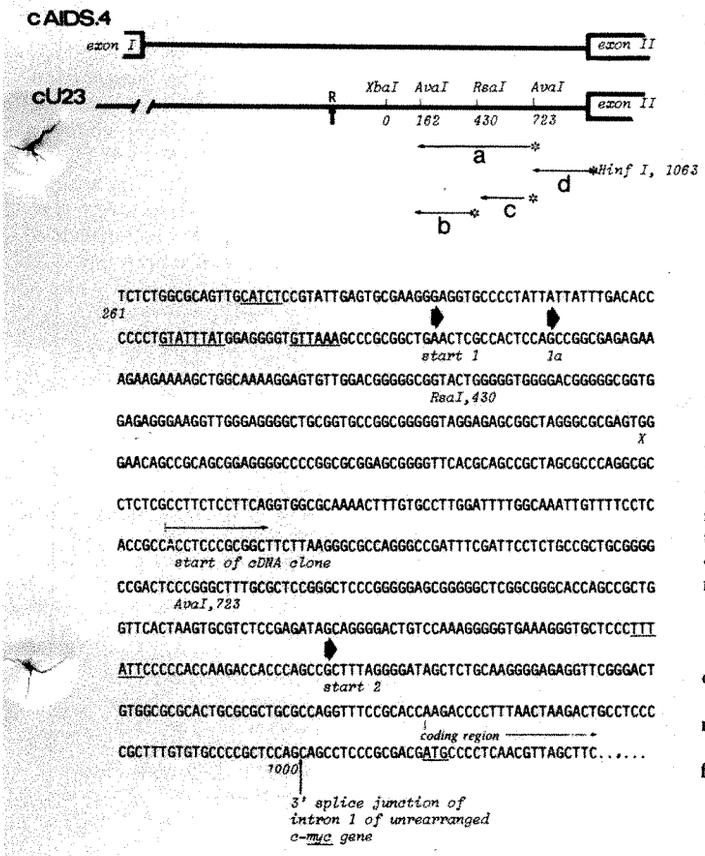


Fig. 3 Shows the nucleotide sequence from intron I of the germ-line c-*myc* gene that contains the initiation sites of the transcripts of the translocated c-*myc* gene. The recombination site of the translocated Manca chromosome is 336 nucleotides 5′ of the start of the sequence shown. Exon II of the germ-line c-*myc* gene starts at nucleotide 1,000. The transcription initiation sites (1, 1a and 2), as deduced by S_1 and primer extension analyses, are marked by the thick arrows. The probes used in these analyses (a–d, a and b being those used in Fig. 2) are shown near the top of the figure. The asterisks mark the ^{32}P-labelled ends of the probes. Underlined sequences are similar to those commonly found 5′ of RNA polymerase II-transcribed genes[27]. The initiator ATG codon of the protein encoded by c-*myc* is also underlined. X is an S_1-sensitive site, and R the recombination site. The sequence data derive from ref. 25 plus our own data in the regions of designated promoters.

Table 1 Transformation efficiency of pSV2.gpt and derivative plasmids

Plasmid	Frequency of transformation to gpt$^+$ phenotype	
	J558L cells*	rat-2 cells†
pSV2.gpt	1×10^{-4}	5×10^{-3}
pSV2.gpt.Xa‡	6×10^{-4}	ND
pSER	5×10^{-6}	ND
pSER.Xa‡	8×10^{-4}	1×10^{-5}
pSER.Xb‡	8×10^{-4}	1×10^{-5}
pSER.Ra§	8×10^{-6}	ND
pSER.Alu.a‖	2×10^{-4}	ND
pSER.Alu.z‖	5×10^{-6}	ND

Escherichia coli cells containing the above-mentioned plasmids were converted to spheroplasts[39], and fused[2] to recipient cells (10^{10} spheroplasts per 2×10^6 cells), after which the cells were plated at 5×10^3 (J558L) cells per well or 1×10^5 (rat-2) cells per culture dish. The selective medium, which contained xanthine (250 μg ml^{-1}), hypoxanthine (15 μg ml^{-1}) and mycophenolic acid (6.5 μg ml^{-1}), was added 48 h after fusion, and colonies were counted after 14 days. The experiments were routinely repeated three times. ND, not done.

* A mouse plasmacytoma cell line.

† A rodent fibroblast cell line.

‡ Xa/Xb plasmids contain an insertion in either orientation (a or b) of the 2.2 kb *Xba*I fragment from cU23.

§ pSER.Ra contains an insertion of a 4 kb *Eco*RI fragment from the mouse immunoglobulin heavy-chain C_α gene.

‖ Alu.a/Alu.z plasmids contain an insertion of either of two *Alu*I fragments (a and z) derived from the 2.2 kb *Xba*I fragment.

heavy-chain locus enhancer, the transcription of the translocated c-*myc* gene was studied in a murine myeloma cell line, J558L. The myeloma cells were transformed by spheroplast fusion with either of two plasmids, pSV.26 or pSV2.Δ4. Plasmid pSV2.26 contains the 11.7 kb *Eco*RI fragment from cU23 (containing the translocated c-*myc* gene plus about 8 kb of 5′ flanking sequences which include the immunoglobulin heavy-chain locus enhancer, see Fig. 1) the translocated c-*myc* gene cloned into the *Eco*RI site of pSV2gpt. Plasmid pSV2Δ4 contains a 9.5 kb *Eco*RI fragment (derived from the 11.7 kb *Eco*RI fragment of cU23 by deletion of the central 2.2 kb *Xba*I fragment) cloned into the *Eco*RI site of pSV2.gpt. Plasmid pSV2Δ4 thus lacks the sequences identified above as having transcriptional enhancing activity.

After transforming with these plasmids, gpt$^+$ transformants of J558L cells were isolated and assessed for the presence of exogenous DNA. Clones 26.1–26.9 were nine cloned isolates of cells transformed by pSV2.26; Δ4.2 is a cell line developed after transformation with pSV2.Δ4; Δ4.1 is a pool of several colonies transformed by pSV2.Δ4. Most cells transformed by pSV2.26 have acquired only single or a few copies of the exogenous DNA, and in most of these cases the integration has occurred within the 11.7 kb *Eco*RI fragment, so that *Eco*RI fragments smaller than 11.7 kb are detected with the c-*myc* probe[31]. Analysis with enzymes that cut within this fragment, however, indicate that in at least four out of nine cases (clones 26.1, 26.2, 26.4 and 26.7) contiguous DNA sequences that extend from the enhancer through the transcriptional initiation sites mapped above, and into the truncated c-*myc* gene, are intact (Fig. 6).

By contrast, the cells transformed in identical conditions by pSV2.Δ4, contain multiple, tandem copies of the exogenous DNA, and in each case the c-*myc* transcription unit and upstream sequences are intact. This difference in copy number of transforming DNA in cells selected for gpt$^+$ phenotype may be due to a positive effect (from over 7 kb away) of the enhancer on *Ecogpt* transcription: in the absence of the enhancer, high levels of *Ecogpt* transcription may only be obtained from an increased copy number. Consistent with this, the transformation frequency of J558L cells by pSV2Δ4 is 5–10-fold lower than the frequency of transformation by pSV2.26 (data not shown). The difference in copy number may also result from a deleterious effect of excess c-*myc* gene product on J558 cells, which already contain an actively transcribed translocated mouse c-*myc* gene[32]. If such an effect exists, and if the immunoglobulin heavy-chain locus enhancer does activate Manca cell c-*myc* transcription, then there may be a selective advantage to cells transformed with pSV2.26 that retain only one or even no intact copies of the Manca cell c-*myc* gene.

Both total and poly(A)$^+$ RNA were isolated from groups of transformants, and were analysed by Northern transfer and hybridization to probe 1 (see Fig. 1). Although transformants receiving pSV2.Δ4 contained multiple intact copies of the transfected c-*myc* gene, none of them showed evidence for much c-*myc* transcription over and above the level of the mouse c-*myc* transcription endogenous to J558L cells (data not shown). By contrast several lines of cells transformed by pSV2.26 showed strongly hybridizing c-*myc* transcripts (data not shown).

Because of the variability in retention of c-*myc* DNA sequences, and the cross-reactivity of the Northern probe with the mouse c-*myc* transcripts in J558L cells, a quantitative com-

parison of human translocated c-*myc* gene transcription was better made by S_1 nuclease mapping. The S_1 probe a (Fig. 3), which detects the major initiation site, was protected to a similar quantitative degree by Manca cell RNA and by RNA of several lines transformed by pSV2.26 (for example, lines 26.1, 26.4 and 26.7, see Fig. 7). That RNA in these cell lines is qualitatively the same as in Manca cells was further indicated by use of S_1 probe b. Use of either probe shows almost no protection by RNA of cell lines Δ4.1 or Δ4.2 (see Fig. 7) even though these cells contain multiple intact copies of the transforming DNA (Fig. 6). It cannot be argued that in either of these cases the transforming DNA has become sequestered in a transcriptionally inactive area, since continual selection for *Ecogpt* gene activity is made. In addition, several isolates of both total and poly(A)$^+$ RNA from these cells gave the same result, and these RNAs were positive in an S_1 nuclease protection assay of *Ecogpt* transcripts (data not shown). It appears then, that the activity of the major transcriptional initiation site for translocated c-*myc* gene transcription is severely reduced by deletion of sequences 360 bp upstream of it. Deletions at this distance upstream of a

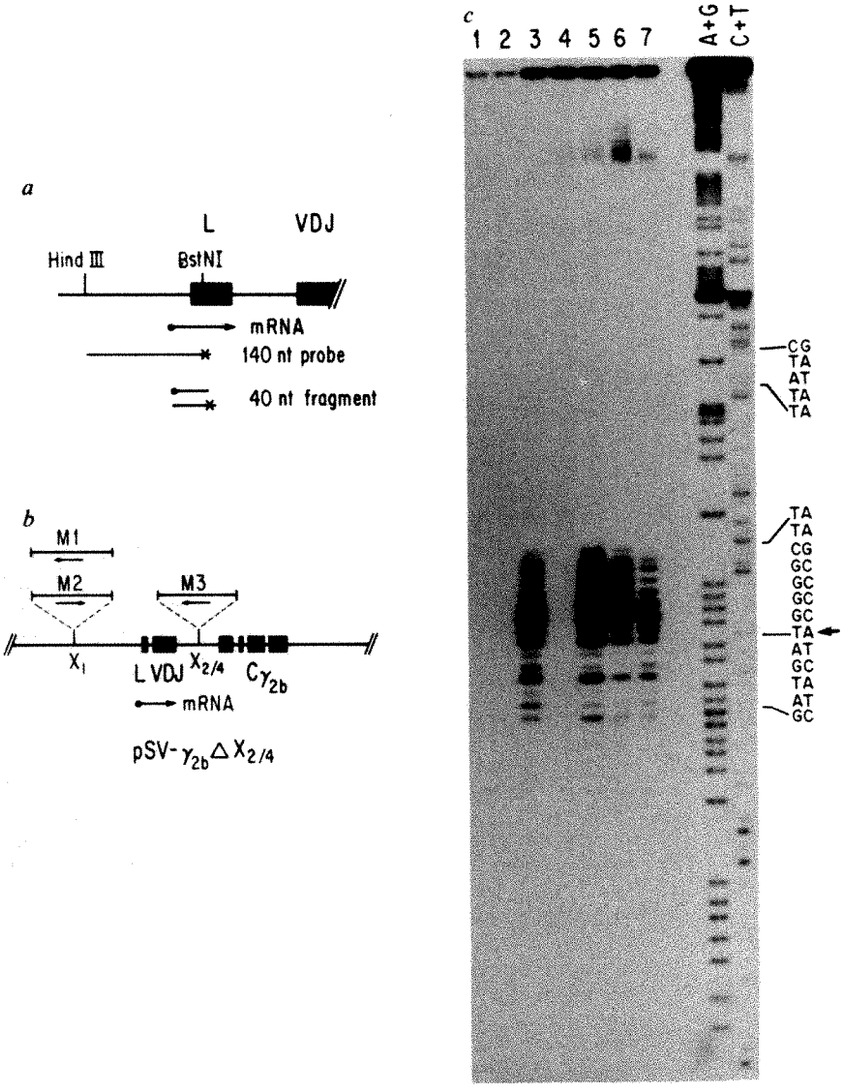


Fig. 4 *a*, Strategy of the S_1 nuclease mapping experiment shown in *c*. The probe is an end-labelled *Hin*dIII–*Bst*NI fragment, which was strand separated and annealed to total cell mRNA before S_1 digestion and electrophoretic resolution, which were carried out as described previously[23,24]. The 40 bp fragment protected by correctly initiated γ26 mRNA is as predicted by primer extension analysis[44]. The solid blocks indicate the leader (*L*) exon and 5′ end of the *V–D–J* exons. ***b*** Shows the derivatives of pSV-γ2bΔ$X_{2/4}$ used for transfection. The 2.2 kb *Xba*I fragment into the X_1 (M1 and M2) or $X_{2/4}$ (M3) sites[2] in the indicated orientations. ***c*** Shows the S_1 nuclease resistant hybrids formed between the probe shown in *a* and: lane 1, 20 μg tRNA; lane 2, 20 μg RNA from J558L cells; lanes 3–7, 20 μg RNA from J558L cells transfected with: lane 3, pSV-γ2bVC[2]; lane 4, pSV-γ2bΔ$X_{2/4}$; lane 5, pSV-γ2bΔ$X_{2/4}$ M1; lane 6, pSV-γ2bΔ$X_{2/4}$ M2; lane 7, pSV-γ2bΔ$X_{2/4}$ M3. In the two right-hand lanes are co-electrophoresed sequencing reactions[34] of the probe, which allow the identification of the transcription initiation site (arrow) and the GATAA box at position −30.

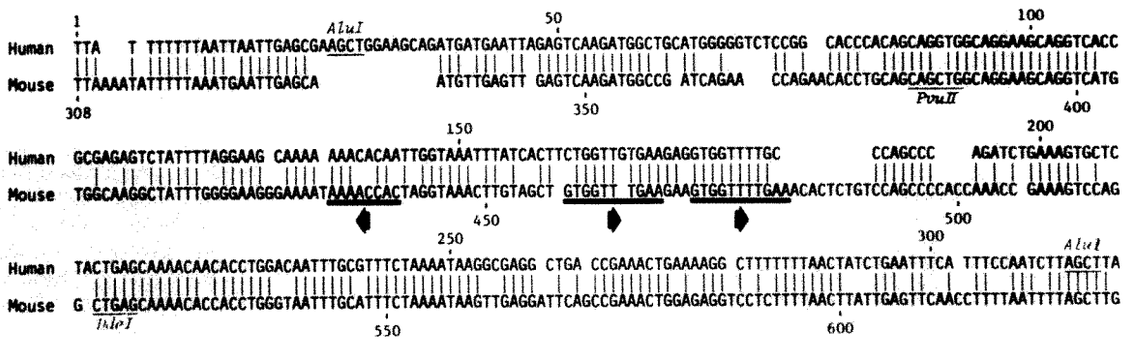


Fig. 5 Sequences of the murine[2] and human enhancer elements, which were compared using the SEQ computer program[45].

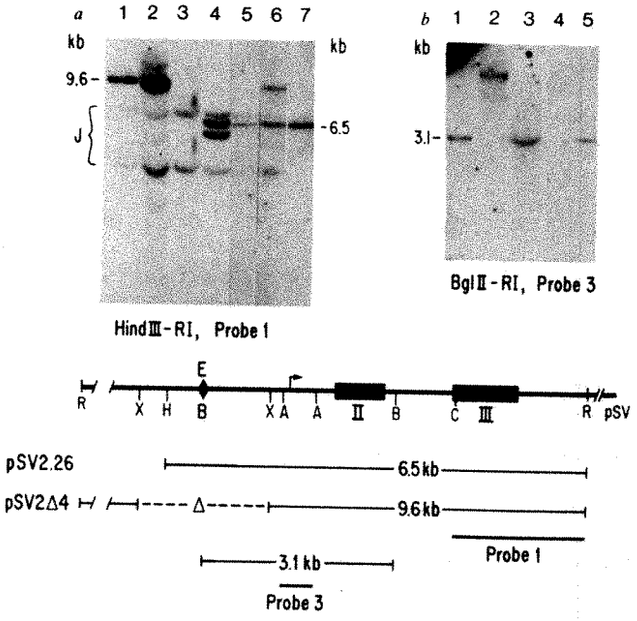


Fig. 6 Southern blot analyses of c-*myc* containing sequences in J558L cells, and in their derivatives transformed with either pSV2.26 or pSV2.Δ4. ***a*** Shows a blot obtained using probe 1 (as indicated in the diagram below) and *Hin*dIII plus *Eco*RI digested DNA from: lane 1, Δ4.2 cells; lane 2, Δ4.1 cells; lane 3, J558L cells; lane 4, 26.7 cells; lane 5, 26.2 cells; lane 6, 26.1 cells; lane 7, 5 pg of pSV2.26 cleaved with *Hin*dIII plus *Eco*RI, co-electrophoresed as a marker, ***b*** Shows a blot obtained using probe 3 and *Bgl*II plus *Eco*RI cleaved DNA from: lane 1, 26.1 cells; lane 2, 26.5 cells; lane 3, 26.4 cells; lane 4, J558L cells; lane 5, as in lane 7 of *a*, but cut with *Bgl*II. The diagram below the blots shows the 11.7 kb *Eco*RI fragment contained in pSV2.26 and also the position of the 6.5 kb *Hin*dIII–*Eco*RI fragment. The deletion (Δ) in pSV2Δ4 removes the *Hin*dIII site, as a result of which a 9.6 kb *Eco*RI fragment is the *Hin*dIII plus *Eco*RI digestion product that hybridizes to the c-*myc* probe. As a further result of this deletion the 3.1 kb *Bgl*II fragment detected with probe 3 (indicated in *b*) is only detected in pSV2.26 transformants. Bands labelled J in *a* are due to the cross-reactivity of probe 1 with normal and rearranged mouse c-*myc* genes in J558L cells[11,32]. Restriction sites marked in the lower diagram: R, *Eco*RI; X, *Xba*I; H, *Hin*dIII; B, *Bgl*II; A, *Ava*I; C, *Cla*I.

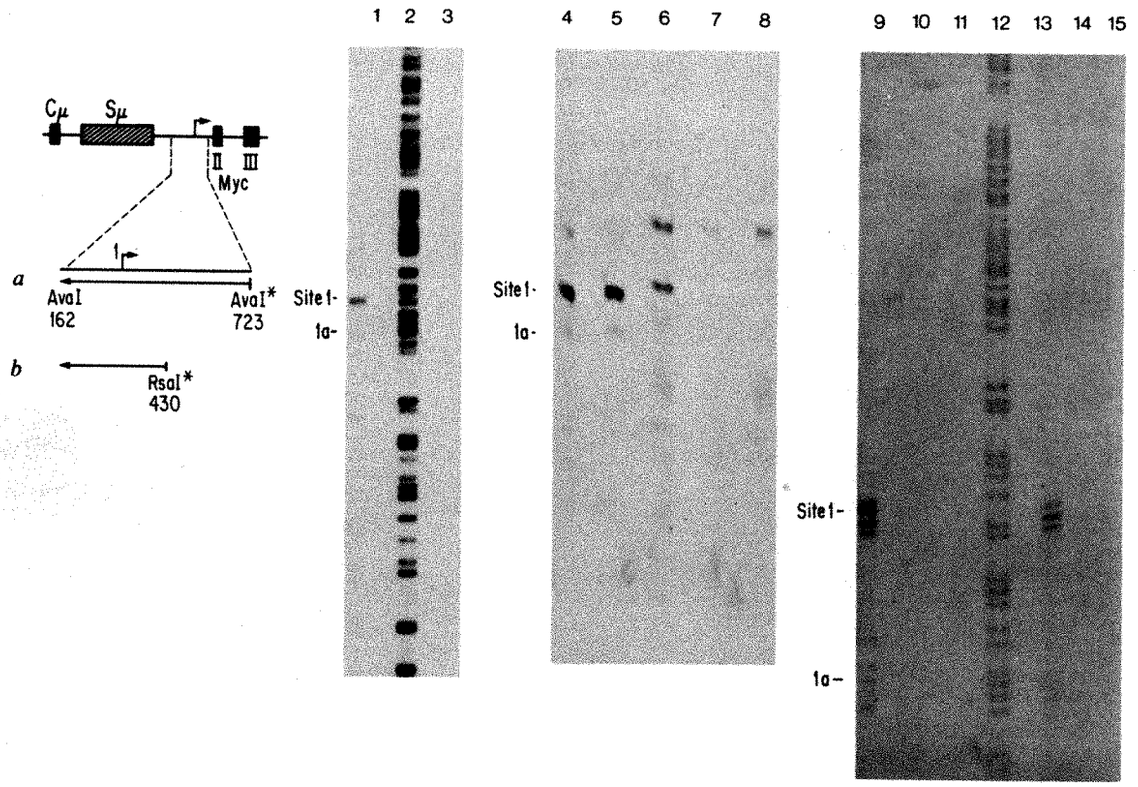


Fig. 7 S_1 nuclease analysis of mRNA from Manca cells, J558L cells, and transformed derivatives of J558L cells. Lanes 1–8 were reactions involving probe a, and lanes 9–15 involved probe b. Lane 2 is a pyrimidine sequencing reaction of probe a, and lane 12 is a similar reaction with probe b. The other lanes are the S_1 reactions, with mRNA from: lane 1, 26.7 cells; lane 3, J558L cells; lane 4, 26.1 cells; lane 5, 26.4 cells; lane 6, Manca cells; lane 7, Δ4.1 cells; lane 8, yeast cells; lane 9, Manca cells; lane 10, 26.3 cells (which retained no human c-*myc* sequences); lane 11, Δ4.1 cells; lane 13, 26.1 cells; lane 14, Δ4.2 cells; lane 15, J558L cells. The end-labelled, strand-separated[41] probes were melted at 85 °C, then annealed to the RNAs at 51 °C for probe a, and at 30 °C for 12 h for probe b. S_1 digestions were carried out at 15 °C for 2 h. Electrophoresis was carried out as described previously, for lanes 1–8 on a 6% gel run for 7 h, for lanes 9–15 on an 8% gel run for 3 h. All reactions contained equal amounts of sample RNA made up to 20 μg with yeast RNA. S_1 nuclease-sensitive sites specific for the presence of lymphoid RNA are indicated.

promoter are characteristic of enhancer mutations[33]. As the enhancing activity of the deleted fragment has been demonstrated and largely localized to one fragment (the *Alu* 'a' fragment), it appears likely that it is the removal of the immunoglobulin heavy-chain locus enhancer that has drastically reduced transcription of the translocated c-*myc* gene.

Multiple mechanisms of c-*myc* activation

We show here that Manca cells contain a translocated c-*myc* gene that is transcribed from previously cryptic start sites that ordinarily form part of intron I of the c-*myc* gene. This transcription is reproduced with fidelity after introduction of the gene into mouse myeloma cells. Use of this system has allowed us to demonstrate that transcription from at least the major cryptic promoter is dramatically dependent on the presence of sequences over 350 bp upstream of the RNA initiation site. These sequences include an element that we demonstrate to be a human immunoglobulin gene-associated enhancer element, and it may be this element itself that is responsible for activating translocated c-*myc* transcription in Manca cells. The remaining possibility, that it was the removal of other sequences (including about 300 bp of DNA that are also ordinarily part of c-*myc* intron I) that drastically reduced transcription from initiation site I is presently being investigated. The unrearranged c-*myc* gene is still present in Manca cells, but our preliminary results from use of Northern probes (probe 2 in Fig. 1), S_1 mapping and cDNA cloning indicate that this allele is barely, if at all, expressed. Such a situation has already been reported for a mouse plasmacytoma and may be true for other Burkitt's lymphomas[11,32,34]. Hence, transcriptional activation of the c-*myc* gene appears to be an important result of translocation and in the case described here, it is possible that such activation is largely contributed to by the immunoglobulin heavy-chain locus enhancer.

The retention of the immunoglobulin associated enhancer element adjacent to the translocated c-*myc* gene is not a common feature of c-*myc* translocations so far documented in either mouse[7,10,11,35] or man[12,36]. In these instances, the translocated gene is still transcribed[7,10,11,37], whilst again the untranslocated gene may be silent[11,32,34]. In these cases, transcription of the translocated c-*myc* gene may be maintained by a cellular enhancer-type element yet to be identified, and which may be a considerable distance from the translocated c-*myc* gene.

Alternatively, translocated c-*myc* genes may not be directly dependent upon immunoglobulin sequences for their transcription, but rather their transcription may be activated by the accumulation of mutations in regulatory regions. For example it has been suggested[34] that the c-*myc* gene product itself may regulate its own transcription, and that, in lymphomas harbouring translocated genes, the translocated but not the normal c-*myc* gene, escapes this repression. At this stage, the hypothesis of positive activation, and that of escaping repression, are not mutually exclusive.

The detachment of c-*myc* exon I sequences from c-*myc* exons II and III is a consequence for all BALB/c and for several human c-*myc* translocations thus far documented[7,10–12,38]. We have previously suggested[22,31] that this event, in itself, leads to increased translation of c-*myc* RNA in cells harbouring such translocations.

In summary, there are probably multiple steps in the activation of the human c-*myc* gene in non-Hodgkin's lymphomas. From this article it is clear that previously cryptic promoters of the c-*myc* gene are activated upon translocation, and that this activation is dependent upon upstream sequences. In Manca cells, the critical element in these upstream sequences may be the human immunoglobulin heavy-chain locus enhancer element. As both the translocated c-*myc* gene from a mouse plasmacytoma and the translocated human c-*myc* gene from Manca cells have detectable biological activity (ref. 46 and our unpublished data) it is apparent that the activation of the c-*myc* gene contributes to either initiation or maintenance of oncogenesis. It is possible that the misuse of a developmentally regulated, cellular enhancer element will be found, in many cases, to be responsible for activating oncogenes.

We thank Lena Angman, John McMaster, Anne Maxwell and Eleanor Basel for their help, and P. Jat for advice concerning S_1 analysis, C. M. Croce for the gift of pH18-cl-10, W. Topp for the kind distribution of rat-2 cells, and B. Clarkson for Manca cells. A.C.H. and K.W. thank ICRF and EMBO respectively for travel and long term fellowships. The work was supported by NIH grants AI-17879 (to S.T.), CA-14051 (a core grant to S. Luria), and CA-34502 (W.H.).

Note added in proof: The integrity of all RNA preparations has been confirmed by succcessful S_1 analysis of gpt transcription.

Received 17 October; accepted 30 November 1983.

1. Tonegawa, S. *Nature* **302,** 575–581 (1983).
2. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33,** 718–728 (1983).
3. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33,** 729–740 (1983).
4. Neuberger, M. *EMBO J.* **2,** 1373–1379 (1983).
5. Hayward, W. S., Neel, B. & Astrin, S. *Nature* **290,** 475–480 (1981).
6. Payne, G. S., Bishop, J. M. & Varmus, H. E. *Nature* **295,** 209–215 (1982).
7. Shen-Ong, G. L., Keath, E., Piccoli, S. P. & Cole, M. D. *Cell* **31,** 443–452 (1982).
8. Dalla-Favera, R., Marinotti, S., Gallo, R., Erikson, J. & Croce, C. M. *Science* **219,** 963–967 (1983).
9. Crews, S., Barth, R., Hood, L., Prehn, J. & Calame, K. *Science* **218,** 1319–1321 (1982).
10. Marcu, K. H. *et al. Proc. Natn. Acad. Sci. U.S.A.* **80,** 519–523 (1983).
11. Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M. & Cory, S. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1982–1986 (1983).
12. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7837–7841 (1982).
13. Cairns, J. *Nature* **289,** 353–357 (1981).
14. Klein, G. *Nature* **294,** 313–318 (1981).
15. Rowley, J. D. *Science* **216,** 749–751 (1982).
16. Nishikori, M. *et al. Cancer Genet. Cytogenet.* (in the press).
17. Lautenberger, J. A., Schulz, R. A., Garon, C. F., Tsichlis, P. N. & Papas, T. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1518–1522 (1981).
18. Neel, B., Jhanwar, S., Chaganti, R. & Hayward, W. S. *Proc. natn. Acad. Sci. U.S.A.* **24,** 7842–7846 (1982).
19. Grosveld, F. *et al. Nucleic Acids Res.* **10,** 6715–6732 (1982).
20. Erikson, J., Finan, J., Nowell, P. C. & Croce, C. M. *Proc. natn. Acad. Sci. U.S.A.* **79,** 5611–5615 (1982).
21. Southern, E. *J. molec. Biol.* **98,** 503–515 (1975).
22. Saito, H., Hayday, A. C., Wiman, K., Hayward, W. S. & Tonegawa, S. *Proc. natn. Acad. Sci. U.S.A.* **80** (in the press).
23. Berk, A. & Sharp, P. A. *Cell* **12,** 721–732 (1977).
24. Weaver, R. F. & Weissman, C. *Nucleic Acids Res.* **7,** 1175–1193 (1979).
25. Colby, W. W., Chen, E. Y., Smith, D. H. & Levinson, A. D. *Nature* **301,** 722–725 (1983).
26. Goldberg, M. thesis, Stanford Univ. (1980).
27. Breathnach, R. & Chambon, P. *A. Rev. Biochem.* **50,** 349–384 (1981).
28. Proudfoot, N. & Brownlee, G. *Nature* **263,** 211–214 (1976).
29. Mulligan, R. C. & Berg, P. *Science* **209,** 1422–1427 (1980).
30. Weiher, H., Konig, M. & Gruss, P. *Science* **219,** 626–631 (1983).
31. Hayday, A. C. *et al. Cold Spring Harb. Symp. quant. Biol.* (in the press).
32. Stanton, L. W., Watt, R. & Marcu, K. M. *Nature* **303,** 401–406 (1983).
33. Benoist, C. & Charbon, P. *Nature* **290,** 304–309 (1981).
34. Nishikura, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 4822–4862 (1983).
35. Calame, K., Kim, S., Lalley, P., Hill, R., Davis, M. & Hood, L. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6994–6998 (1982).
36. Neuberger, M. & Calabi, F. *Nature* **305,** 240–243 (1983).
37. Erikson, J., ar-Rushdi, A., Drwinga, H. L., Nowell, P. C. & Croce, C. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 820–824 (1983).
38. Cory, S. *et al. EMBO J.* **2,** 213–216 (1983).
39. Sandri-Goldin, R. M., Goldin, A. L., Levine, M. & Glorioso, J. C. *Molec. cell. Biol.* 743–752 (1981).
40. Ravetch, J. V., Siebenlist, U., Korsmeyer, S., Waldmann, T. & Leder, P. *Cell* **27,** 583–591 (1981).
41. Maxam, A. & Gilbert, S. *Meth. Enzym.* **65,** 499–560 (1980).
42. Thomas, P. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5201–5205 (1980).
43. Ghosh, P. K., Reddy, V. B., Swinscoe, J., Lebowitz, P. & Weissman, S. M. *J. molec. Biol.* **126,** 813–846 (1978).
44. Gillies, S. D. & Tonegawa, S. *Nucleic Acids Res.* **11,** 7981–7997 (1983).
45. Brutlag, D. L., Clayton, J., Friedland, P. & Kedes, L. *Nucleic Acids Res.* **10,** 279–294 (1982).
46. Land, H., Parada, L. F. & Weinberg, R. A. *Nature* **304,** 576–580 (1983).

A lower limit for the mass of the compact object in SS433—a black hole?

Elia M. Leibowitz

Berkeley Astronomy Department, University of California, Berkeley, California 94720, USA

Tsevi Mazeh & Haim Mendelson

Department of Physics and Astronomy and the Wise Observatory, Tel Aviv University, Israel

The SS433 system ejects two relativistic jets with a velocity of 0.26c. The two jets are in oppsite directions, precessing with a period of 160 days[1]. The system is assumed to include a compact object which rotates around a normal star with a period of 13 days[2,3]. There are strong indications for the presence of an accretion disk around the compact object[4]. Based on study of a photometric light curve obtained during 3 years of observations[5], we suggest here that the compact object might be a black hole.

The photometric characteristics of SS433 have been studied extensively in the past few years[4–12]. Despite the erratic photometric stellar behaviour, a 13 day and 160 day periodicities have been clearly detected (refs 4–12 and 5, 10–12 respectively). The averaged 13-day light curve is highly structural and varies with the 160-day period[4,5,8–10]. However, in one third of the 160-day cycle, when the jets inclination is minimal, the averaged binary light curve is much more smooth and symmetric[5]. This light curve is reproduced in Fig. 1, where one can notice two symmetrical eclipses separated by a 'plateau', very similar to an Algol type light curve observed in many binary systems. This familiar character of the binary light curve has also been recognized by other investigators[9].

If one assumes that Fig. 1 is an Algol-type light curve not only in form but also in essence, that is, it does indeed represent the mutual occultation of two light sources[13], this curve can be used to estimate the mass ratio of the binary system[14]. We considered a system with two components: an accretion disk and a 'normal' star. These two objects are necessary parts of many models of SS433 [5,11,15–18].

In this work we used the following assumptions: (1) The primary minimum is due to the occultation of the disk by the normal star. (2) The binary plane of motion is orthogonal to the precession axis of the jets. (3) The normal to the disk is orientated at the instantaneous jet direction. (4) The normal star fills its critical lobe. (5) The disk is planar.

Consulting Fig. 1, we further assume that: (6) The eclipse of the disk is not total. (7) The width of the disk eclipse is $0.30 \pm 0.02P$, where P is the binary period.

A computer program to calculate the relative occulted area of the disk, with a given radius R_d and orientation, has been coded, using a Roche lobe geometry [assumption (4)]. This geometry is determined by the mass ratio

$$q = M_{\text{normal}} / M_{\text{compact}}$$

where M_{normal} and M_{compact} are the masses of the normal object and the compact star, respectively.

The precessional model for the jets yields $i = 79°$ and $\theta = 20°$, where i is the binary inclination [assumption (2)] and θ is the precessional angle[1]. We use these values for determining our line of sight and the disk orientation [assumption (3)]. For the particular 160-day phase section studied here we assume that the normal to the disk is in the plane which contains the normal to the binary orbital plane and our line of signt. Our numerical results, drawn at the q,R_d plane, are depicted in Fig. 2. Curve a represents the values for which the eclipse width is 0.3 of the binary period P. The dashed line above [under] curve a stands for eclipse width of $0.32P$ [$0.28P$], according to our error estimate. Curve b of Fig. 2 represents the points in which a total eclipse is attained just at mid eclipse. For the points in the domain above curve b, with larger R_d the eclipse is not total. The two constraints together yield

$$q \leq 2.3 \tag{1}$$

To estimate the masses of the two components we further assume that the radial velocity curve of the He II emission line[19] represents the orbital motion of the compact object. Crampton and Hutchings[19] best value for the amplitude of the radial velocity curve is:

$$K = 195 \text{ km s}^{-1} \tag{2}$$

and they put a lower limit of 150 km s^{-1} for this parameter. In Fig. 3, equations (1) and (2) and the lower limit for K have been used to exclude certain domains in the (M_{compact}, M_{normal}) plane. We get

$$M_{\text{compact}} \geq 4.3\ M_\odot$$
$$M_{\text{normal}} \geq 10\ M_\odot \tag{3}$$

All seven assumptions mentioned above are necessary for deriving equation (1). Consider, for example, assumptions (1)–(3). No solid proof can be found for their validity, and other competing assumptions[20,21] cannot be excluded. However, as assumptions (1)–(3) seem to be consistent with the spectroscopic and photometric characteristics of the system, and as they are a necessary part of the 'precessing disk' model[16], these assumptions are adopted here. Assumption (4), on the other hand, is not necessarily essential for all precessing disk models. However, the large mass accretion rate[17], the indication for a stream[8,19] and for a hot spot[5] in the system, all suggest that the mass transfer is through an L_1 overflow[17]. This implies that the normal star fills its critical lobe[17,18] and hence our assumption (4).

Assumption (5) is certainly only an approximation; however, our results would be modified only slightly if the disk structure is somewhere between a planar and a spherical shape.

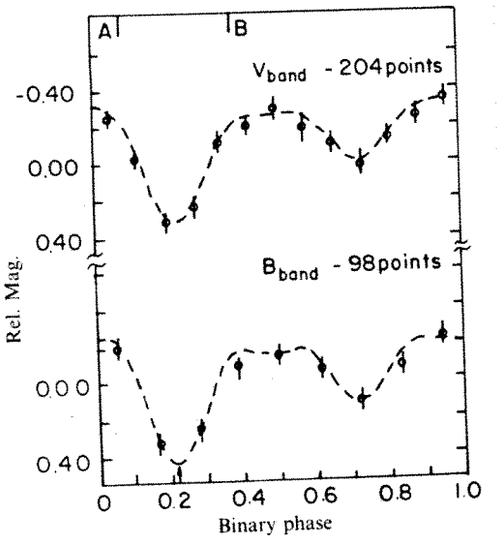


Fig. 1 A reproduction of the binary light curves in V and B bands. The plotted points are the average magnitudes in each of the corresponding bins in the phase axis. The dashed lines were obtained by a least square fitting of all the data points to a 5 term Fourier series in the basic period. The arrow indicates the phase which is a quarter of a cycle after maximum positive velocity of the He II emission lines. The phase interval ($0.3P$) between the A and B bars indicates the eclipse duration. The light curves were obtained for $0.33 \leq \Psi_{164} \leq 0.666$ where $\Psi_{164} = (\text{JD} - 2444068.5)/164$.

© 1984 Macmillan Journals Ltd

0028-0836/84/040341—02$01.00

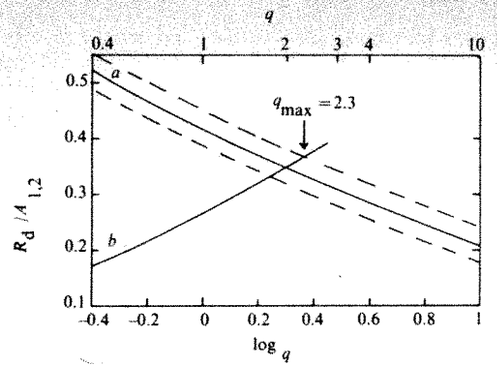


Fig. 2 The constraints on the disk radius R_d, in units of the binary separation $A_{1,2}$ as a function of q, the mass ratio.

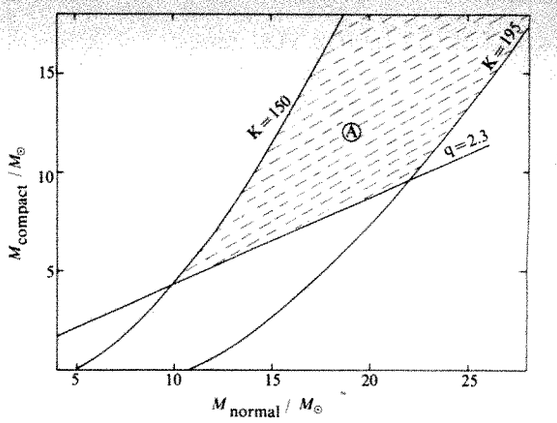


Fig. 3 The constraints on the masses of the two components. A is the allowed domain.

There are several indications that the disk eclipse is not total [assumption (6)]. First, the continuous curves of Fig. 1, which have been adopted to represent the averaged light curves in V and B[5], do not include a flat constant minimum of any length. Secondly, the HeII line appears through the whole binary period, even during mid eclipse[19]. It implies that the source of this emission line (centre of disk, disk's corona?) is not totally eclipsed. As the He II line is probably generated close to the centre of the disk and not at its edge, its appearance during mid eclipse is a strong evidence against the total eclipse hypothesis. Thirdly, the intensity at minimum is larger than half the total luminosity while the disk apparently contributes more than half of the total intensity in V and B[8]. All these arguments make assumption (6) very attractive. We assume, therefore, that if the disk radius is R_d, at least one point on the disk central plane at distance R_d from its center is not occulted. According to this assumption the disk thickness[11,16] did not enter our calculations. The assumption (7) value has been deduced from Fig. 1 and the errors represent our eye estimate.

Our derived constraints on the masses [equation (3)] depend on K to the third power, therefore our conclusion is very sensitive to its exact value. Indeed, Crampton and Hutchings[19] considered the possibility that their value for K does not represent the true orbital motion of the compact object. They estimate the value of 150 km s^{-1} as the lower limit for K when effects that produce spurious high amplitude are taken into account. One important effect of this kind is a partial eclipse of the disk during the phase of maximum radial velocity. The fact that the length of the disk eclipse is less than $0.32P$ significantly smaller than 0.5, excludes this effect in our case. We therefore consider the lower limit for K as real.

The two curves of Fig. 2 were obtained by using a Roche geometry, assuming corotation and alignment of the normal star. However, several works[5,18] suggested that this might not be the case, and therefore the geometry is somewhat different[22,23]. Consider, for example, the case in which $\Omega = \omega_{stellar}/\omega_{orbital} = 1.4$, where $\omega_{stellar}$ and $\omega_{orbital}$ are the rotational frequencies of the normal star and the orbital motion, respectively. In such a case, the upper limit for q should be 3, and the lower limit for $M_{compact}$ would be $3M_\odot$. With $\Omega = 2$, the implied mass of the compact object is $1.5M_\odot$. The last pair of values is, however, very unlikely if one assumes that the basic precession is generated by tidal forces exerted on the normal star. Calculations by Hut and van den Heuvel[18] show that with $\Omega \geq 1.4$ and $M_{compact} = 1.5M_\odot$, a 13d orbital period and a 160d precessional period cannot be accounted for. One can also argue that the possible non-alignment of the star is relatively small ($\sim 20°$) and therefore it will modify our results only slightly[24]. We therefore regard equations 1 and 3 as realistic limits.

An interpretation of the entire light curve of SS433 requires a detailed geometrical and physical model for the system which should include many parameters. In particular the relative intensity of the different sources in the system and the distribution of their surface brightness should be incorporated in such a model. Indeed, such an interpretation is attempted by one of us[25] (E.M.L.) in a separate work, in which a theoretical synthetic light curve is fitted to all the available photometric data. For this purpose Leibowitz[25] assumes a hot spot as an additional source of light characterized by a very special radiation pattern. He also assumes a uniform surface brightness over the disk and neglects limb darkening of the normal star. Our approach, on the other hand, is to analyse a smooth and simple section of the light curve, without introducing any further assumptions or parameters other than those used in the most basic and common precessing disk models. Here we have considered only the disk radius and the mass ratio as free parameters. Despite the difference in approach and in the analyzing technique and despite the many more parameters in Leibowitz's model, his findings are consistent with this work.

Our basic and simple picture has yielded a most likely lower limit of $4.3\,M_\odot$ for the compact object, (equation (3)). This limit is much larger than the mass of the most massive neutron star observed so far[26]. It is also larger than most theoretical upper limits for this value[26]. We therefore conclude, that it is tentatively reasonable to assume a black hole for the compact object of SS433. Further photometric and spectroscopic observations in SS433 are clearly required in order to reaffirm our conclusion and in order to improve the constraints on the mass of the compact object in this system.

We thank M. Milgrom for helpful discussions and N. Brosch, A. Fabian, D. Gies, K. Horne, J. Matese, J. Refaeli, E. van den Heuvel and D. Whitmore for many useful comments.

Received 3 August; accepted 26 October 1983.

1. Margon, B. *Science* **215,** 247–252 (1982).
2. Crampton, D., Cowley, A. P. & Hutchings, J. B. *Astrophys. J. Lett.* **235,** L131–L135 (1980).
3. Crampton, D. & Hutchings, J. B. *Vistas Astron.* **25,** 13–25 (1981).
4. Kemp, J. C., Barbour, M. S., Kemp, G. N. & Hogood, D. M. *Vistas Astron.* **25,** 31–43 (1981).
5. Leibowitz, E. M. *et al. Mon. Not. R. astr. Soc.* (in the press).
6. Kemp, J. C., Barbour, M. S., Arbabi, M., Leibowitz, E. M. & Mazeh, T. *Astrophys. J. Lett.* **238,** L133–L138 (1980).
7. Gladyshev, S. A., Kurochkin, N. E., Novikov, I. D. & Cherepashchuk, A. M. (USSR) *Astron. Tsirk.* No. 1086, 1–8 (1979).
8. Cherepashchuk, A. M. *Mon. Not. R. Astr. Soc.* **194,** 761–769 (1981).
9. Gladyshev, S. A. *Sov. Astron. Lett.* **7,** 330–333 (1981).
10. Mazeh, T., Leibowitz, E. M. & Lahav, O. *Astrophys. Letters* **22,** 185–191 (1981).
11. Anderson, S. F., Margon, B. & Grandi, S. A. *Astrophys. J.* **269,** 605–612 (1983).
12. Henson, G. D., Kemp, J. C., Barbour, M. S., Kraus, D. J., Leibowitz, E. M. & Mazeh, T. *Astrophys. J. Lett.* (in the press).
13. Russell, H. N. *Astrophys. J.* **35,** 315 (1912).
14. Whitmire, D. P. & Matese, J. J. *Mon. Not. R. Astr. Soc.* **194,** 293–299 (1981).
15. Katz, J. I. *Astrophys. J. Lett.* **236,** L127–L130 (1980).
16. Katz, J. I., Anderson, S. F., Margon, B. & Grandi, S. A. *Astrophys. J.* **260,** 780–793 (1982).
17. van den Heuvel, E. P. J., Ostriker, J. P. & Peterson, J. A. *Astron. Astrophys.* **81,** L7–L10 (1980).
18. Hut, P. & van den Heuvel, E. P. J. *Astron. Astrophys.* **94,** 327–332 (1981).
19. Crampton, D. & Hutchings, J. B. *Astrophys. J.* **251,** 604–610 (1981).
20. Kundt, W. *Nature* **282,** 52–53 (1979).
21. Kundt, W. *Vistas Astron.* **25,** 153–164 (1981).
22. Pratt, J. P. & Strittmatter, P. A. *Astrophys. J. Lett.* **204,** L29–L33 (1976).
23. Bahcall, J. H. *A. Rev. Astr. Astrophys.* **16,** 241–264 (1978).
24. Avni, Y. & Schiller, N. *Astrophys. J.* **257,** 703–714 1982).
25. Leibowitz, E. M. *Mon. Not. R. astr. Soc.* (submitted for publication).
26. Joss, P. C. & Rappaport, S. A. *Neutron Stars in Interacting Binary Systems* CSR-AT-82-10 (1982).

Continuing formation of the central star cluster in M87

A. C. Fabian*, H. Itoh†, G. C. Stewart*, C. R. Canizares‡ & P. E. J. Nulsen*

* Institute of Astronomy, Madingley Road, Cambridge CB3 0HA, UK
† Department of Astronomy, University of Kyoto, Kyoto, Japan
‡ Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

The rising velocity dispersion of the stars in the neighbourhood of the central luminosity spike observed[1–3] in the elliptical radio galaxy M87 was originally considered to be evidence for the presence of a massive black hole[4] ($M \sim 5\times10^9 M_\odot$). A later spectrum[3] taken in good seeing, however, shows that most of the light in the spike is due to stars, which restricts any black hole model; the luminosity spike may be a star cluster formed from gas infalling from the outer regions of M87[5,6]. Our analysis[7] of Einstein Observatory X-ray data on M87 revealed a cooling inflow of gas occurring at the rate required to build such a cluster. We show here that the back-reaction on the flow of the nuclear luminosity is small unless M87 was much brighter in the past and we suggest that massive central star clusters may be common in the central galaxies in many clusters and, on a smaller scale, in many elliptical galaxies.

X-ray continuum[5,8] and emission line[5,9,10] measurements of the temperature of the gas in M87 have established the presence of a temperature gradient which implies that the gas is cooling and accreting onto the galaxy[11–14]. We have combined these spectral and imaging[15] data to determine in detail the gas temperature and density profiles and thence the mass flow rate, $\dot{M}$, at each radius[7]. $\dot{M}$ drops from 10 $M_\odot\,yr^{-1}$ beyond about 50 kpc to $\sim$1 $M_\odot\,yr^{-1}$ at 750 pc, or 10 arc s, from the nucleus. At this inner radius, which is determined by the quality of the data, the gas temperature, T, is $\sim10^7$ K. It must fall steeply at some point within this region, liberating an X-ray luminosity $\sim 5/2\,\dot{M}/\mu m_H\,kT$, where μm_H is the mean mass per particle. We have identified this luminosity with the 2–3 arc s extended X-ray source[16] that coincides with the central luminosity spike. Gas is thus cooling and collapsing there at a rate of $\sim1\,M_\odot\,yr^{-1}$ and we presume that most of it forms stars. Up to $\sim10^{10}\,M_\odot$ is added over the age of the galaxy if the flow is steady. The high pressure in a cooling flow ($\sim10^6$ cm^{-3} K) leads to the formation of low mass stars[17,18] and we therefore expect the star cluster so formed to have a large ratio of mass to luminosity ($M/L > 10$). The optical colours[1] are consistent with the models of Sarazin and O'Connell[18].

The optical jet in M87 is evidence for activity in the nucleus and we therefore consider the possible influence of nuclear luminosity on the cooling flow. The flow itself would provide sufficient matter to power even a bright quasar if it were accreted by a compact object. The ionization equilibrium of gas in a cooling flow was calculated numerically by iterating the effects of photo-absorption and collisional ionization due to X-rays from the gas as well as from a nuclear X-ray source ΔL. The important bound–bound, bound–free and free–free radiative processes above 10^6 K of H, C, N, O, S, Si and Fe were included in the calculations. The density and temperature of successive radial shells of gas were interpolated from our solution[7] and extrapolated into the centre assuming constant pressure and $\dot{M}$. The results below are quoted for a radius of 190 pc, which is within the core of M87.

Departures from collisional equilibrium alone are small (less than a factor of 2) for the dominant ions of C, N, O and Fe provided that the $\Delta L_{41} < 10$, where $\Delta L = \Delta L_{41}\times10^{41}$ erg s^{-1} in the 0.5–4.5 keV range (an energy index of 0.75 was used). Radiation pressure is negligible in any reasonable distribution of mass in the core of M87 provided that $\Delta L_{41} < 10^5$. The local heating rate due to photoabsorption and to Compton interactions is found to exceed the local cooling rate in our calculations when $\Delta L_{41} > 10^2$. The cooling flow should be strongly disrupted above that luminosity. The observations[17] indicate $\Delta L_{41} < 4$, which means that the core of M87 must brighten considerably before the cooling flow is stopped. (Increasing $\dot{M}$ does, of course, increase the limits on ΔL.) The smooth X-ray surface brightness profile is observational evidence that the flow has lasted for times comparable with a typical cooling time of $\leqslant10^9$ yr.

Cooling flows are relatively common in clusters surrounding a single cD galaxy[19,20] and we may expect that some of the inferred 5–500 $M_\odot\,yr^{-1}$ of accreting gas forms central star clusters in them. We have re-analysed the X-ray surface brightness profile[21] of NGC1275 in the Perseus cluster to determine $\dot{M}(r)$ within a reasonable gravitational potential. Once again, we find that $\dot{M}$ increases with radius, r, up to 400 $M_\odot\,yr^{-1}$ at 100 kpc and that $\sim40\,M_\odot\,yr^{-1}$ passes into the inner 10 kpc (our best resolution) of the galaxy. It appears that the stars form over a large range of radii, comparable with the extent of the optical filaments[22], and by analogy with M87, it is likely that NGC1275 contains a large star cluster in its core. Scaled-down versions of the flows in M87 and NGC1275 may occur in many elliptical galaxies[23] and could also take place in the bulges of spiral galaxies. The centre of M31 may possibly contain a cluster of low mass stars (ref. 24; but see 25). The evolution of the densest core of a large central star cluster may in turn be related to the nuclear activity seen in many galaxies. Gravitational accretion by any central mass takes place from a hot (10^6–10^7 K) medium.

We thank R. Terlevich for discussion and S. Inagaki for a preprint. A.C.F. acknowledges support from the Royal Society.

Received 4 August; accepted 2 November 1983.

1. Young *et al. Astrophys. J.* **221,** 721–730 (1978).
2. de Vaucouleurs, G. & Nieto, J.-L. *Astrophys. J.* **230.** 697 (1979).
3. Dressler, A. *Astrophys. J. Lett.* **240,** L11–L16 (1980).
4. Sargent *et al. Astrophys. J.* **221,** 731–744 (1978).
5. Lea, S. M., Mushotzky, R. F. & Holt, S. S. *Astrophys. J.* **262,** 24–32 (1982).
6. Okazaki, A. T. & Inagaki, S. *Publs astr. Soc. Japan* (submitted).
7. Stewart, G. C., Canizares, C. R., Fabian, A. C. & Nulsen, P. E. J. *Astrophys. J.* (in the press).
8. Fabricant, D. & Gorenstein, P. *Astrophys. J.* **267,** 535–546 (1983).
9. Canizares *et al. Astrophys. J. Lett.* **234,** L33–L38 (1979).
10. Canizares, C. R., Clark, G. W., Jernigan, J. G. & Markert, T. H. *Astrophys. J. Lett.* **262,** L33–43 (1982).
11. Cowie, L. L. & Binney, J. *Astrophys. J.* **215,** 723–732 (1977).
12. Fabian, A. C. & Nulsen, P. E. J. *Mon. Not. R. astr. Soc.* **180,** 479–484 (1977).
13. Matthews, W. G. & Bregman, J. N. *Astrophys. J.* **224,** 308–319 (1978).
14. Binney, J. & Cowie, L. L. *Astrophys. J.* **247,** 464–472 (1981).
15. Fabricant, D., Lecar, M. & Gorenstein, P. *Astrophys. J.* **241,** 552–560 (1978).
16. Schreier, E. J., Gorenstein, P. & Feigelson, E. D. *Astrophys. J.* **261,** 42–50 (1982).
17. Fabian, A. C., Nulsen, P. E. J. & Canizares, C. R. *Mon. Not. R. astr. Soc.* **201,** 933–938 (1982).
18. Sarazin, C. L. & O'Connell, R. W. *Astrophys. J.* **268,** 552–560 (1983).
19. Stewart, G. C., Fabian, A. C., Jones, C. & Forman, W. *Astrophys. J.* (submitted).
20. Stewart, G. C., Canizares, C. R. & Fabian, A. C. *Astrophys. J.* **272,** 449–455 (1983).
21. Fabian, A. C., Hu, E. M., Cowie, L. L. & Grindlay, J. *Astrophys. J.* **248,** 47–54 (1981).
22. Lynds, R. *Astrophys. J. Lett.* **159,** L151–L154 (1970).
23. Nulsen *et al. Mon. Not. R. astr. Soc.* (in the press).
24. Faber, S. M. & French, H. B. *Astrophys. J.* **235,** 405–412 (1980).
25. Persson *et al. Astrophys. J.* **240,** 779 (1980).

0028-0836/84/040343-01$01.00

© 1984 Macmillan Journals Ltd

Mystery cloud of AD 536

R. B. Stothers

Institute for Space Studies, NASA Goddard Space Flight Center, 2880 Broadway, New York, New York 10025, USA

Dry fogs appear in the atmosphere when large volcanic eruptions inject massive quantities of fine silicate ash and aerosol-forming sulphur gases into the troposphere and stratosphere. Although the ash gravitationally settles out within weeks, the aerosols spread around the globe and can remain suspended in the stratosphere for years. Because solar radiation is easily absorbed and backscattered by the volcanic particles, a haziness in the sky and a dimming of the Sun and Moon are produced. Very dense and widespread dry fogs occur, on the average, once every few centuries[1–3]. The sizes and intensities of some of the largest of them before the modern scientific era have been estimated by several indirect methods[2,4,5]. The densest and most persistent dry fog on record was observed in Europe and the Middle East during AD 536 and 537. Despite the earliness of the date, there is sufficient detailed information to estimate the optical depth and mass of this remarkable stratospheric dust cloud. The importance of this cloud resides in the fact that its mass and its climatic consequences appear to exceed those of any other volcanic cloud observed during the past three millenia. Although the volcano responsible remains a mystery, a tropical location (perhaps the volcano Rabaul on the island of New Britain, Papua New Guinea) can be tentatively inferred.

Four reliable contemporary descriptions of the sky conditions in AD 536–537 exist[6]. Procopius[7], a Byzantine historian who lived in Rome (42° N) at the time, wrote that "the Sun gave forth its light without brightness, like the Moon, during this whole year, and it seemed exceedingly like the Sun in eclipse, for the beams it shed were not clear nor such as it is accustomed to shed... and it was the time when Justinian was in the tenth year of his reign". From Constantinople (41° N) Lydus[8] observed that "the Sun became dim in the course of the recently passed fourteenth indiction, for nearly a whole year, when Belisarius had the highest honour, so that the fruits were killed at an unseasonable time". An anonymous chronicler (associated by tradition with the name of Zacharias of Mytilene[9]) also referred to observations made from Constantinople in the following terms: "in the year fourteen... the Sun began to be darkened by day and the Moon by night... from the 24th of March in this year till the 24th of June in the following year fifteen". According to another contemporary writer (probably John of Ephesus), describing conditions in Mesopotamia (30–37° N) and later quoted by Michael the Syrian[10] and Bar-Hebraeus[11]: "in the year 848 of the Greeks... the Sun was dark and its darkness lasted for eighteen months; each day it shone for about four hours, and still this light was only a feeble shadow... the fruits did not ripen and the wine tasted like sour grapes".

What can be made of such astonishing reports? In ordinary conditions, the Sun's radiance would appear perceptibly diminished only if the Sun lay less than ~2.5° in apparent altitude above the horizon[12]. The normal visual extinction at apparent zenith angle $z = 87.5°$ is 3.4 mag (astronomical magnitudes)[13]. If the Sun shone for only 4 hours a day at a latitude of 33° N during the fruit-growing season (spring and summer) of AD 536, then it must have begun setting at $z \sim 30°$. The total visual extinction produced by the atmosphere in combination with the volcanic dust can be adequately represented by Bouguer's law[14]; in the present case

$$[(\Delta m)_0^D + 0.20] \sec z = 3.4$$

where $(\Delta m)_0^D$ is the zenithal volcanic dust contribution and 0.20 mag refers to the unperturbed atmosphere. It follows that $(\Delta m)_0^D \sim 2.7$ mag, corresponding to an excess optical depth $\tau_D = 0.921\,(\Delta m)_0^D \approx 2.5$. At maximum altitude both the Sun and the full Moon would have appeared ~10 times fainter than normal, which can readily explain their reported 'darkening'. Scattered sunlight, however, would still have illuminated the rest of the sky, which therefore would not have appeared as dark. Although the derived optical depth excess is rather uncertain, it can be checked below by an independent method.

What sort of volcanic eruption could have produced such a dense dust veil? Not a Mediterranean eruption[6], which would almost certainly have been mentioned by Procopius. A clue is found in the duration of the dry fog. Historically, dry fogs have always developed rather quickly, although the dates of their slow disappearances are susceptible to visual impression and have inevitably been uncertain by a few months. The dry fog of AD 536 first appeared in the Mediterranean region in late March. According to the observers at 41–42° N, it lasted 12–15 months, that is, until March–June AD 537; this period agrees in an acceptable way with the variously reported dates. At 30–37° N, however, the duration of the dry fog was 18 months. Its prolonged visibility at the more southerly latitudes suggests that the haze was significantly thicker there, and hence that the eruption which produced the aerosols was situated somewhere to the south. If so, the location was probably in the tropics (23° S–23° N) because aerosols from eruptions south of 23° S do not penetrate the Northern Hemisphere in significant amounts. Only 2 to 3 weeks would be needed for the stratospheric aerosols to reach Mediterranean latitudes. A large volcanic eruption that may be consistent with the year, March season, and possible tropical latitude of the AD 536 event was the explosion of Rabaul (4° S) on the island of New Britain, off New Guinea, which has been radiocarbon-dated to AD 540 ± 90 (ref. 15). This eruption dispersed at least 11 km^3 (bulk volume) of near-source pumice and ash, mostly to the west[16], which suggests that the March–October southeasterly monsoon[17] was blowing at the time. (Most of the distal ash would have fallen into the Bismarck Sea, and could greatly exceed in volume the near-source ejecta.)

A major tropical eruption in AD 536 would also be expected to deposit sulphuric acid aerosols on the polar ice caps, where they would remain preserved in compacted annual snow layers. A very high sulphuric acid signal has in fact been detected in a Greenland ice core by Hammer *et al.*[5], who assigned a date of AD 540 ± 10, and in another Greenland ice core by Herron[18], who estimated ~AD 535. There is little doubt that the acidity signal refers to the same event that produced the dry fog of AD 536, because both kinds of volcanic signal at the exceptionally high levels discussed here occur only once every few centuries on the average. The strength of this ancient volcanic signal relative to the signal obtained from the more recent eruption of another tropical volcano, Tambora (which exploded in April 1815), can be estimated as follows. Herron's data imply relative signal strengths (after subtraction of background) of 0.63 : 1.00 for the AD 536 eruption and the Eldgjá (Iceland, ~AD 934) eruption, while the data of Hammer *et al.*[5] (who did not provide the signal strength for the AD 536 eruption) imply a ratio of 1.00 : 0.37 for the Eldgjá and Tambora eruptions. It follows that the AD 536 signal exceeds that of Tambora by a factor of 1.7. As the eruption of Tambora increased the stratospheric optical depth at north-temperate latitudes in 1815 by $\tau_D = 1.3$ as derived by several methods (ref. 19), the excess stratospheric turbidity at these latitudes in AD 536 must have been $\tau_D = 1.3 \times 1.7 = 2.2$. (If Krakatoa, a tropical volcano that erupted in 1883, is used as the standard for comparison, essentially the same result is obtained.) This is consistent with the preceding result based on the ancient extinction reports, and therefore supports the idea that the eruption was a tropical one because the strength of the ice-core acidity signal depends on eruption latitude[5] and no correction for latitude has been applied here. Peak global aerosol loading of the stratosphere can be computed from the formula $M_D = 1.5 \times 10^{14}\,\tau_D$ g, which is insensitive to the adopted modal particle radius r if $0.15 \leq r \leq$

0028-0836/84/040344—02$01.00

© 1984 Macmillan Journals Ltd

0.7 μm—certainly the case for modern volcanic eruptions (ref. 19). The loading in AD 536 was therefore $\sim 3 \times 10^{14}$ g.

The AD 536 stratospheric 'mystery cloud' presumably decayed exponentially with a typical *e*-folding time of ~1 yr (ref. 19). If so, its optical depth would have dropped to less than 0.1 by November and December AD 539, when a faint cometary tail was visually sighted from Europe[7] and China[20].

Climatologically, the winter of AD 536–537 in the Middle East was an unusually severe one, attended by extraordinary snowfalls in Mesopotamia[9,21]. During the preceding summer (and possibly the following one as well), the fruits, including grapes, did not properly ripen in at least some parts of the Middle East and Europe. Historically, cold weather and poor harvests of grapes and other crops have tended to follow very large explosive volcanic eruptions[2]. The accepted reason is that volcanic dust clouds screen some of the incoming sunlight and thereby cause a reduction of the Earth's average surface temperature. Although it seems that Tambora's large eruption did not catastrophically affect the Middle Eastern growing season[22], the aerosol-producing eruption of AD 536, which was twice as large, apparently did.

I thank M. R. Rampino for useful comments.

Received 22 August; accepted 10 November 1983.

1. Russell, F. A. R. *The Eruption of Krakatoa and Subsequent Phenomena* (ed. Symons, G. J.) 384–405 (Truebner, London, 1888).
2. Lamb, H. H. *Phil. Trans. R. Soc.* A**266,** 425–533 (1970).
3. Stothers, R. B. & Rampino, M. R. *Science* **222,** 411–413 (1983).
4. Deirmendjian, D. *Adv. Geophys.* **16,** 267–296 (1973).
5. Hammer, C. U., Clausen, H. B. & Dansgaard, W. *Nature* **288,** 230–235 (1980).
6. Stothers, R.B. & Rampino, M. R. *J. geophys. Res.* **88,** 6357–6371 (1983).
7. Procopius *History of the Wars* (transl. Dewing, B. H.) 2.4.1–2, 4.14.5–6 (Harvard University Press, Cambridge, Massachusetts, 1916).
8. Lydus, J. L. *On Portents* (ed. Wachsmuth, C.) 9c (Teubner, Leipzig, 1863).
9. Zacharias of Mytilene *Chronicle* (transl. Hamilton, F. J. & Brooks, E. W.) 9.19, 10.1 (Methuen, London, 1899).
10. Michael the Syrian *Chronicle* (transl. (into French) Chabot, J.-B.) 9.296 (Belles-Lettres, Paris, 1901).
11. Bar-Hebraeus *Chronography* (transl. Budge, E. A. W.) 8.79–80 (Oxford University Press, London, 1932).
12. Minnaert, M. *The Nature of Light and Colour in the Open Air* (transl. Kremer-Priest, H. M. & Jay, K. E. B.) 55, 269 (Dover, New York, 1954).
13. Allen, C. W. *Astrophysical Quantities*, 125–127 (Athlone, London, 1973).
14. Blanco, V. M. & McCuskey, S. W. *Basic Physics of the Solar System*, 93 (Addison-Wesley, Reading, Massachusetts, 1961).
15. Heming, R. F. *Bull. geol. Soc. Am.* **85,** 1253–1264 (1974).
16. Walker, G. P. L., Heming, R. F., Sprod, T. J. & Walker, H. R. *Geol. Surv. Papua New Guinea Mem.* **10,** 181–193 (1981).
17. Seltzer, L. E. (ed.) *The Columbia Lippincott Gazetteer of the World*, 1309 (Columbia University Press, New York, 1952).
18. Herron, M. M. *J. geophys. Res.* **87,** 3052–3060 (1982).
19. Stothers, R. B. *Science* (in the press).
20. Ho Peng Yoke *Vistas Astr.* **5,** 127–225 (1962).
21. John of Ephesus *Ecclesiastical History* (transl. (into Latin) van Douwen, W. J. & Land, J. P. N.) 297–298 (Muller, Amsterdam, 1889).
22. Post, J. D. *The Last Great Subsistence Crisis in the Western World*, 55–59 (Johns Hopkins University Press, Baltimore, Maryland, 1977).

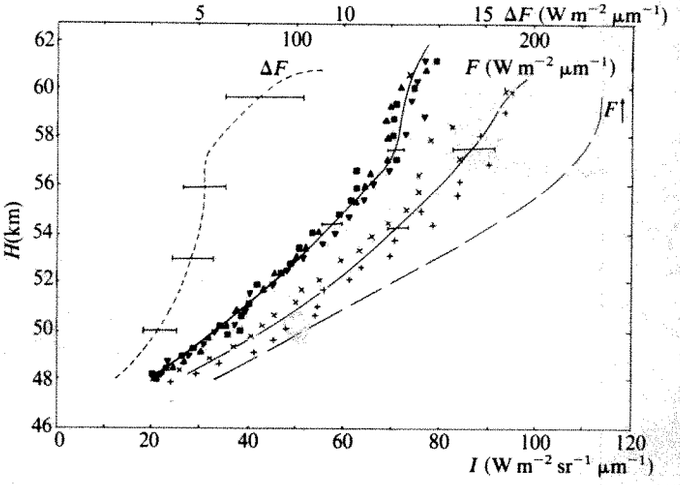


Fig. 1 Observed UV intensities at the zenith angles of 45° (×, +), 133° (▼, ▲, 164° (■) as a function of altitude as well as least square fits. Also shown are the upward flux (— —) and the net flux (– – –) densities, calculated from experimental data.

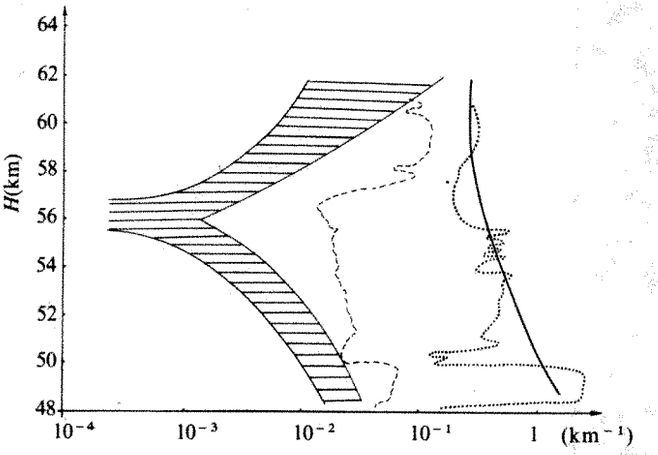


Fig. 2 Observational range in volume absorption coefficient (shaded area) and volume extinction coefficient multiplied by $(1-g)$ (——) as a result of direct computation from the UV photometer data. Also shown are the extinction coefficients multiplied by $(1-g) = 0.2$ (after ref. 13) for mode-1 only (– – –) and total (· · · ·).

Scattered UV solar radiation within the clouds of Venus

A. P. Ekonomov, V. I. Moroz, B. E. Moshkin, V. I. Gnedykh, Yu. M. Golovin & A. V. Crigoryev

Space Research Institute, USSR Academy of Sciences, Moscow, USSR

The spectral albedo curve of Venus shows a steep decline at wavelengths between 0.5 and 0.3 μm (refs 1–3). This is of interest since absorption of solar energy in this spectral region has a strong influence on the thermal balance of the venusian atmosphere. The nature and altitude distribution of the UV absorber have not been determined, although some candidate gases have been proposed. We report here the results of measurements carried out on the last Soviet venusian descent probe Venera-14 which suggest that the UV absorption is by aerosols above an altitude of 57 km and by gases below this level.

It has long been known that some UV absorber(s) is present in the venusian clouds. The same absorbing material seems to be responsible for both the low UV albedo and for producing the UV features on Venus. Gaseous sulphur dioxide is the obvious candidate for the absorber at wavelengths shorter than 0.32 μm (ref. 4), but the nature of the substance responsible for absorption longwards of 0.32 μm is not known. Remote sensing techniques have failed to solve this problem. Pioneer Venus results[5,6] and results from Veneras 9 and 10 (ref. 7) showed that the absorption of sunlight in the visible took place mainly in the upper cloud, which is why one of the principal goals of the Soviet Venera-14 mission was to investigate the distribution of the solar near-UV radiation within the main clouds during the descent of the probe through the atmosphere of Venus on 3 March 1982.

The UV photometer aboard Venera-14 was part of a complex optical instrument with a common angle-scanning device for the near-UV and the visible regions. The detector Ga–P/filter combination provided a bandpass of 0.06 μm at half-maximum centred at 0.37 μm with no appreciable leak beyond bandpass. The photometer had a circular field of view (FOV) with width 10° (FWHM). The instrument scanned a range of zenith angles by rotating the axis of the FOV in a vertical plane with a period of 2.5 s. (The photometer and its operating conditions are described in more detail elsewhere[8,9].)

0028–0836/84/040345—03$01.00

© 1984 Macmillan Journals Ltd

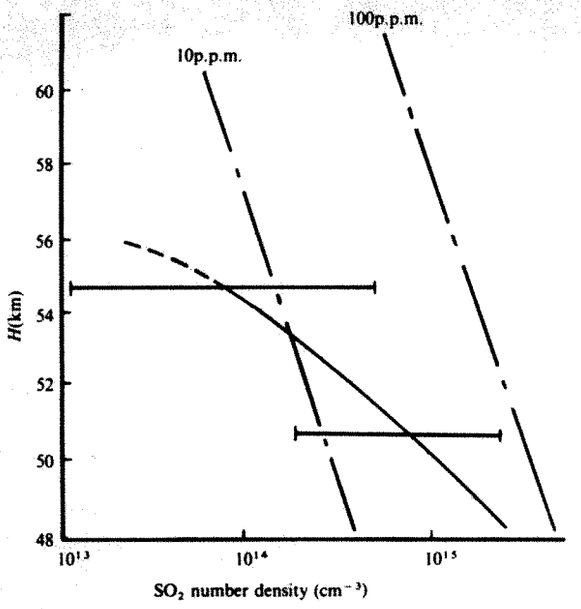


Fig. 3 Sulphur dioxide number density on the basis of the observed data on UV absorption (solid line with bars). Curves of constant mixing ratio are also shown.

The raw data from our photometric measurements as well as the results from the primary data processing are presented in Fig. 1. The data are plotted as a function of altitude (H) above the 6,052 km level. Fits by spline technique were used for least-squares approximation of the data. We define I_{45}, I_{133}, I_{164} as the solar near-UV intensities sampled at zenith angles of 45°, 133°, 164°. $F\downarrow$, $F\uparrow$, ΔF are the downward, upward and net fluxes respectively derived from intensities using the approximation:

$$I(\phi) = I(1)[a + \phi(1-a)]$$

where $I(\phi)$ denotes the angular intensity distribution, ϕ is the cosine of the zenith angle, a is the parameter derived from the least squares fit, $I(1)$ is the downwards intensity. It is a simple matter to show that:

$$F\downarrow = 2\pi I(1)\left(\frac{a}{2} + \frac{1-3}{3}\right)$$

$$F\uparrow = 2\pi I(1)\left(\frac{a}{2} - \frac{1-a}{3}\right)$$

$$\Delta F = \simeq 3(I_{45} - I_{133})$$

The data set in the uppermost portion of the profile (from 62 to 59 km) is characterized by high variability of downward intensities although the upward ones are quite stable. There was no instability of downward intensities below 59 km. Inhomogeneities 1–5 km in length are expected above 59 km which does not seem to be consistent with results obtained from remote sensing orbiters which have failed to discover inhomogeneity at scales of less than 10 km (ref. 10). The above-mentioned phenomenon may resemble the 'silver lining effect'—where the photometer views the illuminated edges of the clouds at horizontally-inhomogeneous upper layers. From about 59 km down to 48 km, the downward flux decreases gradually from 1/5th of the incident solar radiation flux at the top of the venusian atmosphere to 1/35th of this value.

Using ground-based data on the UV albedo of Venus[11] in conjunction with results from our measurements, an effective optical depth above 60 km can be estimated as $\tau_0 = \tau(1-g) \cong 3$ where τ is the optical depth, and g is the asymmetry factor of the phase function. Consequently a thick-atmosphere approximation can be obtained and all calculations may be substantially simplified. For example, altitude profiles of the volume absorption coefficient K and extinction coefficient α of the atmosphere may be derived from $(I_{45} - I_{133})$ and $(I_{45} + I_{133})$ altitude profiles[12]. The results of such an evaluation are presented in Fig. 2. Note, however, that K is extremely sensitive to computational errors which leads to some uncertainty over its value.

The altitude profile of the net flux shows that the true absorption of UV radiation is present everywhere within the cloud structure but most strongly in the upper clouds ($H > 58$ km). The next lowest layer ($58 > H > 55$ km) has the minimum UV volume absorption coefficient. These results are qualitatively consistent with the data Tomasko[6] obtained in the visible region. In the altitude interval between 55 and 48 km the absorption is probably coupled with the increasing gas density.

Figure 2 also provides a comparison between our results and the extinction coefficient from LCPS (cloud particle size spectrometer) measurements[13]. The profiles of K and $\alpha(1-g)$ presented in Fig. 2 lead to the following conclusions:

(1) There are two different absorbers which dominate above and below the 57-km level. This is supported by different altitude dependences above and below this level and the minimum-volume absorption coefficient K observed there.

(2) The most important absorber above 57 km is probably particulate matter. Particles of mode 1 as defined by Knollenberg and Hunten[13] can be identified as the absorbers near the cloud top. The sharp rise of absorption is crudely consistent with the rise in the mode-1 particle concentration above 57 km. Of course, the possibility cannot be ruled out that some absorbing gas has the same type of altitude distribution (due, for example, to some photochemical processes) but this seems unlikely. If mode-1 particles are responsible for the measured K value it is possible by combining Knollenberg and Hunten's data with ours to evaluate κ, the imaginary part of the refractive index. We find that $7\times10^{-4} < \kappa < 7\times10^{-3}$. The existing information is not yet sufficient for identification but now two additional limitations on the absorber are implied: the value of κ and the altitude distribution. Sulphur[4,14], $FeCl_3{\cdot}H_2SO_4$ (ref. 15) and $(NH_4)(S_2O_5)$ (ref. 16) have been considered recently as possible constituents. Toon *et al.*[14] have suggested that even a few per cent of strongly absorbing short-chain sulphur allotropes as particles, such as S_3 (absorption band centred near 4,000 Å) and S_4 (absorption band centred near 5,300 Å) could account for the solar energy deposition profile in the upper clouds at wavelengths longer than 0.32 μm. Our data seem to be consistent with an approximate 1–10% mass of sulphur within the upper clouds. A steep decline of absorption from the beginning of our measurements at 62 km to the minimum absorption detected at 56 km resembles a steep decline from the peak sulphur mass fraction at 58 km calculated by Toon[14] to the small amount present below 56 km. The peak value itself was not detected in our measurements but it could be masked by a mode-1 concentration growth above 58 km.

(3) Probably the most important absorber below 57 km is a gas. The basis for this proposal is the increase of K with decreasing altitude (K increases even faster than does the atmospheric number density). An attractive candidate for this gas is SO_2 which was discovered long ago as a small constituent of the venusian low atmosphere[17,18], whose concentration extrapolated to the altitude ~50 km is about what is needed to provide the observed UV absorption. Figure 3 shows a profile of SO_2 in the altitude interval 50–57 km calculated on the basis of the hypothesis that SO_2 gas is responsible for absorption.

We have used the laboratory cross-section of SO_2 taken from refs 19, 20, although our neglect of the influence of the unresolved line structure of SO_2 bands in the radiative transfer treatment[21] may necessitate a later re-evaluation of the SO_2 concentration. More details concerning results described above and their interpretation are given in ref. 9.

Received 28 July; accepted 3 November 1983.

1. Irvine, W. M. *J. atmos. Sci.* **25**, 610 (1963).
2. Barker, E. S. *et al. J. atmos. Sci.* **32**, 1205 (1975).

3. Moroz, V. I. *et al. Pis'ma Astr. Zh.* **8**, 404 (1982).
4. Esposito, L. W., Winick, J. R. & Stewart, A. I. *Geophys. Res. Lett.* **6**, 601 (1979).
5. Pollack, J. B. *et al. J. geophys. Res.* **85**, 8141 (1980).
6. Tomasko, M. G. *et al. J. geophys. Res.* **85**, 8141 (1980).
7. Ekonomov, A. P. *et al. Kosm. Issled.* **16**, 901 (1978).
8. Moshkin, B. E. *et al. Kosm. Issled.* **21**, 236 (1983).
9. Ekonomov, A. P. *et al. Kosm. Issled.* **21**, 254 (1983).
10. Rossow, W. B. *et al. J. geophys. Res.* **85**, 8107 (1980).
11. Moroz, V. I. in *Venus*, 27 (The University of Arizona Press, 1983).
12. Ustinov, E. A. *Kosm. Issled.* **15**, 768 (1977).
13. Knollenberg, R. G. & Hunten, D. M. *J. geophys. Res.* **85**, 8039 (1980).
14. Toon, O. B., Turco, R. P. & Pollack, J. P. *Icarus* **51**, 358 (1982).
15. Zasova, L. V., Krasnopolsky, V. A. & Moroz, V. I. *Adv. Space Res.* **1**, 13 (1981).
16. Titov, D. V. *Kosm. Issled.* **19**, 794 (1981).
17. Gel'man, B. G. *et al. Kosm. Issled.* **17**, 708 (1979).
18. Hoffman, J. H., Oyama, V. I. & von Zahn, U. *J. geophys. Res.* **85**, 7871 (1980).
19. Vikesland, J. P. & Stricker, S. J. *J. chem. Phys.* **60**, 660 (1974).
20. Sidebottom, H. W. *et al. J. Am. chem. Soc.* **93**, 2587 (1971).
21. Belton, M. J. S. *Icarus* **52**, 149 (1982).

Analysis for rare earth elements in accessory minerals by specimen isolated secondary ion mass spectrometry

J. B. Metson, G. M. Bancroft, H. W. Nesbitt & R. G. Jonasson

Departments of Chemistry and Geology, University of Western Ontario, London, Ontario, Canada N6A 5B7

The distribution of rare earth elements (REEs) in minerals and mineral assemblages is of considerable geochemical significance. However, because of their low levels in most minerals, analysis for REEs is a considerable challenge. The application of secondary ion mass spectrometry (SIMS) to this problem has been limited by the presence of molecular ion interferences in the secondary ion mass spectrum. We describe here the application of the specimen isolation technique[1] to rare earth analysis by SIMS. The method effectively suppresses molecular ions while maintaining sufficient sensitivity to examine REE distribution in a variety of accessory minerals.

REEs have been used to help interpret the genesis of igneous rocks[2], metamorphic rocks[3] and sedimentary rocks[4], as well as the history of the Earth[5]. Their chemistry is predominantly ionic, their properties being controlled by their size. Atomic and ionic radii decrease systematically from lanthanum to lutetium as a result of increasing nuclear charge and imperfect shielding of the 4f electrons. Consequently the properties of these species, including their thermodynamic properties and their concentrations in minerals and rocks, vary sympathetically with changes in ionic radii. Only europium shows variable valency in geological environments and may be dominantly divalent or trivalent, depending on the partial pressure of oxygen. The systematic behaviour of the trivalent lanthanides together with the anomalous behaviour of Eu^{2+}, make them a very powerful tool in geochemistry. However, there are few studies that document the concentration of REEs in the suite of minerals composing a rock[6].

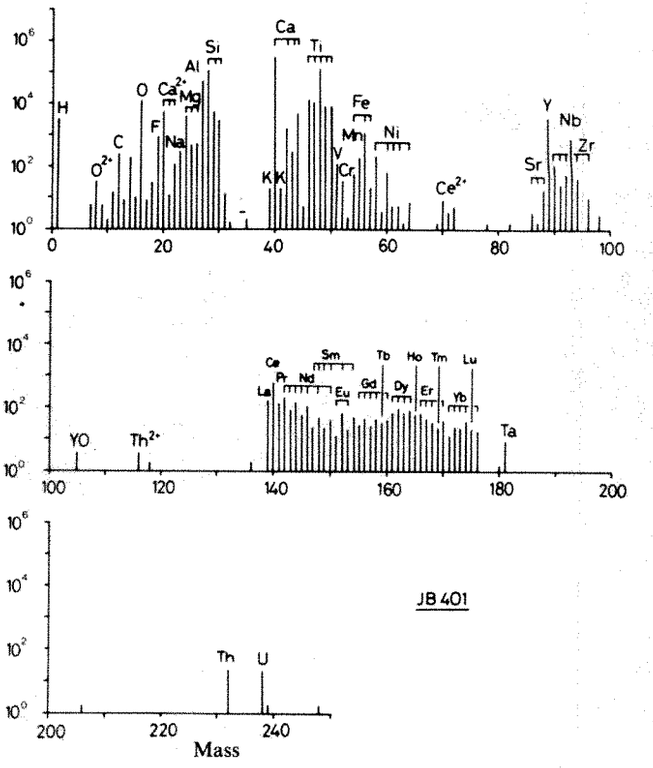


Fig. 1 Secondary ion mass spectrum of a sphene from Cardiff Mine, Ontario, Canada. Count time of 1 s over each integral m/e value.

SIMS has been recognized for some time as the potential 'ultimate weapon' for geochemical analysis[7], however the two major stumbling blocks in realizing this potential have been resolving elemental ions from molecular ions[8], and the inability to quantify secondary ion yields (J.B.M., H.W.N. and G.M.B., in preparation).

There are currently two approaches to removing molecular ion interferences from SIMS spectra. The first involves resolving peaks at the same unit m/e using the small mass difference between elemental and molecular peaks[7–9]. The alternative approach exploits the difference in kinetic energy distribution between molecular and elemental ions to suppress the intensity of the molecular ions. This is possible only with instruments that use a double focusing secondary column geometry such as the Nier design used in the Cameca IMS 3f. Shimizu *et al.*[8,10,11] have refined this technique and shown it to be superior to high mass resolution in a number of applications, particularly at $m/e > 70$.

As discussed recently by Reed[12,13], in the case of the REEs, very high resolution is required to separate the oxide and elemental peaks (for example, a $m/\Delta m > 8{,}000$ is required to separate $^{169}Tm^+$ from $^{153}Eu^{16}O^+$) and the associated intensity loss is prohibitive. Kinetic energy selection fares little better as

Table 1 Observed and natural abundances[17] for rare earth isotopes in Arendal sphene (*a*), Bear Lake sphene (*b*)

Nd				Sm				Gd				Yb			
	Observed		Natural		Observed		Natural		Observed		Natural		Observed		Natural
Mass	*a*	*b*	abundance	Mass	*a*	*b*	abundance	Mass	*a*	*b*	abundance	Mass	*a*	*b*	abundance
142*			27.11	144*			3.09	154*			2.15	170*			3.03
143	12.8	12.8	12.17	147	15.4	15.6	15.0	155	14.0	18.0	14.7	171	14.7	13.5	14.3
144*			23.85	148*			11.2	156	20.7	22.7	20.5	172	22.3	26.6	21.8
145	8.44	8.23	8.30	149	13.1	14.6	13.8	157	16.2	15.3	15.7	173	14.8	19.1	16.1
146	16.8	17.0	17.62	150*			7.44	158	24.8	19.9	24.9	174	31.9	24.8	31.8
148*			5.73	152	26.4	25.3	26.7	160*			21.9	176*			12.7
150*			5.62	154*			22.7								

* Peaks at these m/e values consist of an isotope from more than one element.

0028-0836/84/040347—03$01.00

© 1984 Macmillan Journals Ltd

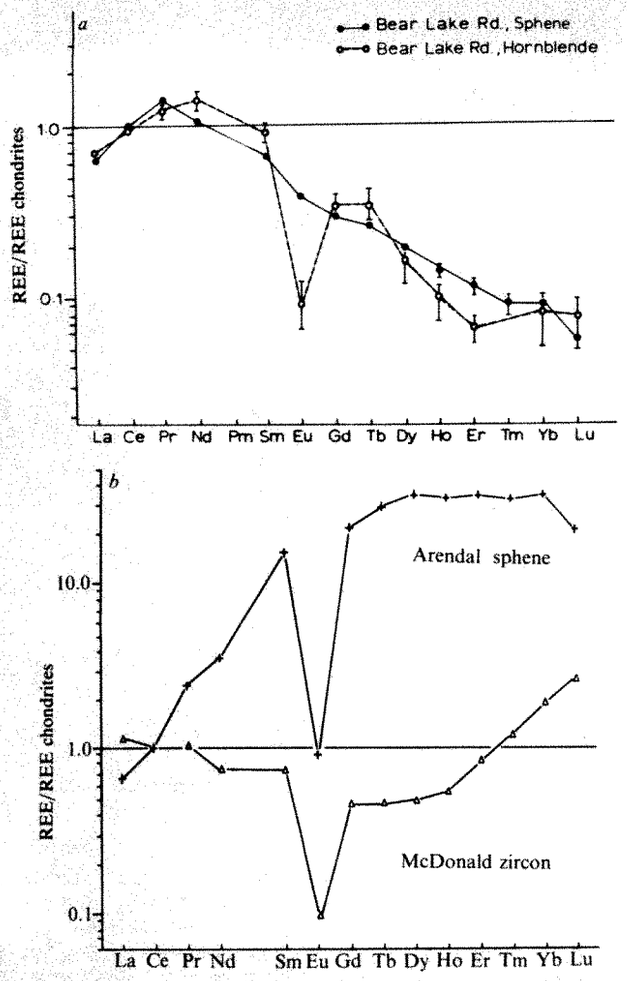


Fig. 2 *a*, Rare earth element distributions from a sphene, hornblende contact from Bear Lake Rd, Ontario, Canada. Intensities are divided by chondritic abundances from ref. 18 and normalized to cerium. In actual abundance $^{140}Ce^+$ sphene/$^{140}Ce^+$ hornblende = 45. Error bars correspond to $\pm 1\sigma$. *b*, Rare earth element distributions from a sphene from Arendal, Norway and a zircon from the McDonald Mine, Ontario, Cadada. Again intensities are compared to chondritic abundances and normalized to cerium.

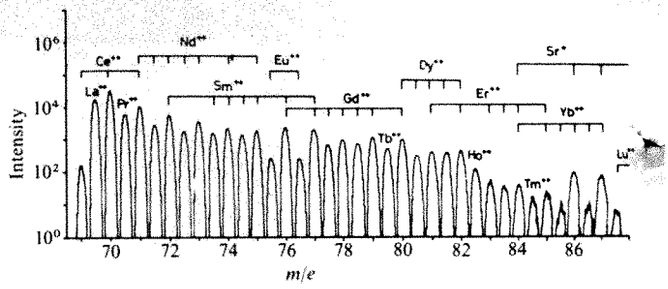


Fig. 3 The complete secondary ion mass spectrum of the monazite sample between $m/e = 69$ and 87, showing the doubly charged ions of the rare earth elements. Total spectrum, 1,000 channels, 5 s per channel.

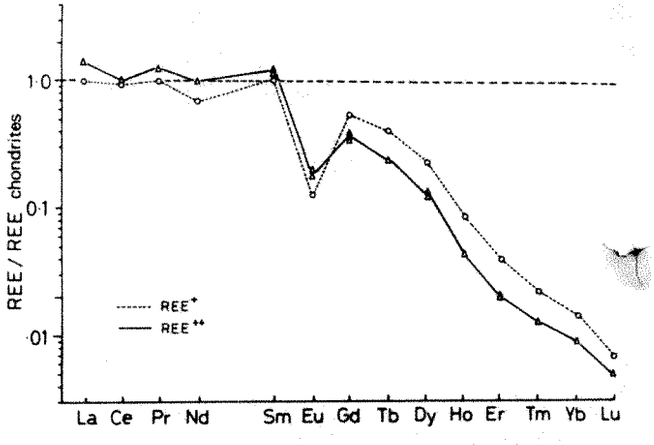


Fig. 4 The distribution of singly and doubly charged ions from the rare earth elements of the New Mexican monazite.

the difference in energy distributions of the REEs and their oxides is not large compared with lighter elements, and the intensity loss with kinetic energy selection is again several orders of magnitude. Clearly, obtaining 'clean' elemental peaks with usable intensities is desirable, and this is the great advantage of the technique described below.

Our results were obtained on a Cameca IMS 3f instrument. A primary beam of $^{16}O^-$ was used exclusively at an accelerating voltage of 11.8–12.2 keV. The specimen isolation technique[1] requires the electrical isolation of the specimen (whether a conductor or insulator) from the sample holder, which in the case of a Cameca IMS 3f carries a secondary accelerating voltage of 4.5 keV. The specimen is thus allowed to charge under the primary beam, creating an extreme kinetic energy selection between the sample surface and the secondary accelerating field. This accounts for the high elemental ion/molecular ion discrimination observed. The lens effect of the sample holder is critical in maintaining usable secondary ion yields in these conditions[1]. The beam conditions necessary to maintain an equilibrium charging rate impose some limitations on spatial resolution. Beam diameters of 40–50 μm in a spot mode, seem to yield optimum secondary ion intensity and stability. In all cases, lens settings in the primary column are determined solely from secondary ion yield and not by reference to the observed primary current. The apertures of the secondary column including the energy slits, remain fully open throughout analysis. This allows a considerable saving in intensity over the standard kinetic energy selection procedure[8].

The six samples used in this study consisted of three sphene ($CaTiSiO_5$) samples, two from Ontario, Canada (Cardiff Mine and Bear Lake Rd) and one from Arendal, Norway, a hornblende in contact with one of the sphenes (from Bear Lake Rd), a zircon crystal from the McDonald Mine, Ontario and a monazite from New Mexico, USA. Count rates for the REEs ranged from 9.5×10^4 c.p.s. from Ce in the monazite to 4×10^2 c.p.s. for Ce in the Cardiff Mine sphene and 36 c.p.s. for Ce in Bear Lake hornblende. The baseline in all cases is <0.1 c.p.s. typical of SIMS measurements.

This study initially concerned the mineral sphene because of its possible use as a host mineral for immobilizing radionuclides from nuclear fuel reprocessing wastes[14,15]. The leaching behaviour of the REEs is considered to be a useful analogue for the behaviour of actinides in such structures. The study of REE distribution and fractionation is also of fundamental geological importance as described above.

Figure 1 shows a complete secondary ion mass spectrum of the Cardiff, Ontario sphene in which the REEs are clearly resolved. To confirm that the rare earth spectra are clear of molecular ions, isotope ratios can be derived for several rare earths (Table 1). The isotope ratios are in very good agreement with literature values[16], particularly for the samples enriched in the heavy REEs (HREEs). There is little interference from hydrides and no measurable contamination of the HREE peaks from oxides of the light REE in the Arendal sphene, although $^{139}La^{16}O^+$ and $^{140}La^{16}O^+$ are probably present in the $^{155,156}Gd^+$ peaks of the Bear Lake sphene.

Despite the multitude of isotopes in the REE mass range and the resulting overlap at many mass units, each element in the

Table 2 Observed abundances for singly and doubly charged REEs from Monazite

Nd				Sm				Gd				Dy			
Mass	1+	2+	Natural abundance	Mass	1+	2+	Natural abundance	Mass	1+	2+	Natural abundance	Mass	1+	2+	Natural abundance
142*				144*			3.09	154*			2.15	160*			2.29
143	12.8	12.9	12.17	147	16.6	15.9	15.0	155	15.8	15.5	14.7	161	19.1	19.8	18.88
144*				148*			11.2	156	22.8	20.8	20.5	162	25.2	25.8	25.53
145	8.23	8.13	8.30	149	14.6	14.4	13.8	157	15.0	15.7	15.7	163	25.0	23.8	24.97
146	17.0	16.6	17.62	150*			7.44	158	22.1	23.6	24.9	164*			28.18
147*				152	24.3	25.1	26.7	160*			21.9				

* Peaks at these m/e values consist of an isotope from more than one element.

series has at least one 'clean' isotope. Assuming similar sputtering yields for the REEs, we can equate relative intensity to relative abundance and normalize to chondritic abundance in the usual way. Ion yields are not in fact equal for the REEs as Reed[12,13] has shown. However, a discussion of ion yields in these conditions is beyond the scope of this paper. The resulting rare earth patterns are still a useful fingerprint of the sample and have semiquantitative significance.

The REE patterns (normalized to chondrites) of two sphenes, a hornblende and a zircon are illustrated in Fig. 2. The Bear Lake Rd. sphene and hornblende are of granitic origin and their patterns are similar to sphene and hornblende patterns obtained by others. For example, both the hornblende and zircon patterns are very similar to those observed by Gromet and Silver in hornblende and zircon samples from a Southern Californian granodiorite[6]. This gives the impression that each mineral displays a distinct REE pattern. However, comparison of the Bear Lake and Arendal sphene samples (Fig. 2*a*,*b*) demonstrates unequivocally that the REE patterns of minerals are determined as much by geological settings and processes as they are by mineral chemistry. This result is not surprising in that the geological history of the environment must influence both amounts and fractionation of constituents available to minerals during their formation.

The REE^{2+} spectra give strong confirming evidence that both REE^{+} and REE^{2+} peaks are indeed clean, and should provide important data for understanding ion yields and the fundamental processes of sputtering and ionization. We have observed the complete REE^{2+} spectrum for both the Arendal sphene and the monazite (Fig. 3). Peaks occur at integral and half mass units between $m/e = 69.5$ ($^{139}La^{2+}$) and $m/e = 87.5$ ($^{175}Lu^{2+}$). Isotope ratios of the 2^{+} ions are once again very close to natural abundances (Table 2), and are in very good agreement with the isotope ratios from 1^{+} ions. In both cases, there is some enhancement of the intensity of the lighter isotopes, as is observed for lighter singly charged ions. These data for the doubly charged ions are contrary to the observations of Shimizu and Hart[17], who suggested heavy isotope enrichment for doubly charged ions from pure silver.

The REE^{2+} pattern when normalized to chondritic abundances (Fig. 4), falls below the REE^{+} pattern for the heavier rare earths, in line with the increasing second ionization potentials for the heavier rare earths.

Reed[12] has shown that ion yields for the REE^{+} ions do not simply follow ionization potentials, despite the chemical similarity of these elements. This affects particularly the light REEs La–Eu, and Lu which has both the lowest first ionization potential and the lowest yield. This pattern is clearly visible when comparing REEs with chondritic abundances, where deviations from a smooth curve (apart from Eu anomalies) are noted for La, Ce, Pr and Lu (Figs 2*a*,*b* and 4).

All our results suggest that rapid and reliable REE patterns of minerals can be obtained directly from SIMS results without evaluation of the absolute concentrations of these elements. Reed[12,13] has shown variations in relative ion yields of the REEs but with suitable standards and/or better understanding of ion yields in specimen isolated conditions, quantitative analysis could be achieved. The method is not competitive with neutron activation in sensitivity but for REE levels above 5–10 p.p.m. it offers significant advantages in speed, convenience and perhaps even in accuracy. The absence of background in the mass spectrum combined with improving stability of magnetic analysers mean count times and thus sensitivity can be increased significantly over the values reported here.

We thank Dr N. S. McIntyre for helpful discussion and D. Johnston and W. J. Chauvin of Surface Science Western for experimental assistance. This work was funded in part by Atomic Energy of Canada Ltd in support of the Canadian Nuclear Fuel Waste Management Program.

Received 2 August; accepted 5 October 1983.

1. Metson, J. B., Bancroft, G. M., McIntyre, N. S. & Chauvin, W. J. *Surface Interface Analys.* **5**, 181–185 (1983).
2. Frey, F. A. Bryan, W. B. & Thompson, G. *J. geophys. Res.* **79**, 5507–5527 (1974).
3. Condie, K. C., Allen, P. & Narayana, B. L. *Contr. Miner. Petrol.* **81**, 157–167 (1982).
4. Taylor, S. R. & McLennan, S. M. *Phil. Trans. R. Soc.* A**301**, 381–399 (1981).
5. McLennan, S. M. & Taylor, S. R. *J. Geol.* **90**, 347–361 (1982).
6. Gromet, L. P. & Silver, L. T. *Geochim. cosmochim. Acta* **47**, 925–940 (1983).
7. Lovering, J. F. *Natn. Bur. Stand. Spec. Publ.* **427**, 135–178 (1975).
8. Shimizu, N. & Hart, S. R. *A. Rev. Earth planet. Sci.* **10**, 483–526 (1982).
9. Steele, I. M., Herviq, R. L., Hutcheon, I. D. & Smith, J. V. *Am. Miner.* **66**, 526–546 (1981).
10. Shimizu, N., Semet, M. P. & Allégre, C. J. *Geochim. cosmochim. Acta* **42**, 1321–1334 (1978).
11. Shimizu, N. *Nature* **289**, 575–577 (1981).
12. Reed, S. J. B. *16th A. Meet. of the Microbeam Analysis Soc.*, Colorado (1980).
13. Reed, S. J. B. *Int. J. Mass Spectrom. Ion Phys.* (in the press).
14. Hayward, P. J. & Cecchetto, E. V. *Scientific Basis for Nuclear Watse Management* (ed. Topp, S.V.) (North Holland, Amsterdam, 1982).
15. Nesbitt, H. W., Bancroft, G. M., Karkhanis, S. N. & Fyfe, W. S. *Scientific Basis for Nuclear Waste Management* Vol. 3 (ed. Moore, J. G.) 131–138 (Plenum, New York, 1981).
16. Weast, R. G. (ed.) *Handbook of Chemistry and Physics* 58th edn (CRC Press, 1977).
17. Shimizu, N. & Hart, S. R. *J. appl. Phys.* **53**, 1303–1311 (1982).
18. Wedopohl, K. H. (ed.) *Handbook of Geochemistry* Vol II/5 (Springer, Berlin, 1970).

Evolution of the geomagnetic secular variation field from the beginning of the century

C. Gire, J. L. Le Mouel & J. Ducruix

Institut de Physique du Globe de Paris, Universités de Paris 6 et 7, 4 place Jussieu, 75230 Paris Cedex 05, France

Here we have monitored the secular variation (s.v.) of the geomagnetic field since the beginning of the century using observatory data. The energy of the s.v. field and the westward drift rate decreased from 1912–13 to 1969. Since 1969 these two parameters have again been increasing. The occurrence of jerks (steps in the second time derivative of the field) has been discovered at these two epochs (1912 and 1969). Moreover, after 1969, the s.v. field tends to have the same geographical distribution it had around 1912.

Several years ago it was shown that in 1969 the secular variation (s.v.) $\dot{\mathbf{B}}(P, t)$ of the geomagnetic field $\mathbf{B}(P, t)$ (P is the current point at the Earth's surface, t is time) exhibited a sudden

0028-0836/84/040349—04$01.00

© 1984 Macmillan Journals Ltd

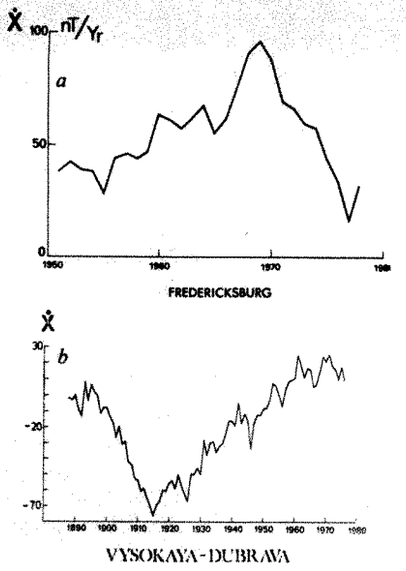


Fig. 1 *a*, The 1969 jerk as seen in the X component at the Fredericksburg observatory. *b*, The 1912 jerk as seen in the X component at the Vysokaya Dubrava observatory. The 1969 jerk at this location is less intense and not as clearly visible as in *a*.

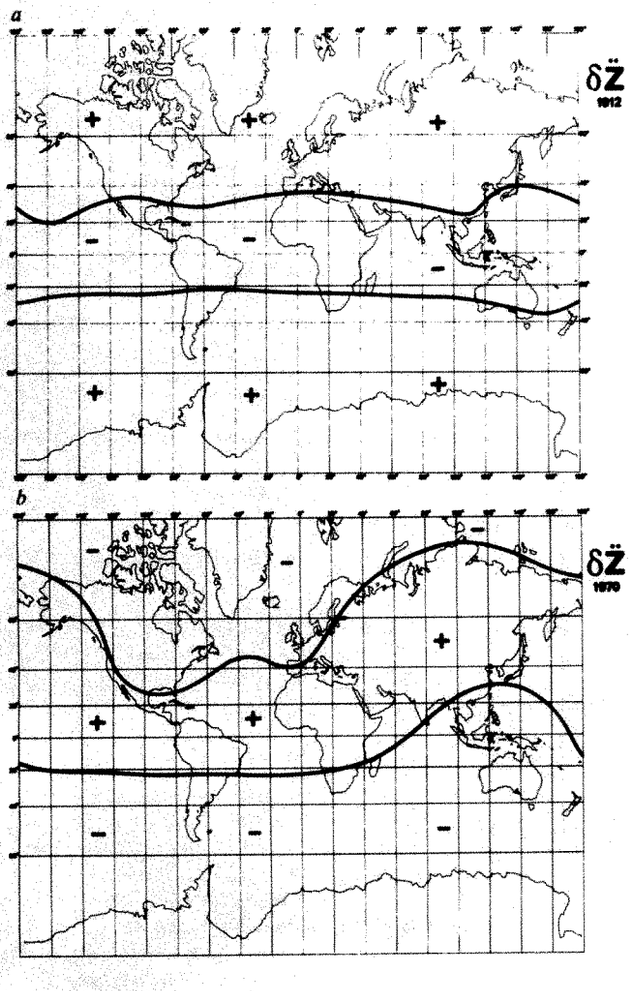


Fig. 2 Comparison between the geographic distributions of the jerks of 1912 and of 1969. The quantity $\delta\ddot{Z}$ is the amplitude of the discontinuity of the second time derivative. Only the zero isopleths are shown.

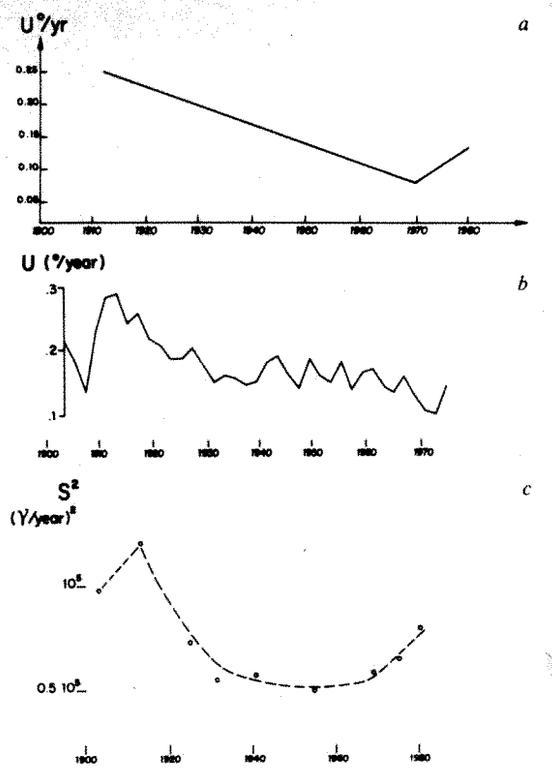


Fig. 3 *a*, Variation with time of the westward drift rate u computed from our simplified model of the geomagnetic secular variation. *b*, Variation with time of the westward drift rate computed using Hodder's method. *c*, Variation with time of the norm of the secular variation as given by spherical harmonic expansions.

change in slope—a step in the second time derivative of the field—of a clearly worldwide character[1–4]. Figure 1*a* illustrates this event, called 'jerk' in English and 'secousse' in French.

More recently Ducruix *et al.*[5] showed that a similar event occurred in 1912–13 (Fig. 1*b*). This event is, of course, less well documented than the 1969 one: only 50 observatories were operating at this time, among which only 10 were in the Southern Hemisphere. Nevertheless, Ducruix *et al.* were able to show that the 1912–13 jerk and the 1969 one have approximately the same geographical distribution, the signs of the second time derivative $\ddot{\mathbf{B}}(P, t)$ jump being reversed. Figure 2 illustrates these geographical distributions.

Ducruix *et al.*, from a close examination of all available secular variation curves, conclude that there is no other similar event over the 1912–80 time interval. Moreover, over this time span, the s.v. field appears to have a rather simple temporal variation: most of the corresponding curves are close to two straight lines, with a discontinuity in slope in 1969. Then, as a first approximation we may write:

$$\begin{aligned} \dot{\mathbf{B}}(P,t) &= \dot{\mathbf{B}}(P,1912) + \vec{\mathbf{b}}(P)\ (t-1912) \qquad 1912 < t < 1969 \\ \dot{\mathbf{B}}(P,t) &= \dot{\mathbf{B}}(P,1969) + \vec{\mathbf{b}}'(P)\ (t-1969) \qquad 1969 < t \end{aligned} \tag{1}$$

In many places, bumps of secular variation, with time constants typically of the order of some tens of years, are superimposed on the schematic s.v. field (1). They tend to obscure the simple broken line pattern which, nevertheless, represents the largest part of the observed s.v. field over the time span considered when studied over the whole Earth's surface.

Let us now choose a single global parameter characteristic of the s.v. field. It is natural to think first of the apparent westward drift (w.d.) rate u. We have computed, from 1912 to 1980, the w.d. rate corresponding to the simplified s.v. field (1) by minimizing quantities such as:

$$\delta^2 = \int \left\{ \left(\dot{\mathbf{B}}(P,t) - u(t) \frac{\partial \vec{\mathbf{B}}}{\partial \phi}(P,t) \right) \right\}^2 \mathrm{d}S \tag{2}$$

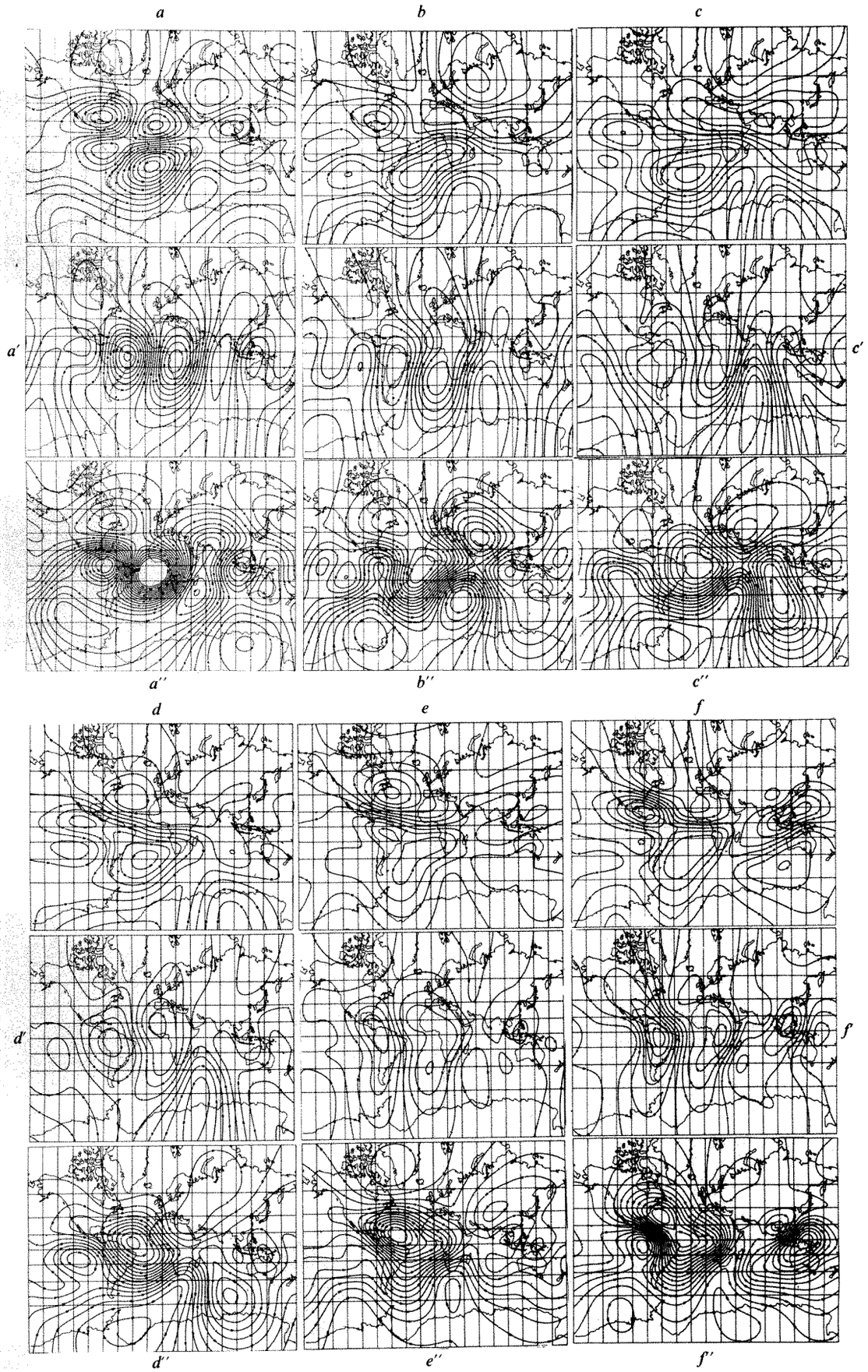


Fig. 4 Evolution with time of the maps of the secular variation for epochs 1913, 1925, 1941, 1955, 1969, 1980 as computed by Hodder. The first row displays the secular variation of X (a–f), the second row s.v. of Y (a'–f'), the third row s.v. of Z (a''–f''). The contour interval is 20 nT $(\text{yr})^{-1}$.

The results are illustrated in Fig. 3*a*. It has previously been shown that the problem of determining u by minimizing quantities such as equation (2) is an ill-posed problem and gives somewhat different values when different techniques and different components of $\vec{\mathbf{B}}$ are used. However, we are only interested here in the trend of the global parameter u from 1912 to 1980—a decrease from 1912 to 1969, then an increase. The 1912–69 decrease also appears in Hodder's estimates[8] derived from raw observatory data (Fig. 3*b*).

Another global parameter is simply the norm $S^2 = \sum_{n,m} (\dot{g}_n^{m^2} + \dot{h}_n^{m^2})(n+1)$ of the s.v. field $\dot{\vec{\mathbf{B}}}$. [$(\dot{g}_n^m, \dot{h}_n^m)$ are the coefficients of the spherical harmonic expansion of $\dot{\vec{\mathbf{B}}}$ with Schmidt's normalization]. Figure 3*c* represents the variation of S^2 with time using s.v. coefficients given by Hodder[9] which rely on observatory data, except in 1980 where the GSFC 1980 s.v. coefficents are used. It appears that the energy decreases steadily from 1912 to 1945, is then nearly constant from 1945 to 1969 (as shown by Fig. 1 of Hodder's paper[9], the lack of homogeneity of the different sets of coefficients can to some extent bias the curve $S^2(t)$, but the general trend can be trusted) and finally increases again after 1969. This confirms the importance and worldwide character of the 1912–13 and 1969 events.

Let us now look at the values of the s.v. field as illustrated by isovalue lines of components $\dot{X}$ (north), $\dot{Y}$ (east) and $\dot{Z}$ (vertical) of $\dot{\vec{\mathbf{B}}}$ for different epochs from 1912 to 1980. The maps in Fig. 4 have been computed, again using s.v. coefficients given by Hodder as above (except for 1980). Despite the uncertainty in the contours of these charts, it is clear that, from the 1912 high of u (or of S^2) to the 1969 low (Fig. 3*a*, *b*), the shape of the s.v. field is evolving monotically; in particular, the isovalue lines get looser and looser, and the s.v. field becomes 'exhausted'. Then the 1969 jerk occurs and the s.v. field increases again and tends to recover the strength it had in 1912. Furthermore, the s.v. field tends to recover, after 1969, the value it had around 1913, at the time of the high of u: the broad lines of maps *a*, *a'*, *a''* and maps *f*, *f'*, *f''* of Fig. 4 indeed present striking similarities (the three components must be considered together) except over the Far-East. Part of the discrepancies could be due to the poor control of 1913 contour lines over these regions.

Unfortunately, these observations are limited to a short time interval. It is, of course, desirable to go back further into the past, but field analysis then relies increasingly heavily on European observatories (19 observatories were operating in 1900, 12 of which were in European countries).

Theoretical studies of the hydrodynamics of the outer core should now account for the occurrence of the 1912–13 and 1969 jerks and for the dramatic change of the secular variation from 1969 to 1980.

Received 19 September; accepted 6 October 1983.

1. Courtillot, V., Ducruix, J. & le Mouel, J. L. *C.r. hebd. Séanc. Acad. Sci., Paris* **D287,** 1095–1098 (1978); **B289,** 173–175 (1979).
2. Ducruix, J., Courtillot, V. & le Mouel, J. L. *Geophys. J. R. astr. Soc.* **61,** 73–94 (1980).
3. Malin, S. R. C. & Hodder, B. M. *Nature* **296,** 726–728 (1982).
4. Backus, G. E. *Geophys. J. R. astr. Soc* **74,** 713–746 (1983).
5. Ducruix, J., Gire, C. & le Mouel, J. L. *C.r. hebd. Séanc. Acad. Sci., Paris* **296** Ser. II, 1419–1424 (1983).
6. Le Mouel, J. L. & Courtillot, V. *J. geophys. Res.* **87,** 4103–4108 (1982).
7. Gire, C., le Mouel, J. L. & Madden, T. *A. Geophys.* (submitted).
8. Hodder, B. M. *Nature* **306,** 136–137 (1983).
9. Hodder, B. M. *Geophys. J. R. astr. Soc.* **65,** 763–776 (1981).

Oldest reliable $^{40}Ar/^{39}Ar$ ages for terrestrial rocks: Barberton Mountain komatiites

M. Lopez Martinez*, D. York*, C. M. Hall* & J. A. Hanes†

* Department of Physics, University of Toronto, Toronto, Ontario, Canada M5S 1A7
† Department of Geological Sciences, Queen's University, Kingston, Ontario, Canada K7L 3N6

We report here the first $^{40}Ar/^{39}Ar$ ages for komatiites and komatiitic basalts from the Barberton Mountain Greenstone Belt on the Transvaal–Swaziland border in southern Africa[1]. Both rock types display remarkable argon retentivity. While some variation is found among the samples, the best argon age estimate for the time of metamorphism is in the 3,450–3,490 Myr range. This age is only slightly less than that found in komatiites from the same area by Sm–Nd dating (3,540 ± 15 Myr)[2]. Within the uncertainties of the decay constants involved, the two age estimates are not different. The results show that the principal pervasive greenschist metamorphism[1] in the area must have occurred within 100 Myr of the eruption of the komatiite. Analytically precise ages were obtained despite potassium contents as low as 80 p.p.m. in the presence of high calcium concentrations (3–5% Ca). To our knowledge, the results represent by far the oldest reliable ages obtained for terrestrial rocks using the K–Ar system.

Komatiites have attracted widespread interest among earth scientists[3] because of their unusual geochemistry and mineralogy, and because they are predominantly confined to the Archaean. The Barberton Greenstone Belt contains, in the Onverwacht Group at the base of the Swaziland Supergroup, one of the earliest and best preserved komatiitic sequences. While the time of eruption of these lavas is usually considered to have been established by Sm–Nd dating[2] (Rb–Sr[4] and U–Pb[5] dates were much less precise), the subsequent tectonothermal history has been uncertain. To shed light on the situation, we have carried out a detailed $^{40}Ar/^{39}Ar$ age investigation on ultramafic and mafic lavas from this area.

Table 1 Plateau and integrated ages of komatiite samples

Sample	Integrated age (Myr)	Plateau age (Myr)	% ^{39}Ar release*
Komatiites			
B40A	3,367 ± 8	3,463 ± 7	72
B40A	3,336 ± 4	3,486 ± 3	59
B40A	3,368 ± 15	3,425 ± 17	54
B40A	3,346 ± 11	3,429 ± 10	63
B40A	2,961 ± 14	3,327 ± 9	75
B40A	3,539 ± 55	3,406 ± 37	92
B41B	3,312 ± 25	3,407 ± 17	63
B40E	2,986 ± 6	3,318 ± 7	44
B40E	3,060 ± 5	3,420 ± 6	33
KT7G	2,905 ± 8	3,225 ± 8	29
KT7G	2,962 ± 7	3,298 ± 10	30
KT16A	2,816 ± 3		
KT16A	2,814 ± 2		
Komatiitic basalts			
KT17B	3,778 ± 2	3,428 ± 2	64
KT17B	3,777 ± 2	3,447 ± 2	54
KT17A	3,053 ± 3	3,111 ± 3	53

Decay constants from ref. 14 were used. Errors are $\pm 1\sigma$.
* Per cent of ^{39}Ar release involved in plateau age calculation.

We made 13 step-heating analyses of five different komatiite specimens, and three similar analyses of two different komatiitic basalts, all from the type area[1] of the Komati Formation of the Onverwacht Group. Whole-rock samples, of ~250 mg mass, were irradiated for ~150 h in the McMaster University research reactor. Samples of hornblende 3 gr[6] were irradiated as standards alongside the unknowns and were fused with the Toronto Laser Probe[7] in one-step runs. Irradiated komatiites and komatiitic basalts were step-heated to 1,200 °C using the detailed temperature control vacuum system described by Berger and York[8]. Residues were then fused in a radiofrequency

0028–0836/84/040352—03$01.00
© 1984 Macmillan Journals Ltd

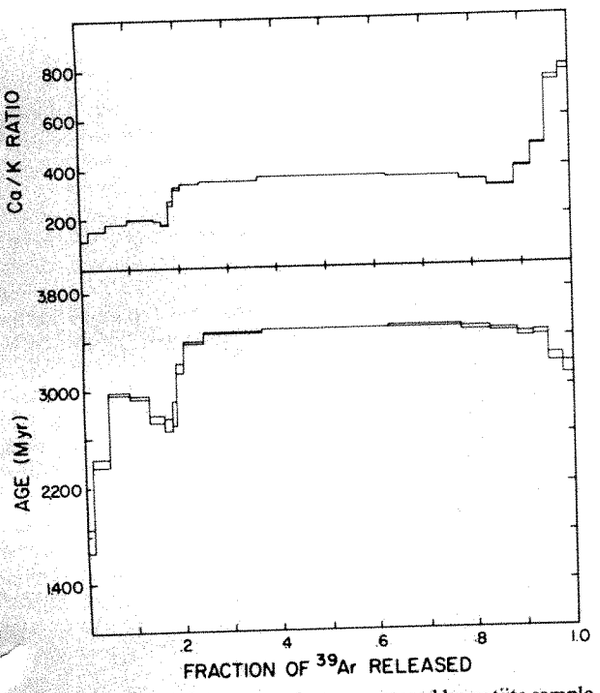


Fig. 1 Age and Ca/K spectra of vacuum-stored komatiite sample B40A. The sizes of the boxes represent 1σ errors, where they are large enough to be shown.

system. Argon isotope ratios in the purified gases were measured with an MS10 mass spectrometer. During the initial analyses it was discovered[9] that the komatiite samples tended to absorb modern atmospheric argon in large quantities after irradiation. A dramatic reduction of this effect was achieved in the later runs by storing the komatiites under vacuum after irradiation until they were required for analysis. These later runs were therefore of higher precision.

The plateau and integrated ages (integrated age = age calculated from the ratio of the total radiogenic ^{40}Ar in the sample to the total potassium-derived ^{39}Ar) are presented in Table 1. Figure 1 shows a typical age spectrum for the komatiites, and the results of duplicate analyses of a komatiitic basalt are shown in Fig. 2. Detailed analytical data will be presented elsewhere.

From Table 1 it can be seen that the komatiites have lost some argon, as the integrated ages are (with one exception out of 13) significantly less than the Sm–Nd age of 3,540 ± 15 Myr. Despite this, plateau ages approaching 3,500 Myr are obtained by the step-heating approach.

The first sample examined, B40A, has been studied in the greatest detail. The fifth and sixth runs on this sample in Table 1 were our earliest[9] and are not of as high a quality as the top four runs listed, which were performed after techniques appropriate to komatiites had been devised. These four superior analyses all yielded integrated ages between 3,300 and 3,400 Myr. It is interesting that two of these four samples, having been vacuum-stored after irradiation, suffered accordingly much lower modern atmospheric argon contamination and yielded the highest plateau ages: 3,463 ± 7 and 3,486 ± 3 Myr. The latter age corresponds to the plateau in the age spectrum illustrated in Fig. 1. In this analysis we extracted gas in several small steps at both ends of the obvious age plateau in order to minimize mixing of adjacent off-plateau gas of low age with the true plateau. Therefore, it may be significant that this extremely precise plateau age (the error in the plateau age = 3 Myr (1σ)) is also the oldest we have yet found.

The shape of the age spectrum in Fig. 1 is characteristic of all those found in the komatiite specimens, except for KT16A, which will be referred to below. This characteristic age spectrum contains three totally distinct portions, with three correspondingly distinct segments in the Ca–K spectrum (shown in the top part of Fig. 1). The low temperature release (600–800 °C)

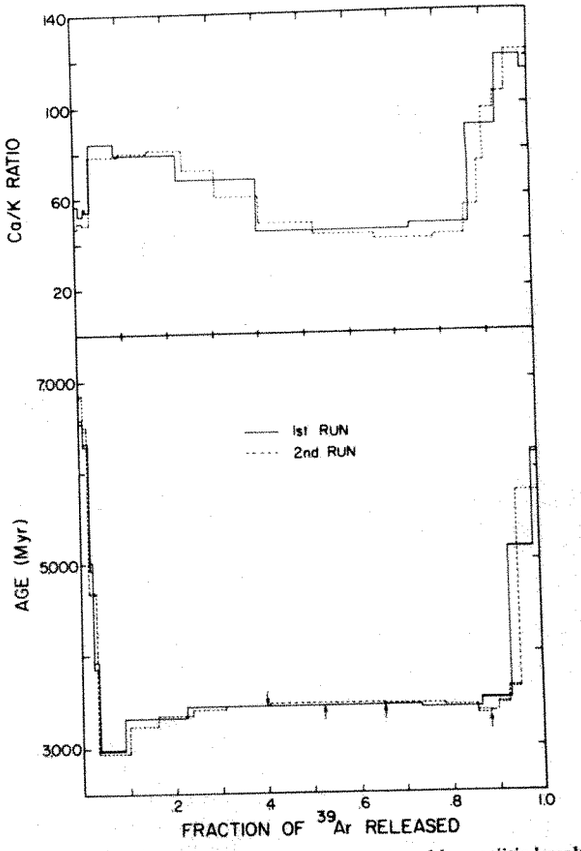


Fig. 2 Age and Ca/K spectra of vacuum-stored komatiitic basalt sample KT17B. Where they are large enough to be shown, the 1σ errors are represented by the sizes of the boxes. Arrows indicate divisions between two fractions of equal age. Note that the Ca/K values and the shape of the Ca/K spectrum are very different from those found in the komatiite samples (see Fig. 1).

displays relatively low ages and Ca/K ratios of ~200. At intermediate temperatures (925–1,035 °C) the typical age plateau appears, together with a new Ca/K regime having a value in the range 350–500 depending on the sample. At the highest temperatures (≥1,100 °C) there is a slight but experimentally reproducible dip in the age along with a large increase in Ca/K ratios. Petrographically, the komatiites retain some primary textures, but consist almost entirely of metamorphic assemblages: serpentine–tremolite–chlorite–magnetite and very minor ferristilpnomelane. Electron microprobe X-ray scans and quantitative analyses show that the only mineral present in sufficient quantity in the komatiite, with the required Ca/K ratio to account for the plateau segment in the age spectrum, is tremolite. The younger ages in the low temperature release range are correlated with the presence of stilpnomelane.

Komatiite specimen B41B also has an integrated age of over 3,300 Myr and displays a plateau age of 3,407 ± 17 Myr, the slightly larger error here being due to the fact that this sample has the lowest potassium content (80 p.p.m.) of all the komatiites examined.

The remaining three komatiites (B40E, KT7G and KT16A) all exhibit greater argon losses from their whole-rock systems, having integrated ages in the 3,060–2,814 Myr range. The lowest such value (2,814 Myr) was obtained for KT16A which, presumably because of its severe disturbance, also displays no plateau in its age spectrum. In contrast, samples B40E and KT7G do show age plateaus, in the 3,420–3,225 Myr range, despite large total argon losses.

The two komatiitic basalts analysed behave completely differently from the komatiite samples and from each other. KT17A shows severe argon loss (integrated age = 3,053 ± 3 Myr) and a ragged age spectrum with a crude plateau at 3,100 Myr. In complete contrast with this, and with all the komatiites so far

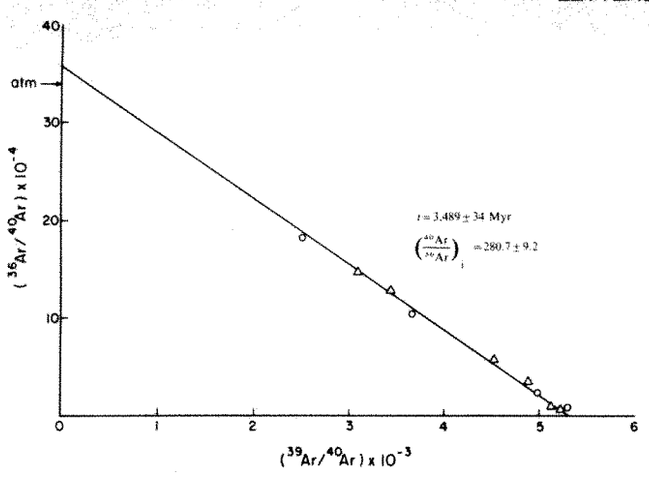


Fig. 3 Isotope correlation plot for the data from the plateaux of the high-precision vacuum-stored analyses of B40A. Except for two points where the errors are approximately the same size as the symbols, the errors in the data are less than the dimensions of the symbols. ○, First run; △, second run.

analysed, komatiitic basalt KT17B contains excess radiogenic argon. Thus, duplicate step-heating analyses gave integrated ages of 3,777 ± 2 and 3,778 ± 2 Myr, ~240 Myr older than the Sm–Nd age. However, as may be seen in Fig. 2, the step-heating experiments are remarkably successful in removing the effect of the excess argon. The well known saddle-shaped spectrum frequently found in samples containing excess argon[10] is observed. A very broad well-defined plateau is revealed in the middle temperature (900–975 °C) fractions, yielding plateau ages of 3,428 ± 2 and 3,447 ± 2 Myr for the duplicates. The latter value was obtained in an experiment in which we took extra, small steps at both ends of the age plateau to reduce the effect of mixing off-plateau gas with the plateau fractions. As with B40A (referred to above) this did seem to produce a slightly higher plateau age.

The komatiitic basalts are comprised of actinolite with rims and patchy intergrowths, crystallographically controlled, of ferro-hornblende, epidote-choked albite, chlorite and altered titanomagnetite with associated minor ferristilpnomelane. Electron microprobe analyses, coupled with the Ca/K spectra from the age runs, show that the age plateaux are dominated by argon release from hornblende, while disturbed portions of the spectra appear related to stilpnomelane. As far as we know, this is the first report of stilpnomelane in either Barberton komatiites or komatiitic basalts, and indicates a condition of low oxygen fugacity during the main metamorphism, with the stilpnomelane having originally formed as ferrostilpnomelane[11]. The later oxidation to ferristilpnomelane may be reflected in the younger ages in the low temperature release range of the komatiites.

With the exception of komatiite sample KT16A which retained some primary olivine, all the samples consist of metamorphic mineral assemblages. We conclude, therefore, that the tremolite in the komatiites and the hornblende in the komatiitic basalt (KT17B) both record the time of the metamorphism of the Komati Formation. From the superior analyses of B40A and KT17B we place this metamorphism somewhere in the 3,450–3,490 Myr age range (within experimental errors). Evidently this principal, pervasive metamorphism[1] occurred within less than 100 Myr of eruption (possibly almost immediately after, when uncertainties in the ^{40}K and ^{147}Sm half lives are considered) of the komatiites. Chauvel *et al.*[12] have recently suggested that in certain circumstances Sm–Nd dates on komatiitic rocks may be anomalously old. Our data indicate that this is not the case here.

All our measurements were corrected for instrumental argon blanks. Any ^{36}Ar remaining after that was used to make a modern atmospheric argon correction. *A priori*, it was, of course, not obvious to us that this would be the correct procedure. Conceivably, the samples may have contained initial argon having a $^{40}Ar/^{36}Ar$ ratio totally different from that found in today's atmosphere. That no significant error has been introduced, however, is apparent from the consistency of the data in the presence of a wide range of ^{36}Ar-based corrections. To test this point further and indeed to look for primordial argon, we examined the distribution of the data from the plateaux of the high-precision vacuum-stored B40A runs (the top two analyses in Table 1) on a graph of $^{36}Ar/^{40}Ar$ versus $^{39}Ar/^{40}Ar$ (Fig. 3). The best straight line[13] allowing for all reasonable sources of correlation in the errors, gives an age of 3,489 ± 34 Myr. While the data are highly linearly correlated, they are more scattered about the best line than would be expected on the basis of experimental error alone. The intercept on the $^{36}Ar/^{40}Ar$ axis indicates initial argon in the samples with a $^{40}Ar/^{36}Ar$ ratio of 280.7 ± 9.2 (1σ, where the error allows for the scatter of the samples about the line). While this is lower than the modern atmospheric value of 295.5, the difference is not statistically significant. Only more detailed studies will show whether this intercept indicates some sort of primordial argon or whether it represents a mixture of initial argon heavily contaminated with modern atmospheric argon, despite our attempt to eliminate this by post-irradiation vacuum storage. Until such measurements have been performed, the data do not allow us to favour any particular one of the theories of mantle–atmosphere evolution.

Finally, as mafic and ultramafic rocks (both komatiitic and tholeiitic) constitute volumetrically the bulk of Archaean greenstone belts, our demonstration here of the precise dating of the greenschist metamorphism of individual komatiitic lavas indicates the potential value of the $^{40}Ar/^{39}Ar$ dating method in elucidating the tectonothermal history of these terrains.

We thank Maarten J. de Wit and Chris J. Hale for supplying the samples, and W. John Kenyon and Peter Kuybida for technical assistance. This research was funded by grants to D.Y. and J.A.H. from the Natural Science and Engineering Research Council of Canada, and by a grant to J.A.H. from the Queen's University Advisory Research Committee. M.L.M. was supported by the Consejo Nacional de Ciencia y Tecnologia (Mexico).

Received 28 July; accepted 26 October 1983.

1. Viljoen, M. J. & Viljoen, R. P. *Geol. Soc. S. Afr. spec. Publ.* **2**, 9–28, 55–85 (1969).
2. Hamilton, P. J., Evensen, N. M., O'Nions, R. K., Smith, H. S. & Erlank, A. J. *Nature* **279**, 298–300 (1979).
3. Arndt, N. T. & Nisbet, E. G. *Komatiites*, 526 (Allen & Unwin, London, 1982).
4. Jahn, B. M. & Shih, C. Y. *Geochim. cosmochim. Acta* **38**, 873–885 (1974).
5. Van Niekerk, C. B. & Burger, A. J. *Trans. geol. Soc. S. Afr.* **72**, 9–25 (1969).
6. Hall, C. M., Walter, R. C., Westgate, J. A. & York, D. *Nature* (in the press).
7. York, D., Hall, C. M., Yanase, Y., Hanes, J. A. & Kenyon, W. J. *Geophys. Res. Lett.* **8**, 1136–1138 (1981).
8. Berger, G. W. & York, D. *Geochim. cosmochim. Acta* **45**, 795–811 (1981).
9. York, D., Hall, C. M., Masliwec, A., Langridge, R. L. & Hale, C. J. *EOS* **62**, 429 (1981).
10. Lanphere, M. A. & Dalrymple, G. B. *Earth planet. Sci. Lett.* **32**, 141–148 (1976).
11. Brown, E. H. *Contr. Miner. Petrol.* **31**, 275–299 (1971).
12. Chauvel, C., Dupre, B., Todt, W., Arndt, N. T. & Hofmann, A. W. *EOS* **64**, 330 (1983).
13. York, D. *Earth planet. Sci. Lett.* **5**, 320–324 (1969).
14. Steiger, R. H. & Jäger, E. *Earth planet. Sci. Lett.* **36**, 359–362 (1977).

Dating diagenesis in a petroleum basin, a new fluid inclusion method

R. S. Haszeldine, I. M. Samson* & C. Cornford

Britoil PLC Stratigraphic Laboratory, 150 St Vincent Street, Glasgow G2 5LJ, UK
* Department of Applied Geology, University of Strathclyde, Glasgow, G1 1XJ, UK

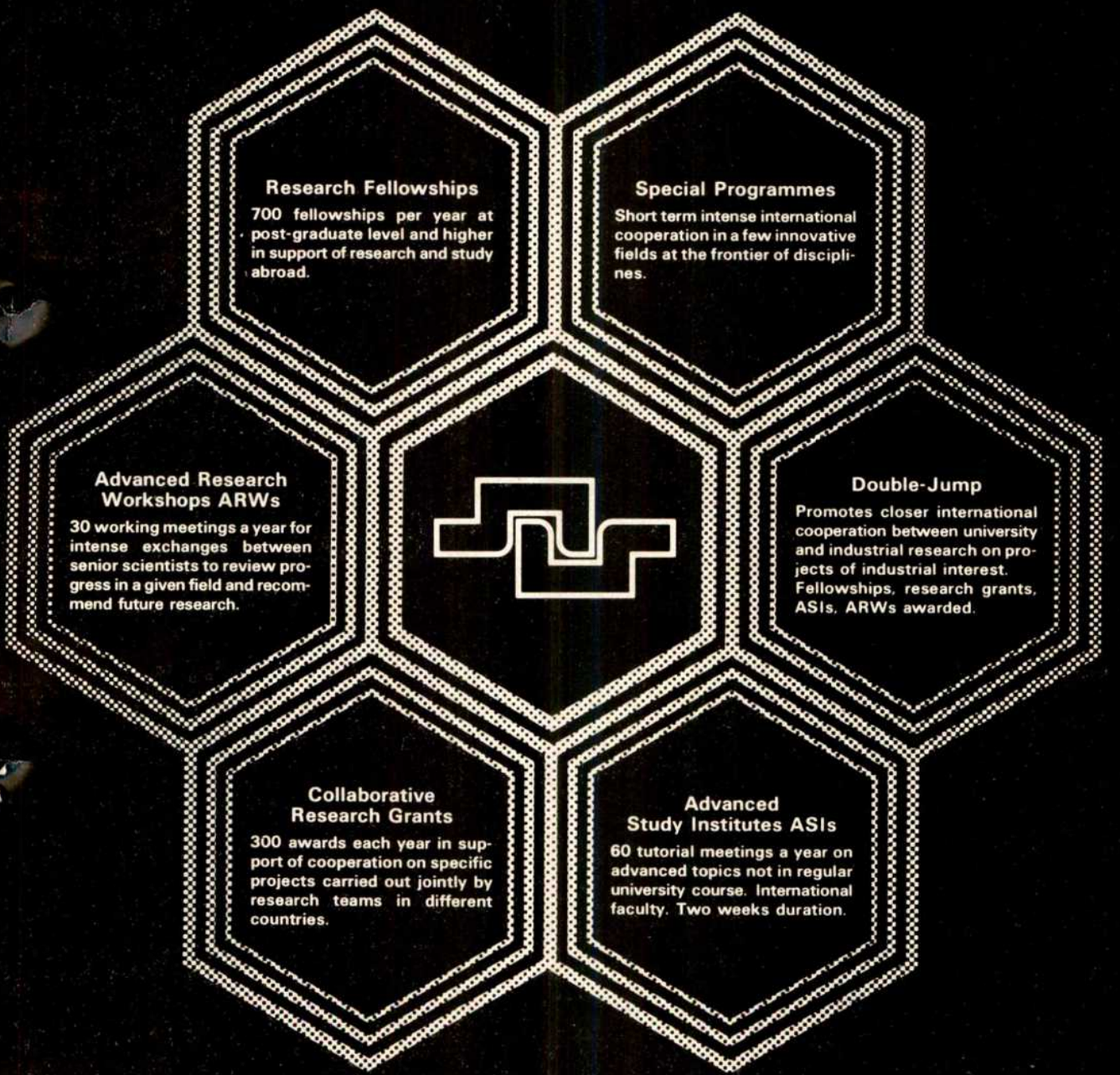
The final porosity and permeability of sandstone petroleum reservoirs is greatly affected by the diagenetic growth of minerals after deposition. For example a sand may be deposited with a porosity of 25% and a permeability of 5,000 mdarcy (mD)[1]; diagenetic growth of quartz around detrital sand grains may leave a rock with only 10% porosity, and later growth of

0028–0836/84/040354—04$01.00

© 1984 Macmillan

Many Activities : One Goal

the advancement of Science & Technology



NATO International Scientific Exchange Programmes

NATO Scientific Affairs Division, B-1110 Brussels, Belgium, Tel.(02) 241.44.00

1984

NATO Advanced Study Institutes

Cours d'été

ADVANCED STUDY INSTITUTES (ASIs) are tutorial courses of two weeks' duration on new, important topics for up to 1 scientists and research students. They aim at the dissemination of advanced scientific knowledge and promotion of internatio contacts among scientists. No tuition fees are requested from participants; some may obtain small grants through the meeti Director to assist with travel and living expenses.

Each meeting is held under the responsibility of its Director, to whom all requests for information, attendance or support should addressed. The meetings marked ● are of particular industrial interest. One meeting is a Training Course sponsored under the Spec Programme on Marine Sciences (MS). **Locations and dates may change.** Titles and addresses have been abridged. Many meetings are an interdisciplinary nature : please check all subject areas.

PUBLICATIONS :
Proceedings of meetings are published in the NATO ASI Series by : **Plenum - Reidel - Nijhoff - Springer Verlag.**

LIFE SCIENCES

MOLECULAR FORM & FUNCTION OF THE PLANT GENOME
Dr. L. VAN VLOTEN-DOTING, Dept. of Biochemistry, P.O. Box 9505, 2300 RA Leiden, The Netherlands
4-14 July 1984 : Renesse, The Netherlands 438/83

CELLULAR & MOLECULAR ASPECTS OF DEVELOPMENTAL BIOLOGY
Mr. R. STORA, Division Théorique, CERN, CH-1211 Geneva 23, Switzerland
1-27 July 1984 : Les Houches, France 444/83

NEW DEVELOPMENTS & METHODS IN MEMBRANE RESEARCH & BIOLOGICAL ENERGY TRANSDUCTION
Prof. L. PACKER, Membrane Bioenerg. Gp., Dept. Physiology/Anatomy, Univ. California, Berkeley, CA 94720, USA
16-29 August 1984 : Spetsai, Greece 449/83

GENOME ORGANIZATION & FUNCTION
Prof. H.G. ZACHAU, Inst. F. Physiol. Chem., Goethestr. 33, D-8000 München 2, Germany
30 August-12 September 1984 : Spetsai, Greece 693/83

CELL TRANSFORMATION
Dr. J.E. CELIS, Inst. of Chemist., University, Langelandsgade 140, DK-8000 Aarhus, Denmark
2-12 September 1984 : Alcabideche, Portugal 760/83

TOXICOLOGY OF THE NERVOUS SYSTEM
Prof. C.L. GALLI, Inst. Pharmacology & Pharmacognosy, Univ., Via Balzaretti 9, 20133 Milan, Italy
10-21 September 1984 : Maratea, Italy 794/83

RADIATION CARCINOGENESIS AND DNA ALTERATIONS
Prof. F.J. BURNS, Dept. Environ. Medicine, NY Univ. Medical Ctr., 550 First Ave., New York, NY 10016, USA
30 September-13 October 1984 : Greece 795/83

CELLULAR AND MOLECULAR CONTROL OF DIRECT CELL INTERACTIONS IN DEVELOPING SYSTEMS
Dr. H.J. MARTHY, Lab. Arago (LA 117 CNRS), F-66 650 Banyuls-sur-Mer, France
10-23 September 1984 : Banyuls-sur-Mer, France 831/83

DRUGS AFFECTING LEUKOTRIENES AND OTHER EICOSANOID PATHWAYS
Prof. F. BERTI, Dept. Pharmacology, Univ., Via Balzaretti 9, 20129 Milan, Italy
3-14 September 1984 : Erice, Sicily, Italy 858/83

SENSORY PERCEPTION AND TRANSDUCTION IN ANEURAL ORGANISMS
Dr. G. COLOMBETTI, CNR Istituto di Biofisica, Via San Lorenzo 26, I-56100 Pisa, Italy
3-14 September 1984 : San Miniato, Italy 911/83

BIOPOLYMERS ●
Dr. E. PIŞKIN, Hacettepe Univ., Chemical Engineering Dept., P.K. 716 Kizilay, Ankara, Turkey
27 August-5 September 1984 : Izmir-Cesme, Turkey 951/83

NMR IN LIVING SYSTEMS
Prof. T. AXENROD, Dept. Chemistry, City College of City Univ. of New York, New York, NY 10031, USA
26 August-7 September 1984 : Maratea, Italy 962/83

PRIMARY PHOTO-PROCESSES IN BIOLOGY AND MEDICINE
Prof. T.G. TRUSCOTT, Paisley College, Dept. Chemistry, Paisley, Renfrewshire, PA1 2BE, Scotland, UK
16-28 September 1984 : Bressanone, Italy 982

EXCITED STATE PROBES IN BIOLOGY AND MEDICINE
Dr. A.G. SZABO, Div. Biol. Sciences, Nat. Research Council, 100 Sussex Drive, Ottawa, Ontario K1A OR6, C
17-28 September 1984 : Acireale, Catania, Italy 98

CHROMOSOMAL PROTEINS AND GENE EXPRESSION
Prof. G.R. REECK, Dept. Biochemistry, Kansas State University, Manhattan, KS 66506, USA
10-19 September 1984 : Sitges, Spain 99

PICTORIAL INFORMATION SYSTEMS IN MEDICINE
Prof. K-H. HÖHNE, Inst. Math. & Computer Sc. in Medicine, Univ., Martinistrasse 52, 2000 Hamburg 20,
27 August-7 September 1984 : Braunlage, Germany 99

ECOLOGY

EPIDEMIOLOGY & QUANTITATION OF ENVIRONMENTAL RISK IN HUMANS FROM RADIATI OTHER AGENTS
Prof. A. CASTELLANI, ENEA, Dept. RAD-PAS, Nuclear Stdy Ctr, POB 2400 AD, Rome, Italy
1-10 September 1984 : San Miniato, Italy 441

STRATEGIES AND ADVANCED TECHNIQUES FOR MARINE POLLUTION STUDIES
Dr. H.J.M. DOU, Labo. Chimique Organique A, Centre Scientif. St.-Jérome, F-13397 Marseille Cedex, I
19-29 October 1984 : France 71

SOCIAL, BEHAVIOURAL AND POLITICAL SCIENCES

ADVANCES IN MEASURING COGNITION AND MOTIVATION
Prof. S.H. IRVINE, Dep. of Psychology, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA, UK
December 1984 : Nice, France 96

VISION AND IMAGE UNDERSTANDING
Dr. A. BORSELLINO, Istituto di Scienze Fisiche dell'Universita, Viale Benedetto XV, 5, 16132 Genova
1-12 July 1984 : Erice, Italy 96

PHYSICS

SOLID-STATE BATTERIES ●
Prof. C.A.C. SEQUEIRA, Lab. de Electroquimica, Inst. Sup. Technico, Av. Rov. Pais, 1096 Lisboa Codex, Po
2-14 September 1984 : Alcabideche, Portugal 22

NATURAL CONVECTION – FUNDAMENTALS & APPLICATIONS ●
Prof. S. KAKAC, Dept. of Mechanical Engineering, University of Miami, Coral Gables, FL 33124, USA
16-27 July 1984 : Izmir-Cesme, Turkey 43

ITICAL PHENOMENA – RANDOM SYSTEMS – GAUGE THEORIES
r. R. STORA, Division Théorique, CERN, CH-1211 Geneva 23, Switzerland
August - 7 September 1984 : Les Houches, France 458/83

ON-EQUILIBRIUM PHONON DYNAMICS
of. W.E. BRON, Depart. of Physics, Indiana University, Swain Hall-West 117, Bloomington, IN 47405, USA
August - 7 September 1984 : Les Arcs, France 682/83

UNDAMENTAL FORCES
R.M. TURNBULL, Dept. of Natural Philosophy, The University, Glasgow, G12 8QQ, UK
August - 1 September 1984 : St. Andrews, UK 683/83

EAVY ION COLLISIONS
of. M. LEVY, Lab. Phys. Théor. & Htes Energ., Tour 16, 4 Pl. Jussieu, F-75230 Paris Cedex 05, France
15 September 1984 : Cargèse, Corsica, France 689/83

ECHNIQUES & CONCEPTS OF HIGH ENERGY PHYSICS
of. T. FERBEL, Dept. of Physics & Astronomy, Univ. Rochester, Rochester N.Y. 14627, USA
13 August 1984 : St. Croix, Virgin Islands, USA 690/83

NDAMENTAL PROCESSES IN ENERGETIC ATOMIC COLLISIONS
of. H. KLEINPOPPEN, Atomic Physics Lab., Univ. Stirling, Stirling, FK9 4LA, UK
-21 September 1984 : Alcabideche, Portugal 755/83

CROSCOPIC THEORY OF STRUCTURAL PROPERTIES AND LATTICE DYNAMICS OF SOLIDS
of. J.T. DEVREESE, D. Natuurkunde, Univ. Instelling Antwerpen, Universiteitsplein 1, B-2610 Wilrijk, Belgium
-29 July 1984 : Antwerp, Belgium 749/83

YSICS OF NEW LASER SOURCES ●
F.T. ARECCHI, Istituto Nazionel di Ottica, Largo e Fermi, 6, 50125 Firenze, Italy
-21 July 1984 : San Miniato, Italy 750/83

YSICS OF PLASMA-WALL INTERACTION PROCESSES IN CONTROLLED FUSION RESEARCH
of. DE POST, Jr., Plasma Physics Lab., Princeton University, PO Box 451, Princeton, NJ 08544, USA
-31 August 1984 : St. Jovite, Canada 830/83

N-EQUILIBRIUM QUANTUM STATISTICAL PHYSICS
of. M.O. SCULLY, Dept. of Physics & Astronomy, Univ. of New Mexico, Albuquerque, NM 87131, USA
16 June 1984 : Santa Fe, New Mexico, USA 952/83

PERSYMMETRY
f. K. DIETZ, Physikalisches Inst., Nussallee 12, D-5300 Bonn 1, Germany
-31 August 1984 : Germany 983/83

PROVED METHODS FOR EXAMINING THE SUBMICRON WORLD
f. J.Wm. MCGOWAN, Nat. Museum of Sc. & T., 1867 St. Laurent Blvd., Ottawa, Ontario K1A 0M8, Canada
y 1984 : Baddeck, Nova Scotia, Canada 991/83

HEMISTRY, GEOPHYSICS AND ASTROPHYSICS

NDAMENTALS & DEVELOPMENTS OF PHOTOCATALYTIC & OTOELECTROCHEMICAL PROCESSES
f. M. SCHIAVELLO, Ist. Ingegneria Chim., Univ. di Palermo, Viale dell Scienze, 90128 Palermo, Italy
May - 3 June 1984 : Palermo, Italy 81/83

ABILITY OF THE SOLAR SYSTEM & ITS MINOR NATURAL & ARTIFICIAL BODIES
f. V. SZEBEHELY, Univ. of Texas, Dept. of Aerospace Engineering, Austin, TX 78712-1085, USA
17 August 1984 : Cortina d'Ampezzo, Italy 680/83

NDAMENTAL & TECHNOLOGICAL ASPECTS OF ORGANO-F-ELEMENT CHEMISTRY ●
f. T.J. MARKS, Dept. of Chemistry, Northwestern Univ., Evanston, IL 60201, USA
-21 September 1984 : Maratea, Italy 694/83

LYMER BLENDS AND MIXTURES ●
D.J. WALSH, Dept. Chemical Eng. & Chem. Techn., Imperial College, Prince Consort Road, London SW7, UK
July 1984 : London, UK 686/83

ING PROVENANCE FROM ARENITES
G.G. ZUFFA, Dip. Scienze della Terra, Univ. Calabria, 87030 Castiglione Cosentino, Stazione Cosenza, Italy
0 June 1984 : Rende (Cosenza), Italy 868/83

SMIC RADIATION IN CONTEMPORARY ASTROPHYSICS
f. M.M. SHAPIRO, Van Allen Hall, Univ. of Iowa, Iowa City, IA 52242, USA
5 November 1984 : Erice, Italy 944/83

VANCES IN CHEMICAL REACTION DYNAMICS
P.M. RENTZEPIS, Phys. & Inorg. Chem. Res., Bell Labs., 600 Mountain Av., Murray Hill, NJ 07974, USA
7 August 1984 : Crete, Greece 948/83

E DEEP PROTEROZOIC CRUST IN THE NORTH ATLANTIC PROVINCES
J. TOURET, Inst. Aardwetenschappen, Vrije Univ., Postbus 7161, 1007 MC Amsterdam, The Netherlands
30 July 1984 : Moi, Nr. Stavanger, Norway 953/83

NDS IN INTERFACIAL ELECTROCHEMISTRY
A.F.S. DA SILVA, Dept. Quimica, Fac. Ciencias, Univ. do Porto, P-4000 Porto, Portugal
3 July 1984 : Viana do Castelo, Portugal 958/83

LOGICAL SYNTHESIS OF THE CALEDONIAN ROCKS OF THE UK
A.L. HARRIS, J. Herdman Lab. Geology, Univ., Brownlow Str., Liverpool, PO Box 147, Liverpool L69 3BX, UK
August - 2 September 1984 : Wales/Scotland, UK 984/83

LICATIONS OF NEW SPECTROSCOPIC TECHNIQUES TO COAL SCIENCES
B.M. LYNCH, Dep. Chemistry, St. Francis Xavier Univ., Antigonish, Nova Scotia, Canada B2G 1C0
8 August 1984 : Antigonish, Nova Scotia, Canada 985/83

PLANETARY PHYSICS
Prof. S.K. RUNCORN, School of Physics, University, Newcastle-upon-Tyne NE1 7RU, UK
26 March - 6 April 1984 : Newcastle-upon-Tyne, UK 1013/83

AIR-SEA TRANFER PROCESSES - TRAINING COURSE (MS)
Dr. K. KATSAROS, Dept. Atmosph. Science AK-40, Univ. Washington, Seattle, Washington 98195, USA
Date to be announced : Lake Washington, USA 1016/83

MATHEMATICS

COMPUTATIONAL MATHEMATICAL PROGRAMMING
Prof. K. SCHITTKOWSKI, Inst. fuer Informatik, Univ. Stuttgart, Azenbergstr. 12, 7000 Stuttgart 1, Germany
23 July - 2 August 1984 : Bad Windsheim, Germany 478/83

OPERATORS AND FUNCTION THEORY
Dr. S.C. POWER, Dept. Mathematics, Univ. Lancaster, Bailrigg Lancaster, LA1 4YL, UK
15 - 26 July 1984 : Lancaster, UK 518/83

RINGS AND GEOMETRY
Prof. R. KAYA, Anadolu Universitesi Fen-Ed., Fakultesi, Ogretim Uyesi, Eskisehir, Turkey
2 - 14 September 1984 : Istanbul, Turkey 696/83

UNIVERSAL ALGEBRA AND RELATIONS
Prof. P. BERTHIAUME, Sémin. Math. Sup., Univ., C.P. 6128, Succ. A, Montreal, Que., Canada H3C 3J7
16 July - 3 August 1984 : Montreal, Canada 697/83

GRAPHS AND ORDERS
Dr. I. RIVAL, Dept. Math. & Stat., Univ., 2500 University Drive N.W., Calgary, Alberta T2N 1N4, Canada
20 May - 1 June 1984 : Banff, Canada 969/83

INFORMATICS

CONTROL FLOW & DATA FLOW – CONCEPTS OF DISTRIBUTED PROGRAMMING ●
Prof. F.L. BAUER, Inst. Inform., Techn. Univ., Arcisstr. 21, Postfach 20 2420, D-8000 München 2, Germany
31 July - 12 August 1984 : Marktoberdorf (Nr Munich) Germany 685/83

NEW SYSTEMS & ARCHITECTURES FOR AUTOMATIC SPEECH RECOGNITION & SYNTHESIS ●
Prof. R. DE MORI, Comp. Sc., 1455 De Maisoneuve Blvd West, Rm H961-22, Montreal, Quebec, Canada H3G 1M8
2 - 14 July 1984 : Bonas, France 712/83

RELATIONAL DATA BASE SYSTEMS DESIGN AND IMPLEMENTATION ●
Dr. C.J. ROGERS, Software & Inform. Syst. Div., TRW Def. Syst. Gp., 1145 Arques Av., Sunnyvale, CA 94086, USA
17 June - 29 July 1984 : Bad Windsheim, Germany 992/83

LOGICS FOR VERIFICATION AND SPECIFICATION OF PROGRAMS ●
Dr. K.R. APT, L.I.T.P., Univ. de Paris VII, 2 Place Jussieu, F-75251 Paris, France
15 - 19 October 1984 : France 995/83

APPLIED SCIENCES & ENGINEERING

GLASS CURRENT ISSUES ●
Dr. J. DUPUY, Dép. Physique des Matériaux, 43 Bd. du 11 Nov.1918, 69622 Villeurbanne, Cedex, France
2 - 13 April 1984 : Tenerife, Spain 10/83

INDUSTRIAL ROBOTIC VISION ●
Dr. A. OOSTERLINCK, Centrum Menselijke Erfelijkheid, Minderbroedersstraat 12, B-3000, Leuven
6 - 17 August 1984 : Leuven, Belgium 135/83

PHYSICS & ENGINEERING OF MEDICAL IMAGING ●
Dr. R. GUZZARDI, Nuclear Measurements Unit, C.N.R., Inst. Clin. Phys., Via Savi, 8, I-56100 Pisa, Italy
23 September - 5 October 1984 : Maratea, Italy 139/83

THERMODYNAMICS & FLUID MECHANICS OF TURBOMACHINERY ●
Prof. A.S. ÜCER, Mechanical Engineering Dept., Middle East Techn. Univ., Ankara, Turkey
17 - 28 September 1984 : Izmir-Cesme, Turkey 435/83

ADAPTIVE METHODS IN UNDERWATER ACOUSTICS ●
Ing. H. URBAN, Fried. Krupp GmbH, Krupp Atlas-Elektronik, PO. Box 44 85 45, D-2800 Bremen 44, Germany
30 July - 10 August 1984 : Lüneburg, Germany 439/83

ADVANCED AGRICULTURAL INSTRUMENTATION ●
Dr. W.G. GENSLER, Dept. of Electrical Engineering, Univ. Arizona, Tucson, AZ 85721, USA
27 May - 9 June 1984 : Italy 736/83

MINERAL PROCESSING DESIGN ●
Prof. Z.M. DOGAN, Mining Engineering Department, Middle East Technical Univ., (METU) Ankara, Turkey
6 - 17 August 1984 : Ankara, Turkey 772/83

OPTICAL METROLOGY ●
Dr. O.D.D. SOARES, Lab. de Fisica, Faculdade de Ciencias, 4000 Porto, Portugal
6 - 17 August 1984 : Povoa de Varzin, Portugal 945/83

MICROARCHITECTURE OF VLSI COMPUTERS ●
Dr. P. ANTOGNETTI, Istituto di Eletrotechnica, Univ. of Genova, Viale Cause 13, 16145 Genova, Italy
July 1984 : Urbino, Italy 970/83

APPLICATIONS OF THE MATERIAL SCIENCES TO THE PRACTICE OF ORTHOPEDIC SURGERY ●
Dr. M. DUJOVNY, Dept. Neurological Surgery, Henry Ford Hospital, 2799 W. Grand Blvd, Detroit, MI 48202, USA
15 - 28 July 1984 : Marbellia, Spain 972/83

rther information on a particular meeting should be obtained from the meeting Director named above.

rther information on the NATO Science Programmes may be obtained from : NATO Scientific Affairs Division, B-1110 Brussels, Belgium.

NATO Advanced Research Workshops

Tables rondes de l'OTAN

ADVANCED RESEARCH WORKSHOPS (ARWs) are working meetings which enable leading researchers, scientists and engineers to review the state-of-the-art in specific topics in fast moving fields and to formulate recommendations for the future. They are of about five days' duration : **attendance is limited and usually by invitation only.**

Each meeting is held under the responsibility of its Director, to whom all requests for information, attendance or support should be addressed. Four of the meetings are being sponsored by the Special Programmes on Global Transport Mechanisms (GTM), Selective Activation of Molecules (SAM), Materials Science (Mat.S) and the Survey Group on Astronomy (Astron.). The meetings marked ● are of particular industrial interest. **Locations and dates may change.** Titles and addresses have been abridged. Many meetings are of an interdisciplinary nature : please check all subject areas.

PUBLICATIONS :
Proceedings of meetings are published in the NATO ASI Series by : **Plenum – Reidel – Nijhoff – Springer Verlag.**

LIFE SCIENCES

MECHANISMS OF PERCEPTION & ORIENTATION TO INSECT PHEROMONES
Dr. M.C. BIRCH, Dept. of Zoology, South Parks Road, Oxford OX1 3PS, UK
5-10 August 1984 : Oxford, UK 75/82

MODELLING THE GROWTH OF WHEAT
Dr. W. DAY, Dept. of Agricult. & Horticult., Univ. Bristol, Long Ashton Research Station, Bristol BS18 9AF, UK
9-12 April 1984 : Bristol, UK 437/83

FUNCTION, STRUCTURE & GENETICS OF PROKARYOTIC & EUKARYOTIC ELONGATION FACTORS
Dr. A. PARMEGGIANI, Laboratoire de Biochimie, Ecole Polytechnique, F-91128 Palaiseau Cedex, France
16-20 July 1984 : Thiverval-Grignon, France 743/83

THE PHYSIOLOGY OF THIRST AND SODIUM APPETITE
Dr. G. DE CARO, Inst. of Pharmacology, Univ. of Camerino, 62032 Camerino, Italy
11-21 July 1984 : Camerino, Italy 767/83

VISUAL SPATIAL PROSTHESES FOR THE BLIND ●
Prof. D.H. WARREN, College Humanities & Social Sciences, Univ. of California, Riverside, CA 92521, USA
30 April-3 May 1984 : San Bernardino, USA 710/83

HUMAN IN VITRO FERTILIZATION - PRESENT PROBLEMS AND PROSPECTS
Prof. J. TESTART, Inserm 187, Gynéc.-Obstét., Antoine-Béclère, 157 rue Pte-de-Trivaux, 92141 Clamart, France
19-22 September 1984 : Cargèse, Corsica, France 757/83

MECHANISMS OF SECONDARY BRAIN DAMAGE
Prof. A. BAETHMANN, Inst. Surgical Research, Univ., Klinikum Grosshardern, 7000 München 70, Germany
19-23 February 1984 : Mauls/Sterzing, Italy 774/83

MULTIDISCIPLINARY ANALYSIS OF EVOLUTIONARY RELATIONSHIPS AMONG RODENTS
Dr. W.P. LUCKETT, Dep. Anatomy, Univ., Medical Sciences, G.P.O. Box 5067, San Juan, Puerto Rico 00936, USA
2-6 July 1984 : Paris, France 788/83

SYMPOSIUM ON BIOLOGY OF INVERTEBRATE AND LOWER VERTEBRATE COLLAGENS
Dr. S. BAIRATI, Dept. General Physiology & Biochemistry, Univ. of Milano, Via Celoria 26, 20133 Milano, Italy
3-6 June 1984 : Como, Italy 956/83

THE IN VITRO EFFECTS OF MINERAL DUSTS
Prof. E.G. BECK, Inst. of Hygiene, Justus-Liebig Univ., 63 Giessen, Germany
11-14 September 1984 : Schluchsee-Hochschwarzwald, Germany 976/83

STANDARDIZATION OF METHODOLOGY FOR THE MYCOLOGICAL EXAMINATION OF FOODS
Dr. J.E.L. CORRY, Min. of Agriculture Fisheries & Food, (Food Sc. Div.), 65 Romney Str., London SW1P 3RD, UK
July 1984 : Boston, USA 980/83

COORDINATE REGULATION OF CHOLESTEROL METABOLISM IN THE LIVER
Dr. A. SANGHVI, Dep. of Pathology, Univ. of Pittsburgh, School of Medicine, Pittsburgh, PA 16261, USA
October 1984 : Santa Fe, New Mexico, USA 990/83

ECOLOGY

ENVIRONMENTAL MONITORING FOR ARCHITECTURAL CONSERVATION
Dr. R.A. LIVINGSTON, RD-677, Res. & Dev., EPA, 401 M St SW, Washington, DC 20460, USA
11-15 June 1984 : Rome, Italy 459/83

POPULATION BIOLOGY - THE INTERFACES
Dr. P. JACQUARD, Dep. Physiologie Ecologique, C.N.R.S., B.P. 5051, 34033 Montpellier, France
28 May-2 June 1984 : Montpellier, France 876/83

SOCIAL, BEHAVIOURAL & POLITICAL SCIENCES

RESEARCH IN USER-COMPUTER INTERACTION ●
Prof. B. SCHACKEL Dept. of Human Sciences, University of Technology, Loughborough, UK
9-14 September 1984 : Cambridge or Loughborough, UK 968/83

PHYSICS

CONDENSED MATTER RESEARCH USING NEUTRONS, TODAY & TOMORROW
Dr. S.W. LOVESEY, Rutherford Appleton Lab., Chilton, Oxfordshire, OX11 0QX, UK
26-29 March 1984 : Abingdon, Nr. Oxford, UK 479/83

CHEMISTRY, GEOPHYSICS AND ASTROPHYSICS

THE MORPHOLOGY & DYNAMICS OF THE POLAR CUSP
Prof. A. EGELAND, Inst. of Physics, Univ. Oslo, P.O. Box 1048, Blindern, N-Oslo 3, Norway
20-27 May 1984 : Lillehammer, Norway 434/83

COMPOSITION & INTERACTIONS OF SOIL COLLOID SURFACES
Prof. M.F.L. DE BOODT, Fakult. Landbouw, RUG, Coupure Links 533, B-9000 Gent
2-8 September 1984 : Gent, Belgium 515/83

MOLECULAR ASTROPHYSICS – STATE OF THE ART & FUTURE DIRECTIVES
Dr. G.H.F. DIERCKSEN, Inst. Astrophysik, Karl-Schwarzschild-Str. 1, D-8046 Garching bei München, Germany
8-14 July 1984 : Bad Windsheim, Germany 741/83

EVOLUTION OF STRUCTURE IN THE UNIVERSE
Prof. M.J. REES, Inst. of Astronomy, Madingley Road, Cambridge CB3 0HA, UK
January 1984 : Santa Barbara, California, USA 768/83

CHAOS IN ASTROPHYSICS
Prof. J.R. BUCHLER, Dept. of Physics, Univ. of Florida, Gainsville, FL. 32611, USA
8-11 April 1984 : Palm Coast, Florida, USA 797/83

PHASE TRANSITIONS IN THE VERY EARLY UNIVERSE
Prof. R. BAIER, Fakultät für Physik, Univ. Bielefeld, Postfach 8640, D-4800 Bielefeld 1, Germany
4-8 June 1984 : Bielefeld, Germany 859/83

TRANSPORT IN NON-STOICHIOMETRIC COMPOUNDS ●
Prof. G. SIMKOVICH, Dept. Mat. Sc. & Eng., 206 Steidle Bldg, Penn. State, University Park, PA 16802, USA
10-16 June 1984 : University Park, Pennsylvania, USA 861/83

POLYDIACETYLENES - SYNTHESIS, STRUCTURE AND ELECTRONIC PROPERTIES ●
Dr. R.R. CHANCE, Corporate Technology, Allied Corporation, P.O. Box 1021R, Morristown, NJ 07960, USA
12-17 August 1984 : Stratford-upon-Avon, UK 959/83

COMPUTER SIMULATION OF CONDENSED MATTER (Mat.S)
Dr. C.R.A. CATLOW, Dep. Chemistry, Univ. College London, 20 Gordon Street, London WC1H 0AJ, UK
22-27 July 1984 : Norwich, UK 758/83

ICE IN THE SOLAR SYSTEM (Astron.)
Prof. A. DOLLFUS, Observatoire de Paris, 92190 Meudon, France
16-19 January 1984 : Nice, France 450/83

BIOGEOCHEMICAL CYCLING OF S & N IN REMOTE AREAS (GTM)
Dr. J.N. GALLOWAY, Environmental Studies Dept., Univ. Virginia, Charlottesville, VA 22903, USA
8-12 October 1984 : St. Georges, Bermuda 771/83

THE CHEMISTRY OF WEATHERING (GTM)
Dr. J.I. DREVER, Geology Dep., Univ. of Wyoming, Laramie, WY 82071, USA
25-29 June 1984 : Bonas, France 977/83

LASER STUDIES IN REACTIONS KINETICS VIA THE SELECT. ACTIVAT. OF MOLECULES (SAM) ●
Prof. E.W. SCHLAG, Inst. F. Physik. und Theoret. Chemie, Lichtenbergstrasse 4, 8046 Garching, Germany
24-27 September 1984 : Bavaria, Germany 1000/83

SELECTIVE ACTIVATION OF ORGANIC COMPOUNDS - ELECTROSYNTHESIS (SAM) ●
Dr. J.H.P. UTLEY, Dept. Organic Chem., Queen Mary Coll., Univ. of London, Mile End Road, London E1 4NS, UK
9-13 September 1984 : Oxford, UK 1011/83

UTILIZ. OF ACTIVATED DIOXYGEN SPECIES IN THE SELECT. OXIDAT. OF SUBSTRATES (SAM) ●
Prof. G. MODENA, Ist. di Chimica Organica dell'Universita, via Marzolo, 1, 35131 Padova, Italy
25-29 June 1984 : Galzignano (Padua), Italy 770/83

ASYMMETRIC CATALYSIS (SAM)
Prof. B. BOSNICH, Dept. of Chemistry, Univ. Toronto, 80 St. Georges St., Toronto M5S 1A1, Canada
2-7 January 1984 : Florida, USA 756/83

MATHEMATICS

DIFFERENTIAL GEOMETRY IN STATISTICAL INFERENCE
Prof. D.R. COX, Dept. Mathematics, Imperial College, Huxley Building, 180 Queen's Gate, London SW 7 2BZ, UK
9-11 April 1984 : London, UK 701/83

COMBINATORIAL ALGORITHMS ON WORDS
Ing. A. APOSTOLICO, Ist. di Scienza dell'Inform., Fac. di Scienze, Univ. di Salerno, I-84100 Salerno, Italy
18-22 June 1984 : Salerno, Italy 804/83

INFORMATICS

FORMAL SOFTWARE DEVELOPMENT METHODS ●
Prof. D. BJORNER, Dansk Datamatik Center, Lundtoftevej 1 C, DK-2800 Lyngby, Denmark
21-25 May 1984 : Denmark 979/83

APPLIED SCIENCES & ENGINEERING

PARTIAL PRESTRESSING – FROM THEORY TO PRACTICE ●
Prof. M.Z. COHN, Dept. Civil Engineering, Univ. Waterloo, Waterloo, Ontario, N2L 3G1, Canada
18-22 June 1984 : St. Rémy-lès-Chevreuse, France 764/83

PROBLEMS IN SERVICE LIFE PREDICTION OF BUILDING MATERIALS (Mat.S) ●
Dr. G.J. FROHNSDORFF, Bldg 226, Room B-368, National Bureau of Standards, Washington, DC 20234, USA
10-12 September 1984 : Paris, France 429/83

SEMICONDUCTOR DEVICE RELIABILITY (Mat.S) ●
Dr. C.M. MELLIAR-SMITH, Bell Telephone Labs, 2525 North 12th St., Reading, PA 19604, USA
Date & Place to be announced 534/83

APPLICATION OF FRACTURE MECHANICS TO CEMENTITIOUS COMPOSITES (Mat.S)
Prof. S.P. SHAH, Dept. Materials Eng., Univ. Illinois, Box 4348, Chicago, IL 60680, USA
June 1984 : Evanston, Illinois, USA 2/82

clays may partly fill these remaining pores and block inter-pore connections, reducing permeability to 100 mD (ref. 2). If the depth and timing of such diagenetic alteration can be measured and the extent of diagenesis estimated, then prediction of the diagenetic state of undrilled sandstones may become possible and diagenesis related more closely to the timing of hydrocarbon migration and the formation of hydrocarbon traps. We present an example of a new method for estimating the date of quartz diagenesis using a combination of techniques from thin section petrography, fluid inclusion thermometry, organic geochemical thermometry and sedimentary basin stratigraphic analysis. These results suggest that quartz in the Beatrice oilfield was precipitated from moving and cooling pore fluids, at a temperature between 68 °C and 94 °C in the late Jurassic.

The Beatrice oilfield, operated by Britoil, lies in the Inner Moray Firth of the UK North Sea and contains oil reservoired in middle Jurassic sandstones[3]. Thin section and scanning electron microscope (SEM) studies of the diagenetic sequence in Bajocian reservoir sandstones indicate a specific diagenetic sequence: early-formed concretionary calcite, minor feldspar leaching and kaolinite formation, syntaxial quartz overgrowth (in at least two phases), widespread feldspar leaching and coeval kaolinite formation with minor illitization of some muscovite, minor late aggressive calcite and finally minor very late cryptocrystalline silica. The details of diagenesis will be described in more detail elsewhere[4], but the general sequence is typical of many sandstones both in the North Sea, and in other petroleum basins[5,6].

Thin section observations show that the primary permeability in some sandstone layers has been low (1–3 mD) since deposition, due to blockage of pore connections by detrital clay. These sandstones show very little diagenetic alteration and contain 3–4% potassium feldspar. By contrast, compositionally similar sandstones with high (>100 mD) primary permeabilities have experienced dissolution of potassium feldspar, and growth of authigenic quartz to form about 13%, by area, of the whole rock. Only 1% of grains in all these sandstones have suffered any pressure solution. Quartz overgrowth has uniformly affected the permeable sandstones, except in areas immediately beneath thin (5–50 cm), laterally persistent, mudstone beds, where diagenetically overgrown quartz forms a pervasive 3–20 cm thick layer, so that quartz forms about 99% of the rock. Similar layers do not occur above these mudstones. Such features, where diagenesis is limited to permeable sandstones and enhanced below impermeable layers, suggest that the diagenetic pore fluids moved through the sandstones and precipitated quartz as they cooled beneath the mudstones. It may be noted that in neutral to acid fluids, the solubility of quartz at diagenetic temperatures and pressures is dominated by temperature[7], rather than being dependent on ionic concentration[6,7], salinity[8], pressure[7], or *p*H changes[7].

During the formation of diagenetic quartz overgrowths, samples of pore fluids were trapped as fluid inclusions and as they show no signs of leakage, each is assumed to have been constant in volume and composition since its formation. These 'primary' inclusions were examined by heating and freezing fluid inclusion microscopy[9,10]. Briefly, in the case of a simple low salinity aqueous fluid, this method involves cooling an inclusion to freeze its fluid, and then warming it. The salinity of the fluid[10,11] is given by the temperature at which the ice crystals melt, and other chlorides present can be expressed as equivalent NaCl[10]. Further heating causes the inclusion to revert to a one-phase system at the 'homogenization temperature', which is the minimum trapping temperature of the inclusion and hence the fluid density[10] is determined. The true trapping temperature of an inclusion was hotter and lay somewhere along the constant density 'isochore' path through the liquid or fluid phase for the inclusion concerned. This line can be drawn from pressure-volume-temperature properties of the NaCl–H_2O fluid[10,15,16] and its measured density. In this paper the size of the upward temperature correction is constrained using an independent organic geochemical measurement of the maximum temperature

Table 1 Polycyclic alkane data used to establish maximum palaeotemperatures for the interbedded mudrocks

Sterane and triterpane ratios	Ratio* (%)	Equivalent† temperature (°C)	Comments on plots used‡
Ratio Tt No. 5	22.4	78	Well defined linear low scatter plot (Fig. 2).
Ratio St No. 5	40.8	94	Linear portion of moderate scatter plot
Ratio St No. 6	50.1	97	On shoulder of moderate scatter plot
Ratio St No. 9	38.6	87	Well defined sigmoidal low scatter plot

The following ratios have been measured for the interbedded mudstones. Triterpanes: Ratio Tt 5: 17β(H), 21α(H), 30-normoretane ÷ [17β(H), 21α(H), 30-normoretane]+[17α(H), 21β(H), 30-norhopane]. Steranes: Ratio St 5: 5α,14α,17αmethyl cholestanes (20R ÷ 20R+20S). Ratio St 6: 5α,14α,17αethyl cholestanes (20R ÷ 20R+20S). Ratio St 9: 5α,14β,17β, 20R+20S ethyl cholestanes ÷ (5α, 14β, 17β, 20R+20S)+(5α, 14α, 17α, 20R+20S) ethyl cholestanes.

* Quantitations based on *m/e* 191, 386, 217 and 218 (ref. 12, Table 1).

† Error on measurements ±6.5 °C (see text).

‡ Taken from calibration plots in ref. 12.

experienced by interbedded mudstones. This enables a pressure measurement at the time of trapping to be derived, rather than the usual stratigraphical method being used, of estimating a pressure to give a temperature. The maximum temperature recorded by the organic geothermometer may have been reached later than the time of trapping; however, since the organic temperature is only used as a constraint on the upward limit of the homogenization-to-trapping temperature correction, this will tend to give maximum trapping temperatures and maximum pressures.

In the Beatrice Field, inclusion salinities range from 8 to 17 equivalent weight per cent NaCl[11] and homogenization temperatures show a unimodal distribution around 60–78 °C, $\bar{x}$ = 68 °C (Fig. 1), and show no variation with position in the overgrowths or between different parts of the reservoir sandstones. In this case organic rich mudrocks are interbedded with the reservoir sandstones, so that the maximum burial temperature can be estimated from changes in the molecular ratios of triterpanes and steranes[12,13]. Four organic geothermometer polycyclic alkane ratios have been measured for the interbedded mudstones (Table 1). These ratios have been compared with trends established from a depth (temperature) sequence of Kimmeridge Clay Formation (North Sea 'hot shales') to determine the maximum temperature of burial for the interbedded mudstones. Although the depositional environment, and to some extent the kerogen type of the middle Jurassic interbedded mudstones in Beatrice are not identical to the Kimmeridge Clay Formation, both contain kerogens of an algal origin, bacterially degraded to a lesser or greater extent.

It should be noted that the polycyclic alkane ratios used are those that give a relatively low level of scatter in the calibration plots (Table 1, Fig. 2), and are sensitive to temperature in the range under consideration. Within our laboratories a duplicate analysis on a split sample of powdered sediment generally gives a reproducibility of ±5% on the ratios used, which corresponds to an average difference in temperature of ±6.5 °C over the temperature range under discussion. The use of the calibration curves to define the maximum palaeotemperature is entirely pragmatic, and does not rely on, or imply, any precursor-product relationship between the ratioed molecules (contrast ref. 14). Use of the Kimmeridge Clay Formation calibration plots for the middle Jurassic shales is justified by the good agreement

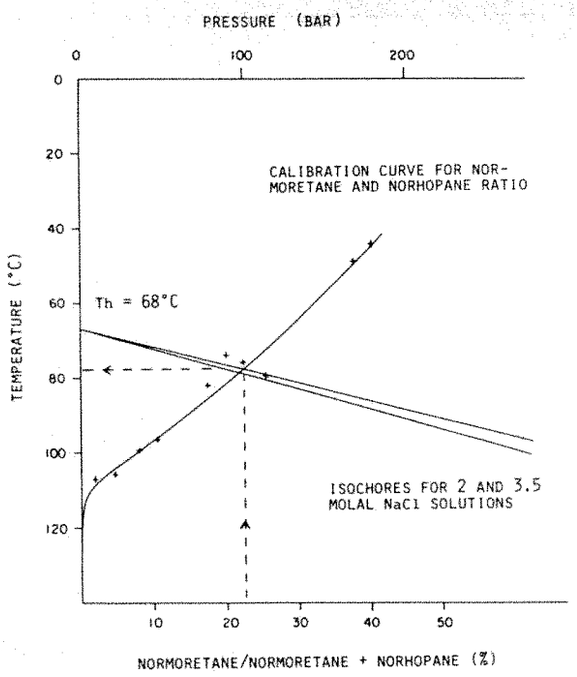


Fig. 1 Homogenization temperatures of fluid inclusions trapped in diagenetic quartz overgrowths from middle Jurassic oil reservoir sands of the Beatrice field, North Sea. Inclusions at different positions within overgrowths give similar homogenization temperatures. The spread of values may be due to slight 'necking' of some inclusions. Blank = aqueous inclusions; black = hydrocarbon bearing inclusion. Number of inclusions = 38, $\bar{x}$ (total) = 67.6 °C, $\bar{x}$ (central peak, 55 ≤ Th ≤ 80) = 67.1 °C, σ = 5 °C.

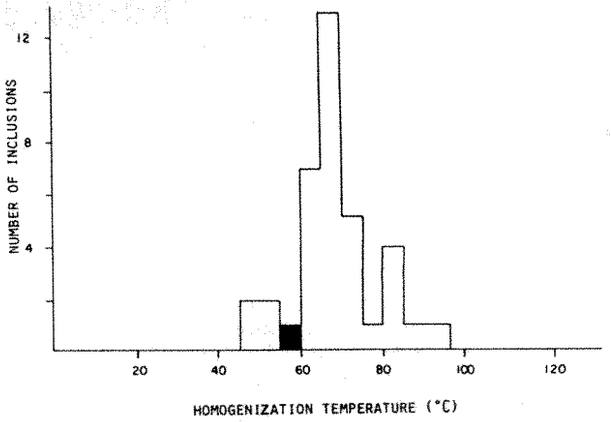


Fig. 2 Limits on pressure and temperature of diagenetic quartz overgrowth formation from volumetric properties[10] of system NaCl–H_2O. Mean inclusion homogenization temperature of 68 °C shown, together with dashed line for maximum sediment temperature of 78 °C derived from ratio Tt 5 (Table 1), calibrated using North Sea Kimmeridge Clay Formation (+) isomer data[12]. Note that triterpane ratios are calibrated against temperature rather than pressure, the correspondance of values on the pressure and triterpane scales being expected to vary for different sedimentary basins.

between the predicted temperatures for the four essentially independent ratios. Use of these four ratios indicates a minimum temperature of 78 °C and a maximum of 97 °C with a mean of 89 °C. However, the lower values derive from the calibration curves showing the least degree of scatter and are hence in practice likely to be more accurate.

We are now in a position to constrain the maximum trapping temperature at which fluid inclusions formed, and to derive the pressure at which they formed. The polycyclic alkane ratio with the best calibration curve gives a maximum temperature of 78 °C (Table 1). Thus the most probable correction of fluid inclusion homogenization temperature is from 68 °C to 78 °C, i.e. 10 °C. As the density of the trapped fluid can be calculated[15,16], and the volume of an inclusion has remained constant from its time of formation, this correction to a maximum temperature of 78 °C equates to a maximum burial pressure of 100 bars (Fig. 2)[15,16] which is equivalent to a depth of 1.0 km under purely hydrostatic loading, or of 0.4 km under purely lithostatic loading (mudrock density 2.4 g cm^{-3}). However, pressures at 0.4 km are unlikely to be lithostatic[17] and the necessary geothermal gradient of about 195 °C km^{-1} is very unlikely. Thus we are forced to conclude that diagenetic quartz overgrowth occurred at about 1.0 km depth under hydrostatic pressure, which enabled free circulation of diagenetic fluids. If the less reliable temperature of 94 °C is used (Table 1) as a maximum constraining estimate, the maximum temperature correction becomes 26 °C and so maximum pressure of quartz overgrowth increases to 240 bar, equivalent to between 1.0 km (lithostatic) and 2.4 km (hydrostatic). However, we suggest that since hydrostatic pressures are unlikely below 1.5 km (ref. 17), quartz overgrowth formation did not occur below this depth.

The shape[18] and stratigraphy[3] of the basin are known from seismic and drilling evidence. Subsidence in the Beatrice area was slow during deposition of the reservoir sandstones and remained slow until the earliest Oxfordian[19], accumulating 180 m of sediment (present-day thicknesses). Subsidence rates increased dramatically in the Oxfordian, culminating in late Oxfordian sedimentation rates of about 100 m per Myr, and accumulating 340 m of Oxfordian mudstones in all. Subsidence slowed during the Kimmeridgian[19], accumulating 200 m of mudrock, but became rapid again during the Tithonian[19], preserving 320 m of mudrock. During this late Jurassic basin subsidence a total of 860 m of mudrock accumulated in about 19 Myr (ref. 19).

We considered two extreme cases, maximum and minimum organic geothermometric temperatures, with overburden pressures derived from fluid inclusions, and equated these with overburden burial pressures generated by the present-day sediment thicknesses. Case 1: an organic geothermometric temperature of 78 °C implies, from fluid inclusion data, an overburden pressure of 100 bar. We know from stratigraphical evidence (above) that at the end of the Oxfordian, 180 m of sandstone and mudstone (density 2.6 g cm^{-3}) plus 340 m of mudrock (density 2.4 g cm^{-3}) had accumulated, giving a lithostatic load of 128 bar, plus an arbitrary additional 10 bar from seawater pressure in a sediment-starved basin, totalling 138 bar. Thus the earliest time that quartz diagenesis could have taken place was in the late Oxfordian.

Case 2 utilizes an organic geothermometer value of 94 °C (Table 1) implying an overburden pressure of 240 bar from fluid inclusion data. Stratigraphical evidence shows that a lithostatic overburden of 252 bar would have been generated by the end of the Tithonian, plus 10 bar from seawater in a sediment-starved basin, totalling 262 bar. Thus the latest time for quartz diagenesis was during the late Tithonian, the mean of these ages[19] is 150 ± 6 Myr.

To estimate geothermal gradients during quartz diagenesis we must infer sediment overburden thickness at the time[20]. In both cases we estimate that the middle Jurassic section is increased from 180 m to 200 m, as the reservoir sands at the base must have been compacted to their cemented thickness, and a 10 °C seafloor temperature is assumed. In Case 1, 340 m of mudrock would represent an initial thickness[20] of about 500 m, giving a 700 m section and a geothermal gradient of 97 °C km^{-1}. In Case 2, mudrock layers of 340 m, 200 m and 320 m would represent 370 m, 250 m and 500 m respectively[20] giving a 1,320 m section and a geothermal gradient of 71 °C km^{-1}. Although these figures are simply guidelines, they do imply a very high geothermal gradient at the end of a short period of very rapid basin subsidence (up to 100 m per Myr). We know from organic geochemistry (Table 1) that the maximum sediment temperature did not exceed 97 °C, and from electric log sonic velocity data that the maximum burial depth

of these sediments was about 3.0 km (present-day thicknesses), so that the geothermal gradient at maximum burial was only 29 °C km^{-1}. The present-day geothermal gradient from borehole measurements is 38 °C km^{-1}. Thus evidence in Beatrice suggests that the geothermal gradient varied through time[21], with very high temperatures immediately following the most rapid phase of basin subsidence, increasing only slightly, if at all[22], during the subsequent Cretaceous thermal subsidence[22] of the basin. It may be that rock microfracturing associated with basin rifting and subsidence encouraged rapid circulation of diagenetic fluids.

We have seen that diagenetic quartz forms about 13% of the reservoir sandstones; possible sources of this quartz could be: pressure solution, dissolution of silt-size quartz or of biogenic silica, feldspar breakdown, or illitization of smectite clays. The first three alternatives are inconsistent with petrographic observations. Feldspar breakdown is also insufficient, because even if dissolved feldspar had formed 10% of original grains, silica released could only have contributed 2% of the diagenetic quartz; thus we are left with a quartz supply from the illitization reaction[23–25]. This reaction occurs at about 70–90 °C[23–25], a similar temperature to that recorded by the fluid inclusions. In addition, Pearson *et al.*[26] have argued that smectite-rich muds were present in the Inner Moray Firth. Thus it seems probable that illitization in surrounding mudrocks slightly deeper in the half graben[18] released silica-rich fluids which travelled up-dip through permeable sandstone beds, and authigenic quartz was precipitated as the aqueous fluids cooled beneath mudstone roofs—with a fall in temperature from 100 °C to 80 °C the solubility of quartz decreases by 56%[7].

Thus diagenetic quartz growth occurred at several times at temperatures between 68 °C and 94 °C and pressures of 100–240 bar. Assuming that lithostatic pressure is achieved at all depths greater than 1.5 km the quartz grew in a very high geothermal gradient of 71 to 97 °C km^{-1}, immediately following episodes of rapid basin subsidence during the Oxfordian and Tithonian. Sediments reached their maximum temperatures near the end of this rifting and the geothermal gradient decreased sympathetically with postrifting subsidence. Silica was released by illitization of smectite clays and precipitated as quartz in permeable sandstone aquifers when the transporting aqueous solutions cooled.

I. M. S. was funded by the NERC and the fluid inclusion equipment supplied through NERC grant GR3/3726A. We thank M. J. Russell (University of Strathclyde) for suggesting examination of fluid inclusions, R. Archer and J. Sargeant of Britoil for helpful discussions, and Britoil and Beatrice partners for permission to publish. We thank A. S. Mackenzie for a constructive review.

Received 23 September; accepted 17 October 1983.

1. Pryor, W. A. *Bull. Am. Ass. Petrol. Geol.* **57** 162–189 (1973).
2. Hayes J. B. *Soc. Econ. Paleont. Mineral. Spec. Publ.* **26,** 127–139 (1979).
3. Linsley, P. N., Potter, H. C., McNab, G. & Racher, D. *Mem. Am. Ass. Petrol. Geol.* **30,** 117–130 (1980).
4. Haszeldine, R. S., Samson, I. M. & Cornford, C. *Clays Clay Miner.* (submitted).
5. Loucks, R. G., Babout, D. G. & Galloway, W. E. *Trans. Gulf-Cst Ass. geol. Soc* **27,** 109–120 (1977).
6. Curtis, C. D. in *Geochemistry and Exploration of Europe* (ed. Brooks, J.) 113–125, (Blackwells, London, 1983).
7. Holland, H. D. & Malinin, S. D. in *Geochemistry of Hydrothermal Ore Deposits* 2nd Edn (ed. Barnes, H. L.) 461–508 (Wiley, New York, 1979).
8. Fournier, R. O. & Marshall, W. L. *Geochim. cosmochim. Acta* **47,** 587–596 (1983).
9. Burruss, R. C. *Am. J. Sci.* **281,** 1104–1126 (1981).
10. Crawford, M. L. in *Fluid Inclusions: Applications to Petrology* (eds Hollister, L. S. & Crawford, M. L.) *Min. Ass. Canada Short Cse, Handbk* **6,** 75–100 (1981).
11. Potter, R. W. II, Clyne, M. A. & Brown, D. C. *Econ. geol.* **73,** 284–285 (1978).
12. Cornford, C., Morrow, J. A., Turrington, A., Miles, J. A., & Brooks, J. in *Petroleum Geochemistry and Exploration of Europe.* (ed. Brooks, J) 175–194 (Academic, London, 1983).
13. Mackenzie, A. S. & Maxwell, J. G. in *Organic Maturation Studies and Fossil Fuel Exploration* (ed. Brooks, J.), 239–254 (Academic, London, 1981).
14. McKenzie, D., Mackenzie, A. S., Maxwell, J. R. & Sajgo, C. *Nature* **301,** 504–506 (1983).
15. Potter, R. W. II & Brown, D. C. *Bull. U.S. geol. Surv.* **1421-C** (1977).
16. Haas, J. L. Jr *Bull. U.S. geol Surv.* **1421-A** (1976).
17. Fyfe, W. S., Price, N. J. & Thomson, A. B. *Fluids in the Earth's Crust* (Elsevier, Amsterdam, 1977).
18. Chesher, J. A. & Bacon, M. *Inst. geol. Sci. Rep. 75/11* (HMSO, London 1975).
19. Harland, W. B. *et al. A Geologic Timescale* (Cambridge University Press, 1982).
20. Perrier, R. & Quiblier, J. *Bull. Am. Ass. Petrol. Geol.* **58,** 507–520 (1974).
21. Price, L. C. *J. Petrol Geol.* **6,** 5–38 (1983).
22. McKenzie, D. *Earth planet. Sci. Lett.* **55,** 87–98 (1981).
23. Hower, J., Eslinger, E. V., Hower, M. E. & Perry, E. A. *Bull. geol. Soc. Am.* **87,** 725–737 (1976).
24. Boles, J. R. & Franks, S. G. *J. sedim. Petrol.* **49,** 55–70 (1979).
25. Dypvik, H. *Bull. Am. Ass. Petrol. Geol.* **67,** 160–165 (1983).
26. Pearson, M. J., Watkins, D. & Small, J. S. *Develop. Sedimentol.* **35,** 665–675 (Elsevier, Amsterdam 1982).

Did the Dalradian slides originate as extensional faults?

N. J. Soper

Department of Geology, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK

R. Anderton

Department of Applied Geology, University of Strathclyde, 75 Montrose Street, Glasgow G1 1XJ, UK

Rocks of the Dalradian Supergroup[1,2] of Scotland and Ireland were deposited in late Proterozoic–early Cambrian times on what became the southern margin of the Laurentian plate, and underwent metamorphism and deformation when this margin was affected by the Grampian orogeny in the late Cambrian[3,4]. The resulting complex structure is still not fully understood, and a continuing problem is presented by the origin and significance of the 'slides', a series of ductile faults which lie sub-parallel to bedding[5]. Although numerous slides have been recognized within the Dalradian terrain[6] the term has seldom been used outside the British Caledonides. It is generally assumed that these slides are synmetamorphic and were formed during Grampian deformation. However, a consideration of their geometry, stratigraphic relationships and context now suggests that they may have been initiated as syndepositional extensional faults which were only modified, and not formed, during the Grampian orogeny.

The numerous local stratigraphic successions recognized in the Scottish Dalradian have been consolidated into a fourfold sequence comprising the Grampian (oldest), Appin, Argyll and Southern Highland Groups[1]. These have a combined stratal thickness of some 25 km and the Dalradian thus represents one of the major supracrustal sequences of the Caledonides[2]. It was deposited on a basement composed of crystalline gneisses (Lewisian) and a cover sequence (Moine) which had undergone orogenic reworking during the Grenville cycle (~1,000 Myr). Much of the Dalradian is characterized by distinctive lithologies, faciliting subdivision and correlation, and leading in turn to the recognition of many stratigraphic and structural complexities. One such is the problem posed by the 'slides'. This term was introduced by Bailey in 1910[7] for planes along which parts of the stratigraphic succession in the Ballachulish district had undergone thinning or complete excision. Slides were subsequently widely mapped within the Dalradian terrain (Fig 1) and in the Moine and Lewisian rocks of the North-West Highlands[8]. A recent tendency has been to expand the usage, inadvisably in our view, to include platy high strain zones without demonstrable stratigraphic separation[9,10].

Grampian structure is characterized by large, often recumbent primary folds which face north-west and south-east away from a central zone. Current interpretations involve upward and outward expulsion of material from this zone, the so-called 'fountain of nappes' hypothesis[11,12]. Attempts to integrate the slides into this scheme by interpreting them as ductile thrusts associated with the primary folding have not been successful (Fig. 2). As Bailey[13] was well aware, important slides such as Ballachulish and Fort William (Fig. 1) emplace younger rocks over older and thus have the geometry of 'lags' not thrusts. In

0028-0836/84/040357-04$01.00

© 1984 Macmillan Journals Ltd

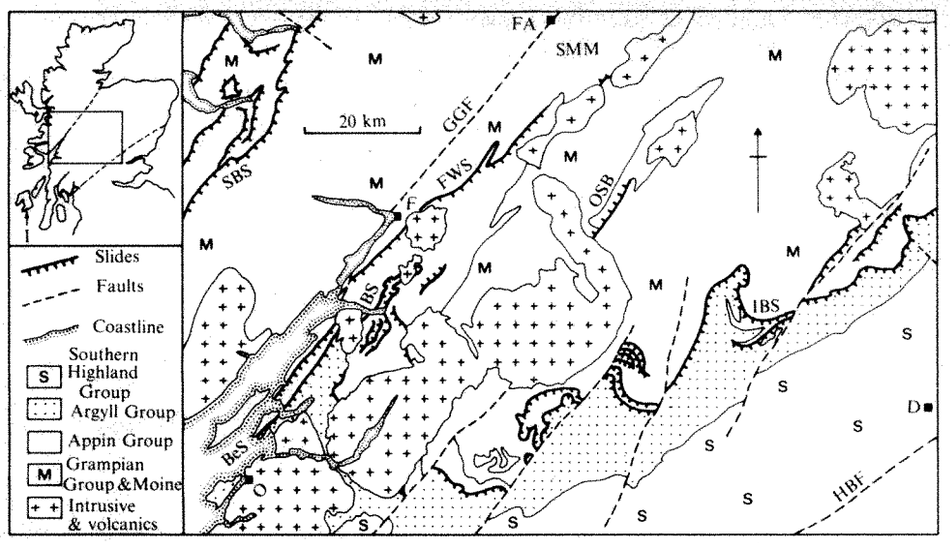


Fig. 1 Simplified geological map of part of the southwestern Highlands of Scotland showing some of the slides in the Dalradian and Moine: BeS, Benderloch slide; BS, Ballachulish slide; FWS, Fort William slide; IBS, Iltay Boundary slide; SBS, Sgurr Beag slide. Ticks are on down-dip side of slides. Box inset shows location of map. Towns: D, Dunkeld; F, Fort William; FA, Fort Augustus; O, Oban. Other localities: I, Islay; OSB, Ossian steep belt; SMM, southwestern Monadhliath Mountains. Faults: GGF, Great Glen Fault; HBF, Highland Boundary Fault. Compiled from refs 12, 27, 37–39.

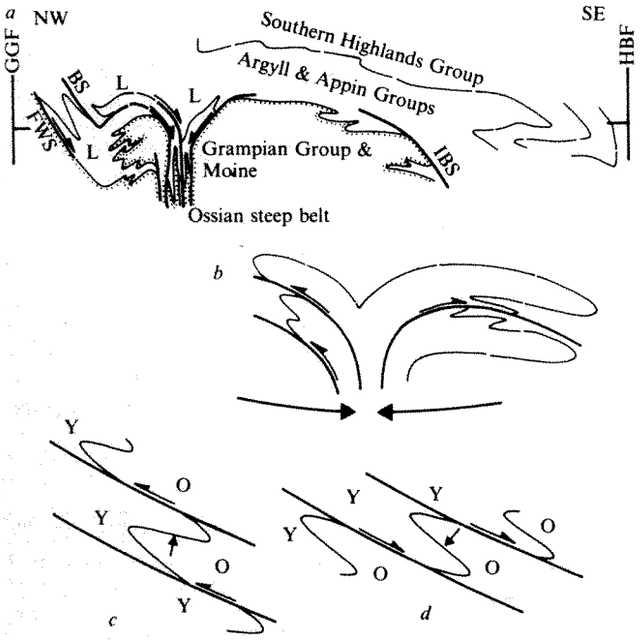


Fig. 2 *a*, Diagrammatic profile across the Grampians[12], between Fort Augustus and Dunkeld (Fig. 1). Major slides are shown by bold lines, with their displacement directions inferred from the stratigraphy indicated by half arrows and labelled as in Fig. 1. GGF, Great Glen Fault; HBF, Highland Boundary Fault; L, Lochaber Sub-group. *b*, The 'fountain of nappes' hypothesis[12] showing slide displacements opposite in sense to those shown in *a*. *c*, Lag or extensional fault geometry, as in *a*. *d*, Contractional thrust geometry, as in *b*. Y and O refer to younger and older beds respectively.

addition, associated folds often show attentuation of their right-way-up, not their inverted limbs (Fig. 2).

The concept of a lag as a thrust plane on which the upper plate was displaced less far forward than the lower seems improbable and the term is now rarely used. Lag geometry is in fact extensional, not contractional. We propose a radical solution to the problem illustrated in Fig. 2 by suggesting that the slides which show extensional geometry were initiated during Dalradian sedimentation as growth faults, not during the Grampian orogeny as thrusts.

During the late Proterozoic the Dalradian terrain evolved from a slowly subsiding stable shelf on which sediments of the Grampian and Appin Groups were deposited, into a series of rapidly subsiding fault-bounded basins (Argyll Group). This increasing instability is thought to have reflected an increasing rate of crustal stretching which eventually led, at about the beginning of the Cambrian, to rupture of the Proterozoic Super-continent[14] and birth of the Iapetus Ocean[15]. The time of rupture was marked by the extrusion of the tholeiitic Tayvallich volcanics[16] although this vulcanism appears to have been peripheral to the site of continental splitting which lay further to the south-east. It is now considered that during Argyll Group times the Dalradian terrain of the southwestern Highlands developed into a type of quasi-oceanic crust similar to that now forming at the head of the Gulf of California[17]. In this, development of a mature spreading centre is suppressed by the rapid influx of sediment and spreading takes place on a limited scale by sill and dyke injection. Thus, it is thought that there was an attempt to form oceanic crust within the present area of Dalradian outcrop, but that this was aborted and the final split took place further to the south-east.

Dalradian deposition can be viewed in the context of the McKenzie model for basin subsidence by lithosphere stretching and thermal relaxation[18–20]. During Appin Group times stretching and thinning was insufficient to allow volcanic activity, and sedimentation was able to keep pace with subsidence. More rapid stretching produced the deep Argyll Group basins[21,22]. By end-Argyll times the sediment pile had attained a maximum thickness of some 12 km and the lithosphere had thinned by a factor of about four, permitting the injection of basic magma towards the top of the sediment column. An interpretation of the lateral thickness and facies changes within the Argyll Group suggests that this stretching was accomplished by major *syn*-depositional faults, with throws of up to several kilometers and an across-strike spacing of tens of kilometres, dividing the area into blocks and basins[22]. Current ideas on extensional faulting suggest that such faults can often be listric in form, flattening out at a depth of 8–12 km[19,20,23,24]. This would be the depth of the lower part of the Appin Group by mid-Argyll times. Figure 3 illustrates these faults schematically; some of them we suggest are now represented by slides. Later subsidence to accommodate the Southern Highland Group may have been largely thermal, following abandonment of the incipient rifting centre, no further extension being required.

It has always been assumed that the slides are of Grampian age, associated with the primary deformation. Many carry a penetrative fabric and some coincide with a change in facing of early folds[6,25,26]. These must either have been initiated during the Grampian deformation or, if earlier, suffered reactivation. On the other hand the Benderloch slide, for example, has the regional fabric superimposed obliquely across it[27] and appears to be pre-Grampian. As Litherland[28] pointed out, the slide coincides with a major facies change in the Argyll Group which thickens rapidly across it to the south-east. Our contention is

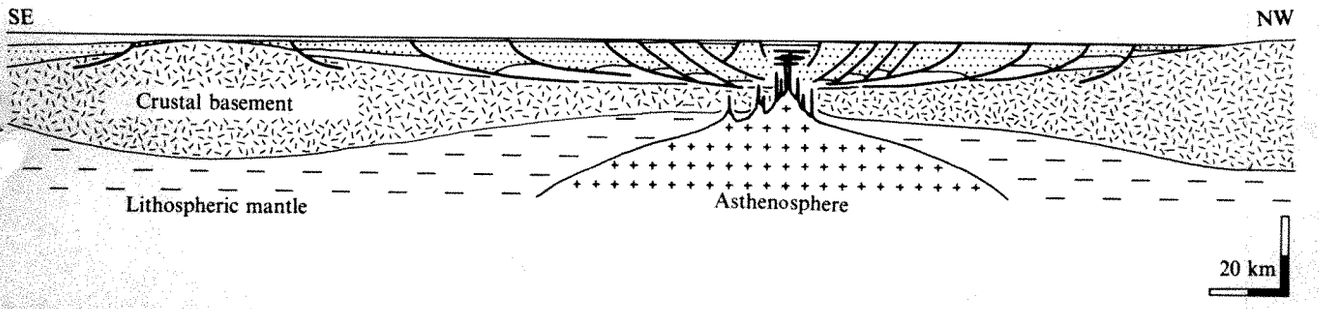


Fig. 3 Sketch illustrating the crustal structure of the Dalradian terrain, in late Argyll Group times, showing listric and other normal faults. Continental crust is on the verge of rupturing with the formation of quasi-oceanic crust by sill and dyke emplacement. Crustal stretching subsequently ceased here, the complete failure of the Laurentian–Baltic plate taking place at another site of crustal attenuation to the south-east of the area shown on this diagram. Ornament for Appin and Argyll Groups is as shown on Fig. 1. Older basement rocks are not differentiated.

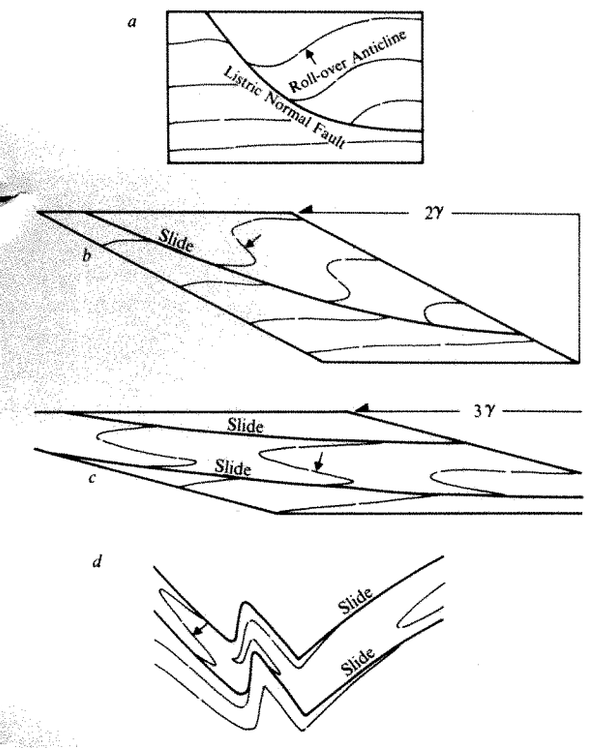


Fig. 4 Diagrams illustrating the geometrical possibility of deriving Grampian slide and primary fold geometry by the superimposition of simple shear strain on early extensional structures. *a*, Listric normal fault and associated roll-over anticline[29]. *b*, Passive rotation of slide plane and bedding under a shear strain of 2γ. *c*, Under 3γ 'primary' recumbent folds are produced. Two slide zones are shown. *d*, Secondary refolding of *c* produces a profile comparable to that of the southwestern Monadhliath Mountains (compare ref. 30, Fig. 3).

that slides such as the Benderloch, which emplace younger rocks over older and thus have extensional geometry, started life as listric growth faults and either behaved entirely passively during Grampian deformation, or were reactivated. Studies are needed to test whether displacements inferred from fabrics and folds associated with particular slides are compatible with the displacements required by the stratigraphy.

Figure 4 illustrates that the hypothesis is geometrically realistic, and can also account for those associated folds which have their 'wrong' limb attenuated. We start with a listric normal fault and roll-over anticline, as seen in many examples revealed by reflection profiling of extensional continental margins[29]. Accepting the view that Grampian deformation in Scotland did involve the expulsion of material north-west and south-east from a central zone (which coincided approximately with a major depocentre) we model the deformation as simple shear with an opposite sense of displacement to that of the growth fault. Passive rotation of the material planes (bedding and fault) under quite modest shear strains produces folds with their right-way-up limbs apparently attenuated at the slide. Secondary refolding generates a geometry comparable with that of the published profile of the southwestern Monadhliath Mountains[30,31].

Our hypothesis could throw light on unexplained features of Highland geology. For example the Ossian steep belt[12] (Fig. 2) could represent a highly compressed graben. The Loch Skerrols 'thrust' in Islay[32] marks the western margin of the Grampian orogenic belt against the north-west foreland, a position analogous to that of the Moine thrust zone to the north, a major contractional structure. However, the Loch Skerrols displacement brings Argyll Group rocks in the hanging wall against older Bowmore sandstone and thus appears to be extensional. It may have developed on the site of a major basin-margin fault.

Structures referable to the major phase of extension which affected the sub-Dalradian basement in the late Proterozoic, and which might have survived Grampian–Caledonian overprinting, should be sought. Extensional displacements at depth would generate ductile fabrics difficult to distinguish from those associated with subsequent compressional events. The reactivation of syndepositional faults in compression may well represent an important mechanism of upper lithosphere shortening in zones of continental convergence[33].

Finally, we point to the apparent coincidence in time between the Morarian pegmatite 'event' which affected the Old Moine basement at around 750 Myr[9,34], and the onset of Grampian–Dalradian sedimentation. The traditional interpretation of the Morarian as a late Grenville, pre-Grampian orogenic event (equivalent to the now-discredited Carolinides of north-east Greenland[35]) is difficult to sustain without clear evidence of compressional structures or syntectonic metamorphism. Although it has been proposed that these pegmatites are associated with ductile shear zones[9], they are clearly dilational and lack synkinematic crystallization fabrics[36]. We suggest that they were formed during a major phase of crustal stretching and enhanced heat flow associated with the onset of cover sedimentation.

Received 10 August; accepted 31 October 1983.

1. Harris, A. L. & Pitcher, W. S. *Geol. Soc. Lond. Spec. Rep.* No. 6, 52–75 (1975).
2. Harris, A. L., Baldwin, C. T., Bradbury, H. J., Johnson, H. D. & Smith, R. A. *Geol. J. Spec. Issue* No. 10, 115–138 (1978).
3. Lambert, R. St. J. & McKerrow, W. S. *Scott. J. Geol.* **12**, 271–292 (1976).
4. Bradbury, H. J., Smith, R. A. & Harris, A. L. *J. geol. Soc. Lond.* **132**, 677–684 (1976).
5. Fleuty, M. J. *Geol. Mag.* **101**, 452–456 (1964).
6. Hutton, D. W. H. *Earth Sci. Rev.* **15**, 151–172 (1979; *Geol. Soc. Lond. Spec. Publ.* No. 9, 261–265 (1981).
7. Bailey, E. B. *Q. Jl geol. Soc. Lond.* **66**, 586–608 (1910).
8. Tanner, P. W. G. *J. geol. Soc. Lond.* **126**, 435–463 (1971).
9. Piasecki, M. A. J. & van Breemen, O. *Nature* **278**, 734–736 (1979); *Trans. R. Soc. Edinburgh: Earth Sci.* **73**, 119–134 (1983).
10. Mendum, J. R. *Geol. Soc. Lond. Spec. Publ.* No. 8, 291–297 (1979).
11. Roberts, J. L. *J. geol. Soc. Lond.* **130**, 93–124 (1974).
12. Thomas, P. R. *Geol. Soc. Lond. Spec. Publ.* No. 8, 205–211 (1979).
13. Bailey, E. B. & Maufe, M. A. *Mem. Geol. Surv. Scotl.* 113 (1960).
14. Piper, J. D. A. *Earth planet. Sci. Lett.* **59**, 61–89 (1982).
15. Anderton, R. J. *J. geol. Soc. Lond.* **139**, 421–431 (1982).
16. Graham, C. M. *J. geol. Soc. Lond.* **132**, 61–84 (1976).

17. Moore, D. G. *Bull. geol. Soc. Am.* **84,** 1883–1906 (1973).
18. McKenzie, D. *Earth planet. Sci. Lett.* **40,** 25–32 (1978).
19. Le Pichon, X. & Sibuet, J.-C. *J. geophys. Res.* **86**B, 3708–3720 (1981).
20. Dewey, J. F. *J. geol. Soc. Lond.* **139,** 371–412 (1982).
21. Knill, J. L. in *The British Caledonides* (eds Johnson, M. R. W. & Stewart, F. H.) 99–121 (Oliver & Boyd, Edinburgh, 1963).
22. Anderton, R. *Geol. Soc. Lond. Spec. Publ.* No. 8, 483–488 (1979).
23. Profett, J. M. *Bull. geol. Soc. Am.* **89,** 1389–1403 (1978).
24. Montadert, L., Roberts, D. G. & De Charpal, O. *Init. Rep. DSDP* **Leg 48,** 1025–1060 (1979).
25. Sanderson, D. J., Andres, J. R., Phillips, W. E. A. & Hutton, D. W. H. *J. geol. Soc. Lond.* **137,** 289–302 (1980).
26. Hutton, D. W. H. *Trans. R. Soc. Edinburgh: Earth Sci.* **73,** 151–171 (1983).
27. Litherland, M. *Scott. J. Geol.* **18,** 205–225 (1982).
28. Litherland, M. *Scott. J. Geol.* **16,** 105–123 (1980).
29. Wernicke, B. & Burchfiel, B. C. *J. struct. Geol.* **4,** 105–115 (1982).
30. Haselock, P. J., Winchester, J. A. & Wittles, K. H. *Scott. J. Geol.* **18,** 275–290 (1982).
31. Roberts, J. L. & Treagus, J. E. *Scott. J. Geol.* **13,** 237–254 (1977).
32. Westbrook, G. K. & Barradaile, G. J. *Scott. J. Geol.* **14,** 213–224 (1978).
33. Jackson, J. A. *Nature* **283,** 343–346 (1980).
34. van Breemen, O., Halliday, A. N., Johnson, M. R. W. & Bowes, D. R. *Geol. J. Spec. Iss.* No. 10, 81–106 (1978).
35. Jepsen, H. F. & Kalsbeek, F. *Grønlands geol. Unders. Rapp.* **106,** 7–14 (1981).
36. Powell, D., Brook, M. & Baird, A. W. *J. geol. Soc. Lond.* **140,** 813–832 (1983).
37. Roberts, J. L. & Treagus, J. E. *Scott. J. Geol.* **13,** 165–184 (1977); *Geol. Soc. Lond. Spec. Publ.* No. 8, 199–204 (1979).
38. Hickman, A. H. *Scott. J. Geol.* **14,** 191–212 (1978).
39. Powell, D. *J. geol. Soc. Lond.* **130,** 577–593 (1974).

The gradual decline of the dinosaurs—fact or fallacy?

Dale A. Russell

Paleobiology Division, National Museum of Natural Sciences, Ottawa, Canada K1A OM8

Both great dinosaurian orders (Saurischia and Ornithischia) were major components of terrestrial vertebrate faunas for ~150 Myr. The prevailing view is that dinosaurs attained an evolutionary acme late in the Cretaceous, after which they gradually declined in taxonomic diversity over ~10 Myr to their extinction 63 Myr ago[1–6]. The postulated decline is usually supported by comparing diversity levels in 76 Myr-old and 64 Myr-old dinosaurian assemblages from North America. The resulting differences in diversity have never been compared, however, with those observed between older dinosaurian assemblages, when their extinction was not imminent. I show here that, taken as a whole, the known fossil record of North American dinosaurs shows no evidence of a decline in taxonomic diversity lasting several million years or more before their extinction.

The fossil record of dinosaurs preserved in Triassic to Cretaceous strata in North America is unsurpassed on other continents in terms of the number of specimens that have been recovered, and the degree to which they are representative of successive phases of dinosaurian evolution[7]. Thus, a possible terminal Cretaceous decline in diversity is assessed here through a compilation of all of the known families and genera of North American dinosaurs[8] (see Table 1 and Fig. 1). The data are based on skeletal materials collected largely from the eastern Rocky Mountains and adjacent plains, and from sediments associated with the Newark Rift System extending from Nova Scotia to North Carolina. Only late Triassic, late Jurassic and late Cretaceous terrestrial reptilian faunas have been relatively well sampled. These circumstances, together with the fact that many available collections are inadequately studied, imply that fewer than one-half of the dinosaurian genera which at one time or another were indigenous to North America have been recorded. Nevertheless, the compilation suggests that dinosaurs increased in diversity through the Mesozoic era. Data based on mammalian material collected from the same lithostratigraphic units[9] show similar trends (the differences in slopes of linear regressions for dinosaurian and mammalian genera and families against time are not statistically significant). Dinosaurs show no more evidence of a late Mesozoic decline than do mammals.

Rarefaction analyses[10,11] should be carried out on dinosaurian (and contemporary mammalian) assemblages to remove the effect of sample size on observed diversity. This can seldom be done because of the lack of available data. It is possible, however, in the case of the most diverse dinosaurian assemblage known, that occurring in sediments deposited 13 Myr before the end of the Cretaceous in the vicinity of Dinosaur Provincial Park, Alberta. This locality has also yielded the largest number of dinosaurian skeletal fragments known from any area of badlands of comparable size[12–14]. A rarefaction trend (Fig. 2) was plotted, based on the number and proportions of generically identified, articulated specimens from the Alberta locality.

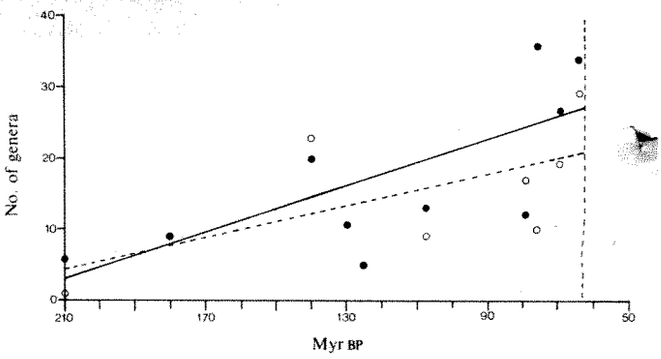


Fig. 1 Generic diversity of North American dinosaurs (closed circles) and mammals (open circles) plotted against time measured in Myr BP. The regression line for dinosaurs (solid line) is $g = 37.1 - 0.16t$, $r = -0.71$, and for mammals (broken line) $g = 27.2 - 0.11t$, $r = -0.61$, where g = number of genera and t = time in Myr. The vertical broken line represents the Cretaceous–Tertiary boundary plotted at 63 Myr BP.

Collections of dinosaur skeletal materials from younger assemblages are smaller[15] and less well studied. The diversity contained within one relatively well studied collection of 20 specimens in the Los Angeles County Museum, from sediments deposited in eastern Montana ~1 Myr before the extinctions[16–20], falls within the 95% confidence interval for the rarefaction curve calculated for the older Canadian site (asterisk, Fig. 2). This is despite the fact that the older locality has been shown to contain an ecological gradient and therefore may have yielded a more than usually diverse assemblage[12–14]. Diversity levels were evidently maintained in the terminal Cretaceous sample from Montana despite environmental changes in the wake of a major retreat of the midcontinental sea which occurred ~1 Myr previously[21,22], but did not recur at the end of the dinosaurian era[23]. The diversity contained within another small collection of a more inland facies from the Scollard Formation of Alberta[24,25] (with apparently 7 genera distributed among 17 specimens) may be similar to that of the Montana sample. No other assemblage of terminal Cretaceous dinosaurs is sufficiently well studied to serve as a useful basis for comparison. The

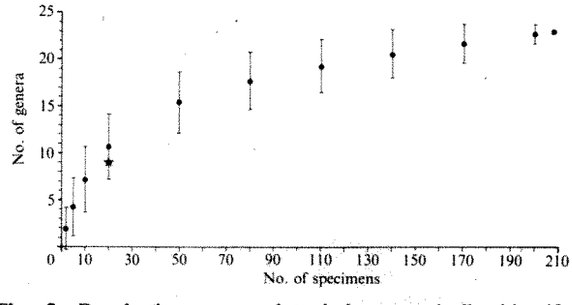


Fig. 2 Rarefaction curve plotted for generically identified dinosaur specimens from Dinosaur Provincial Park, Alberta, dating from ~13 Myr before the terminal Cretaceous extinctions. The asterisk indicates the position of the Hell Creek Fossil Field subsample in the collections of the Los Angeles County Museum dating from ~1 Myr before the terminal Cretaceous extinctions.

0028–0836/84/040360—02$01.00

© 1984 Macmillan Journals Ltd

Table 1 Total numbers of dinosaurian and mammalian genera and families identified in Mesozoic sediments of North America

Marine stage (continental stage)	Absolute age	No. of genera		No. of families	
		Dinosaurs	Mammals	Dinosaurs	Mammals
Upper Maastrichtian (Lancian)	~64	34	29	19	16
Upper Campanian–Lower Maastrichtian (Edmontonian)	~70	27	19	13	13
Upper Campanian (Judithian)	~76	36	10	15	8
Lower Campanian (Aquilan)	~80	12	17	8	11
Aptian–Albian (Paluxian)	~108	13	9	10	7
Valanginian	~125	5	—	5	—
Kimmeridgian–Portlandian	~140	20	23	13	8
Hettangian–Pleinsbachian	~180	9	—	7	—
Carnian	~210	6	1	5	1

The absolute age is given in Myr BP. Mammalian generic diversity levels are probably exaggerated for Kimmeridgian–Portlandian time due to the assignment of separate names to disassociated upper and lower dentitions[39].

Cloverly assemblage from Wyoming and Montana[26–29], which predates the end of the Cretaceous by ~45 Myr and contains 6 genera distributed among at least 50 specimens, is evidently of low diversity for reasons other than its proximity in time to the Cretaceous–Tertiary boundary.

The record of dinosaurian diversity through Mesozoic time in other parts of the world is less complete. A sequence of continental strata in Mongolia, although not well dated, is clearly of late Cretaceous age[30–32]. A succession of three dinosaurian assemblages in these sediments shows no evidence of a decline in diversity[30]. Other potentially excellent continental records[33–35] will require more study before broad trends in dinosaurian diversity become apparent. It is clear, however, that dinosaurs were distributed around the world during terminal Cretaceous time[36].

This compilation of dinosaurian families and genera from Mesozoic strata in North America seems to be in general conformity with contemporary diversity trends seen in terrestrial mammals, marine invertebrates[37] and terrestrial plants[38]. There is no compelling evidence for a terminal Cretaceous decline in dinosaurian diversity on a time scale of millions of years.

I thank F. Asaro for suggesting that a compilation similar to that of Table 1 be made and D. M. Raup for the rarefaction analysis shown in Fig. 2. I also thank W. Alvarez, P. J. Currie, P. Dodson, J. S. McIntosh and K. A. Pirozynski for critically reviewing the manuscript. D. Baird, M. K. Brett-Surman, K. Carpenter, P. Dodson, P. M. Galton, J. R. Horner, W. Langston Jr, R. A. Long and J. S. McIntosh generously provided information on the affinities of numerous dinosaurian specimens.

Received 9 August; accepted 28 October 1983.

1. Archibald, J. D. & Clemens, W. A. *Am. Scient.* **70,** 377–385 (1982).
2. Bakker, R. T. in *Patterns of Evolution as Illustrated by the Fossil Record* (ed. Hallam, A.) 439–468 (Elsevier, Amsterdam, 1977).
3. Buffetaut, E. *Mém. Soc. géol. Fr.* **139,** 47–52 (1980).
4. Schopf, T. J. M. *Geol. Soc. Am. spec. Pap.* **190,** 415–422 (1982).
5. Rozhdestvensky, A. K. in *Evolution and Change of the Organic Realm at the Mesozoic–Cenozoic Boundary* (in Russian) (ed. Menner, V. V.) 57–82 (Academy of Sciences of the USSR, Moscow, 1978).
6. Van Valen, L. & Sloan, R. E. *Evol. Theory* **2,** 37–64 (1977).
7. Colbert, E. H. *Dinosaurs, their Discovery and their World,* 300 (Dutton, New York, 1961).
8. Russell, D. A. *Syllogeus* (in the press).
9. Clemens, W. A., Lillegraven, J. A., Lindsay, E. H. & Simpson, G. G. in *Mesozoic Mammals* (eds Lillegraven, J. A. *et al.*) 7–58 (University of California Press, Berkeley, 1979).
10. Raup, D. M. *Paleobiology* **1,** 333–342 (1975).
11. Tipper, J. C. *Paleobiology* **5,** 423–434 (1979).
12. Béland, P. & Russell, D. A. *Can. J. Earth Sci.* **15,** 1012–1024 (1978).
13. Russell, D. A. *Terra* **21,** 3–9 (1983).
14. Dodson, P. *Mosasaur* **1,** 89–118 (1983).
15. Russell, D. A. *Geol. Ass. Canada spec. Pap.* **13,** 119–136 (1975).
16. Galton, P. M. & Sues, H. D. *Can. J. Earth Sci.* **20,** 462–472 (1983).
17. Molnar, R. E. *J. Paleont.* **52,** 73–82 (1978).
18. Molnar, R. E. *J. Paleont.* **54,** 102–108 (1980).
19. Morris, W. J. *Los Angeles County Mus. Contr. Sci.* **193,** 1–14 (1970).
20. Morris, W. J. in *Athlon, Essays on Palaeontology in Honour of Loris Shano Russell* (ed. Churcher, C. S.) 93–113 (Royal Ontario Museum, Toronto, 1976).
21. Gill, J. R. & Cobban, W. A. *U.S. geol. Surv. prof. Pap.* 776 (1973).
22. Jeletzky, J. A. *Geol. Surv. Canada Pap.* 77-18 (1978).
23. Frye, C. I. *Bull. N. Dak. geol. Surv.* **54,** 65 (1969).
24. Sternberg, C. M. *Geol. Surv. Canada Pap.* 47-1 (1947).
25. Russell, D. A. *Natn. Mus. Canada nat. Hist. Pap.* 36 (1967).
26. Ostrom, J. H. *Bull. Peabody Mus. nat. Hist.* **30,** 165 (1969).
27. Ostrom, J. H. *Bull. Peabody Mus. nat. Hist.* **35,** 234 (1970).
28. Ostrom, J. H. *Mus. Comp. Zool. Breviora* **439** (1976).
29. Sues, H. D. *Palaeontographica* **A169,** 51–72 (1980).
30. Osmolska, H. *Mém. Soc. géol. Fr.* **139,** 145–150 (1980).
31. Fox, R. C. *Geol. Ass. Canada spec. Pap.* **18,** 577–594 (1978).
32. Kielan-Jaworowska, Z. & Sloan, R. E. *Acta palaeont. pol.* **24,** 187–197 (1979).
33. Bonaparte, J. F. *El. Mesozoico de America del Sur y sus Tetrapodes* (Fundacion Miguel Lillo, Tucuman, 1978).
34. Taquet, P. *Mém. Soc. géol. Fr.* **139** (1980).
35. Jacobs, L. L. (ed.) *Aspects of Vertebrate History,* 210 (Museum of Northern Arizona Press, Flagstaff, 1980.
36. Russell, D. A. *Geol. Soc. Am. spec. Pap.* **190,** 401–405 (1982).
37. Sepkoski, J. J. Jr, Bambach, R. K., Raup, D. M. & Valentine, J. W. *Nature* **293,** 435–437 (1981).
38. Knoll, A. H., Niklas, K. J. & Tiffney, B. H. *Science* **206,** 1400–1402 (1979).
39. Prothero, D. R. *Bull. Am. Mus. nat. Hist.* **167,** 277–325 (1981).

Courtship feeding increases female reproductive success in bushcrickets

Darryl T. Gwynne*

Department of Zoology, University of Western Australia, Nedlands, Western Australia 6009

Parental investment theory suggests that investment by males through courtship feeding of their mates may represent an important source of nutrition which increases female fitness and ultimately influences patterns of sexual selection[1,2]. In certain insects males provide nutritional products from reproductive accessory glands during mating; these are either eaten by the female or are absorbed in her genital tract[2,3]. Male bushcrickets (Orthoptera: Tettigoniidae) feed their mates with an elaborate spermatophore consisting of a spermatophylax, which is eaten by the female after mating, and a sperm ampulla, eaten after the ejaculate has emptied[4,5]. Studies of bushcricket mating systems have indicated that spermatophore nutrients are important to females—females prefer to mate with males able to supply larger spermatophores[6]—and field studies of species with very large spermatophores have revealed a role-reversal in reproductive behaviour, with females aggressively competing for males capable of producing spermatophores[7,8]. Although radiolabelling experiments (with several insect species) have demonstrated that male-derived nutrients are incorporated into eggs[3,9–11], these studies do not demonstrate that this sort of courtship feeding enhances female fitness. Here I report the results of an experiment which shows that feeding on the spermatophore enhances the reproductive success of female bushcrickets (*Requena verticalis* Walker) by increasing the numbers and size of eggs produced.

In *R. verticalis*, radiolabelled nutrients from the spermatophore are incorporated to a large degree into maturing eggs[3]. A previous study of this species showed no influence of the amount of male-derived nutrition on fecundity[5]. All females

* Present address: Department of Biology, Erindale Campus, University of Toronto, Mississauga, Ontario, Canada L5L 1C6.

0028–0836/84/040361–03$01.00

© 1984 Macmillan Journals Ltd

Table 1 Effect of number of spermatophylaxes eaten on egg number

No. of spermatophylaxes eaten	0	1	3	7
No. of eggs ($\bar{x}$+s.e.)	31.9±2.5 (32.9)*	45.8±3.1 (46.3)*	59.8±5.1 (59.7)*	70.7±4.7 (69.3)*
Sample size	14	12	12	13

Analysis of variance	Slope	SS	d.f.	MS	F	P
Treatment		9,274.8	3	3,091.6	23.3	<0.001
Covariates:						
Day mated	−0.56	1,513.8	1	1,513.8	11.4	0.002
Pronotum length	9.5	1,089.2	1	1,089.2	8.2	0.006
Explained		14,683.9	5	2,936.8	22.2	<0.001
Residual		5,966.0	45	132.6		

Subjects for the experiment were collected as late instar females in Perth, Western Australia, and reared to adulthood in the laboratory (14 h light: 10 h dark at 25 °C). Nymphs were all fed on a diet of rolled oats, tropical fish food and pollen (17% crude protein) and fresh carrot. Adult females were measured (pronotum length in mm (±0.01 mm)) and mated 7–15 days after the adult moult, over a 41-day period in February–March 1983. For treatment 0, the intact spermatophylax was removed from the ampulla with forceps before the female grasped it (females remove the spermatophylax from the ampulla a few minutes after mating[5]). The female was then placed in a glass tube for 4–5 h (sufficient time to allow sperm supplies to empty) to prevent her from removing the sperm ampulla prematurely[5]. After releasing the female from the tube, I removed the empty ampulla before she could consume it. Additional spermatophylaxes for treatments 3 and 7 were obtained from both treatment 0 matings and other matings set up at the same time. These spermatophylaxes were fed to females within an hour of being produced. Spermatophylaxes received by each female from treatments 3 and 7 were derived from different males. Females from all matings were housed in 1-l jars placed over the oviposition substrate (dry sand) and fed on rolled oats (12% crude protein) and fresh carrot. Eggs were collected from the sand at 7, 14, 21, 28 and 30 days post-mating. Dissection of females revealed that they all had extensive fat body in the abdominal cavity. SS, sum of squares; MS, mean of squares, d.f., degrees of freedom.

* Mean adjusted for the effects of covariates, day mated and pronotum length.

in that study received a high protein diet, with some females receiving one extra spermatophylax at mating. For bushcrickets, limited access to high protein food sources (several subfamilies)[12,16] and up to 12 matings, and thus spermatophores, per female (*Anabrus simplex*)[16] have been documented in nature. Therefore, in the present study females were given a wide range of the male nutrient contribution and were maintained on a lower (12%) protein diet.

Four treatments were established in which the amount of male-derived nutrition was manipulated but the amount of sperm and other ejaculatory materials received by females was controlled. Individuals were all mated once and all matings were conducted over a 41-day period. Treatment numbers represent the number of spermatophylaxes females consumed: hence, treatment 0, the intact spermatophylax was removed after mating and the empty ampulla after insemination (that is, females received no oral nutrition from the male); treatment 1, normal matings in which females were allowed to consume the spermatophylax and ampulla; treatment 3, females were fed two additional spermatophylaxes, one every 6 or 7 days, during the first 14 days after a normal mating; and treatment 7, females were fed six additional spermatophylaxes, one every 2 or 3 days, during the first 14 days following a normal mating. All individuals were allowed to oviposit in dry sand for 30 days after mating. This time period was sufficient to allow the incorporation of a large amount of male-derived nutrition into mature eggs[3]. Females were then killed, numbers of mature ovarian eggs (those with a chorion) were added to the counts of eggs laid and the spermathecae were checked for the presence of sperm. Of 54 individuals, 51 had ample sperm supplies; the three females without sperm were discarded from the analysis (additional details of methods are presented in Table 1 legend).

Analysis of variance of egg number by treatment with size (pronotum length) and day mated (1–41) as covariates revealed a highly significant effect: the number of eggs produced increases with the amount of male-derived nutrition eaten (Table 1). All pairwise comparisons of treatment means were significantly different (Student–Newman-Keuls (SNK) tests, $P<0.05$). The

Table 2 Effect of number of spermatophylaxes eaten on egg weight

No. of spermatophylaxes eaten	0	1	3	7
Mean weight of 5 eggs (mg) ($\bar{x}$+s.e.)	9.7±0.16	10.0±0.18	10.3±0.18	10.6±0.21
Sample size	14	12	12	13

Analysis of variance	Slope	SS	d.f.	MS	F	P
Treatment		4.44	3	1.48	3.48	0.024
Covariates:						
Day mated	−0.005	0.12	1	0.12	0.27	0.604
Pronotum length	0.22	0.59	1	0.59	1.40	0.245
Explained		5.78	5	1.16	2.72	0.031
Residual		19.2	45	0.426		

consumption of the spermatophore meal in the female's first mating represents an increase in numbers of eggs produced of some 40% (compare treatments 0 and 1, Table 1). The covariates, size and day mated, each had a significant effect (positive and negative, respectively) on egg number (Table 1). A preliminary analysis of covariance demonstrated that the slopes of size and day mated on egg number did not differ between treatments ($P>0.05$).

Analysis of variance of egg weight (mean weight of groups of five eggs for each female) by treatment with size and day mated controlled revealed a significant effect of treatment; egg weight increases with the amount of male-derived nutrition eaten (Table 2). Only one pairwise comparison (0 and 7) showed a significant difference (SNK test, $P<0.05$). Neither size nor day mated had a significant effect on egg weight (Table 2). The slopes of these two covariates on egg weight did not differ between treatments (analysis of covariance, $P>0.05$).

Thus, an increase in the number of spermatophores eaten not only enhances female fecundity but also increases the size of the eggs she produces. Larger egg size in insects is known to correlate with several components of offspring fitness[13]. Although an increase in either egg size or number *per se* may, in certain circumstances, not represent an increase in reproductive success[13,14], a concomitant increase in both probably represents increased fitness of females.

Investment in the spermatophore can be regarded as 'parental'[15] as the investment not only enhances female fecundity but is also a cost to the male[1,15]. A spermatophore represents a weight loss of up to 40% for the male bushcricket[7]; in *Orchelimum delicatum* there is a reduction in the size of the spermatophore produced in a second mating 24 h after the first[7]. A cost would be reflected in a decreased ability to invest in further offspring[1] if a reduction in this food donation to the female reduced the numbers and size of those eggs subsequently fertilized by the male.

The advantage to females of obtaining additional spermatophore nutrients provides an adaptive basis for female discrimination of males observed in some species; female *R. verticalis* prevent insemination when no spermatophylax is received from the male[5] and female *Conocephalus nigropleurum* prefer to mate with males able to supply larger spermatophores[6]. Thus, both selection on males to produce fitter offspring and intersexual selection (female choice) may have contributed to the evolution of one of the most elaborate sperm packages known.

I thank W. Bailey, R. Black, C. Codd, I. Dadour, D. Roberts, R. Thornhill for comments on the paper, J. Pryce for help in the laboratory, and R. Black for statistical advice. The work was supported by ARGS (Australia) grant D1-77/15071.

Received 24 May; accepted 7 November 1983.

1. Trivers, R. L. in *Sexual Selection and the Descent of Man, 1871–1971* (ed. Campbell, B.) 136–179 (Aldine, Chicago, 1971).
2. Thornhill, R. *Am. Nat.* **110**, 153–163 (1976).
3. Bowen, B., Codd, C. & Gwynne, D. T. *Aust. J. Zool.* (in the press).
4. Boldyrev, B. T. *Horae ent. Soc. Ross.* **41**, 1–245 (1915).
5. Gwynne, D. T., Bowen, B. & Codd, C. *Aust. J. Zool.* (in the press).
6. Gwynne, D. T. *Anim. Behav.* **30**, 734–738 (1982).

7. Gwynne, D. T. in *Orthopteran Mating Systems: Sexual Competition in a Diverse Group of Insects* (eds Gwynne, D. T. & Morris, G. K.) 337–366 (Westview, Boulder, 1983).
8. Gwynne, D. T. *Science* **213**, 779–780 (1981).
9. Boggs, C. & Gilbert, L. E. *Science* **206**, 83–84 (1979).
10. Mullins, D. E. & Keil, C. B. *Nature* **283**, 567–569 (1980).
11. Schal, C. & Bell, W. J. *Science* **218**, 170–172 (1982).
12. Gangwere, S. K. *Trans. Am. ent. Soc.* **87**, 67–203 (1961).
13. Capinera, J. L. *Am. Nat.* **114**, 350–361 (1979).
14. Smith, C. C. & Fretwell, S. D. *Am. Nat.* **108**, 499–506 (1974).
15. Gwynne, D. T. in *Sperm Competition and the Evolution of Animal Mating Systems* (ed. Smith, R. L.) (Academic, New York, in the press).
16. Gwynne, D. T. *Evolution* (in the press).

Stress-induced alignment of division plane in plant tissues grown *in vitro*

Philip M. Lintilhac & Thompson B. Vesecky

Botany Department, University of Vermont, Burlington, Vermont 05405, USA

The architectural nature of cell patterns in plants, which are visible under the light microscope, offers strong intuitive evidence for a structural relationship between mechanical forces acting through the tissue and cell wall orientation at cytokinesis. However, a direct relationship between the two has never been convincingly established. We find that compressive forces at intensities between 500 and 800 mg mm^{-2} can induce a conspicuous spatial ordering of division activity when applied to sterile plant tissues during continued growth in culture; this results in cambium-like areas of cell wall co-planarity which are clearly distinguishable from the characteristically disorganized growth of unstressed regions of the explant, and implies the presence of an intracellular structural–mechanical sensor capable of directing the installation of the new cell partition. The experiment reported here, in which compressive forces were applied to plant tissue growing *in vitro*, provides an example of mechanical control of division plane.

Cell division in multicellular plant tissues is characterized by a telophase structure known as the cell plate[1] which is an early stage of partition wall formation. The role of cell plate orientation in the development of form in plants is clear as repeated parallel divisions increase the number of cells in a file, thereby contributing to the directional accumulation of tissue mass. Such file cells also tend to elongate perpendicular to the new cross walls, which means that the overall cross-wall orientation of the cells comprising an unexpanded organ also predicts the direction of elongation of the organ as a whole.

We propose that the final orientation of the cell plate is adjusted according to extracellular stress–mechanical cues until it assumes an orientation which is architecturally functional, that is, maximally protected from shear[2]. According to our hypothesis, mechanical forces transmitted through the tissue are directly sensed at the cellular level, so that each dividing cell responds to its own stress–mechanical environment with the erection of an appropriately oriented partition wall.

If this is true, then dividing cells subjected to external forces acting in a given direction should respond by orienting new partition walls consistently with respect to the direction of force propagation. This has been shown for whole plants *in vivo*[3], but never for isolated tissues growing *in vitro*. Cultured plant tissues subjected to exogenous forces should provide particularly clear-cut support for this hypothesis because: (1) They may be considered to be largely stress-relieved after explanting and transfer to sterile culture, so that any induced order can be unequivocally attributed to the stresses and strains generated by the experimentally applied forces. (2) The physiological environment of growth can be more closely controlled, making it more difficult to attribute induced order to concerted hormonal responses and morphogen gradients which could be organized by a whole plant. (3) Tissues can be chosen which are virtually homogeneous and devoid of structurally specialized cell types which could modify the transmission of forces through the tissue. The freshly explanted pith tissue used here is homogeneous and regular, but not ordered into ranks and files as are the products of an *in vivo* vascular cambium (Fig. 1*A*). Thus, the experimentally induced co-planarity cannot be ascribed to pre-existing order in the explant.

Fresh pith cores weighing ~25 mg were taken from the second internode of greenhouse-grown *Nicotiana tabacum L.* (Fig. 1*A*) with a square coring tool, providing a square pith explant 1 mm on a side and of indefinite length. The apical centimetre of this explant was removed and 'planted' in its original orientation in an agar-solidified medium, which was then layered with 50 ml of identically constituted liquid medium. We used a standard defined medium[4] with added indole 3-acetic acid (IAA; 10 mg l^{-1}) and kinetin (0.4 μg l^{-1}), and incubated the explants at 28 °C.

After a 24-h initial accommodation period to allow for a transient burst of elongation growth, compressive forces were applied to the tissue by means of a pneumatic forcing frame

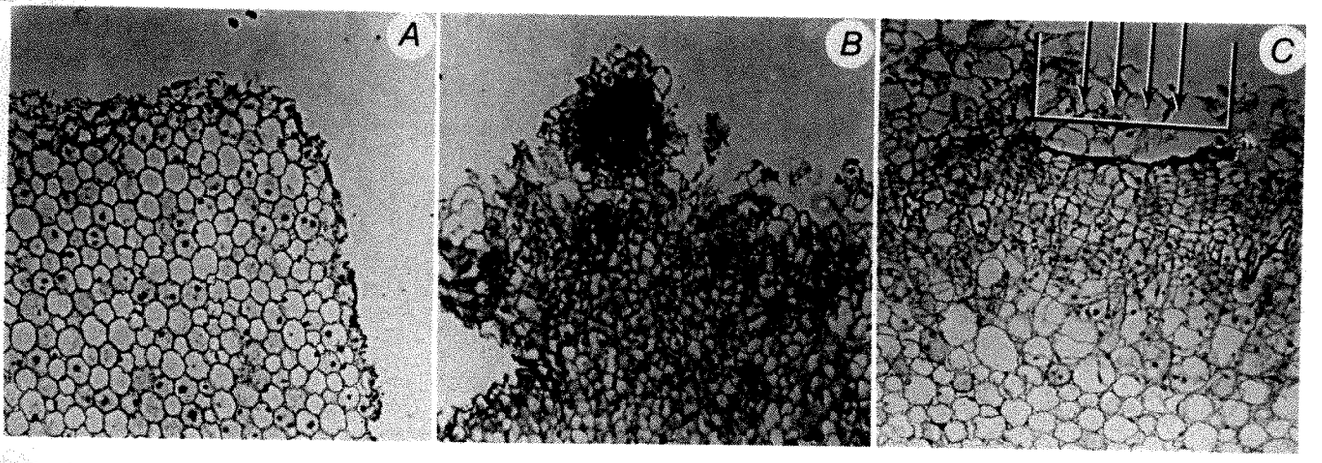


Fig. 1 Transverse sections of cultured and uncultured explants of *Nicotiana* pith. ×100. *A*, The initial condition of the explant before transfer into sterile culture. Note the lack of differentiated structural or vascular elements and of regular, co-oriented partition walls. Interfascicular cambium is absent from this level of the stem. *B* And *C* show aspects of an equivalent explant after 5 days in a standard defined medium. *B* Shows unrestrained callus proliferating from one corner of the explant; note the random proliferation, with little evidence of repeated divisions in any one plane. *C* Shows another area of the same section, growing under continuous compressive stress applied through a narrow Teflon interface (arrows). Note the radially aligned files of cells extending from the original pith core out to the interface surface. These files of cells result from repeated, co-planar divisions of pith-derived cells, constrained by 0.075 bar compressive stress. Parallel development gives the region a cambium-like appearance. Some evidence of xylem differentiation can be seen at a level 6–8 cells internal to the interface. Some loose callus cells have slumped into the interface area during fixation.

0028-0836/84/040363—02$01.00

© 1984 Macmillan Journals Ltd

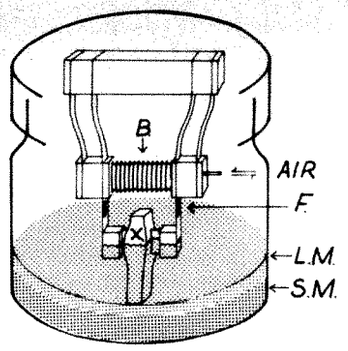


Fig. 2 Schematic representation of a pneumatic forcing-frame developed for continuous, long-term compressive loading of tissues in sterile culture. The sterilizable assembly hangs from the lid of a standard culture dish. Bellows B is the actuator, connected to an external peristaltic pump which reversibly meters small amounts of air into the bellows. A continuous matching of the applied force to a preset force set-point is accomplished by an external feedback control circuit (not shown). Forces applied to the tissue are measured by resistive strain gauges at F, which act as force transducers. The tissue explant is partly embedded in a layer of solidified medium (SM), over which is poured a layer of liquid medium (LM). Partly submerged Teflon interface pieces attached directly to the force transducers apply forces directly to the tissue at the meniscus. Each of the opposed contact surfaces measures 10.0×0.5 mm.

(Fig. 2). In the experiment reported here the frame squeezed opposing vertical faces of the explant.

A rapidly developing callus growth at the air–liquid meniscus soon supported the entire load generated by the forcing frame. At the end of the experiment, forces of $0.75g$ were supported by an area of callussed explant surface somewhat larger than 1 mm^2, generating compressive stress intensities of 0.075 bar at the contact surface. After 5 days of active proliferative growth while being subjected to continuous, controlled compressive stress, the explant, now weighing ~250 mg, was fixed and sectioned in a plane perpendicular to its long axis.

The effective resolution of force measurement in our system is ±10 mg. There is also a cumulative error due to instrumental drift, which in the experiment presented here resulted in the gradual increase in applied force from an initial value of 500 mg to a final value of 750 mg after 5 days. These instrumental difficulties, together with the fact that not all explants are completely alike or grow equally rapidly and uniformly, have prevented us from obtaining a true picture of the repeatability of our result. Nevertheless, in all explants where compressive stresses between 500 and 800 mg mm^{-2} were applied to actively dividing cells, co-planar divisions were evident, although not always extending uniformly across the interface surface. Further development of our instrument and methods should allow us to routinely effect cell alignments like those illustrated here.

Fig. 1*C* shows that co-planar divisions immediately internal to the interface surface accumulated to a depth of 10 ranks of cells, giving the load-bearing region of the explant an appearance similar to that produced by cambial activity. By comparison, unloaded regions of the same section show the largely disorganized callus growth behaviour typical of this tissue in this medium (Fig. 1*B*).

The proliferation of the explanted tissue at the meniscus is presumably due to an interplay of factors from the medium and from the air which provide an optimum of nutrition, gas exchange and surface effects. However, the proliferation encircles the explant at the meniscus, whereas the region of co-planar divisions is restricted to those parts of the proliferation supporting the directional compressive forces generated by the forcing frame. This implies that mechanical stimulation is the principal effector responsible for the co-planar orientation of newly installed cell partitions in this experimental system.

Although many plant tissues, when explanted into sterile culture, can be induced to undergo a normal morphogenesis, most will revert temporarily to a disorganized growth form termed callus. Tobacco pith is one of these. We propose that this behaviour is due to the loss of the *in vivo* mechanical environment of growth, which no amount of *in vitro* nutritional or hormonal manipulation can restore. The experiment reported here is a direct attempt to re-establish this lost mechanical environment. The distribution, coherence and regularity of divisions in the example presented here are difficult to explain by a diffusively maintained prepattern or positional signal[5] which would have to be focused through the tissue and interpreted by the individual cells in such a way as to orient new partition walls with strict regard to external landmarks.

A mechanism responding directly to stress–mechanical stimuli can refer to the intracellular physical effects of the stresses and strains propagating through the tissue for local spatial cues, and requires only that each cell is provided with an intracellular sensor which can react in predictable ways to the slight cellular dimension changes resulting from the directional transmission of force through the tissue[2]. The regularity of the response then becomes simply a function of the predictability of stress propagation through the tissue, which in turn depends on the quality of the mechanical coupling of the tissue provided by the shared cell walls. The need to assign unique positional values to each cell[5] disappears, as the stress–mechanical environment of each cell is necessarily location-specific.

Throughout the plant kingdom, growth and development provide repeated examples of highly ordered and conserved division sequences which offer *prima facie* evidence of structural adaptation at the tissue level. Although this is one of the oldest and most recurrent themes of plant science[6,7], a solid experimental and theoretical basis for understanding the structural sensitivities of individual plant cells is absent from the literature.

We hope that the direct manipulation of the plane of division in actively growing plant tissues *in vitro* will lead to a more precise understanding of developmental events in plants by providing a tool for the dissection of the constraints which govern cell wall patterns.

Received 25 August; accepted 2 November 1983.

1. Hepler, P. K. *Protoplasma* **111,** 121–133 (1982).
2. Lintilhac, P. M. in *Positional Controls in Plant Development* (ed. Barlow, P.) (Cambridge University Press, in the press).
3. Lintilhac, P. M. & Vesecky, T. *Am. J. Bot.* **67,** 1477–1483 (1980).
4. Murashige, T. & Skoog, F. *Physiologia Pl.* **15,** 473–497 (1962).
5. Holder, N. *J. theor. Biol.* **77,** 195–212 (1979).
6. Kny, L. *Jb. wiss. Bot.* **37,** 55–98 (1902).
7. Sinnott, E. W. *Plant Morphogenesis,* 43–54 (McGraw-Hill, New York, 1960).

Prevention of gastrulation but not neurulation by antibodies to fibronectin in amphibian embryos

Jean Claude Boucaut*, Thierry Darribère*, Habib Boulekbache† & Jean Paul Thiery‡

* Laboratoire de Biologie Expérimentale, Université René Descartes, 45, rue des Saint Pères, 75270 Paris, Cedex 06, France
† Equipe de Biologie du Développement, Université Paris 7, 2, place Jussieu, 75221 Paris, Cedex 05, France
‡ Institut d'Embryologie du CNRS et du Collège de France, 49 bis avenue de la Belle Gabrielle, Nogent-sur-Marne 94130, France

Gastrulation and formation of the neural plate are major steps in early vertebrate embryogenesis. Although morphogenetic movements leading to the formation of the primary germ layers have been extensively described[1–3], the mechanisms governing migration of mesodermal cells and their interactions with ectoderm remain ill-defined. A large body of evidence indicates that fibronectin (FN), a high molecular weight cell-surface-associated glycoprotein, promotes cell adhesion and cell migration throughout embryogenesis[4–6]. FN has been detected at an early blastula stage in *Pleurodeles waltlii*[7]. We now show that FN is a component of a dense fibrillar matrix underlying the blastocoel roof; in contrast, the exterior surface of the embryo

0028–0836/84/040364—04$01.00
© 1984 Macmillan Journals Ltd

is devoid of FN. Microsurgical inversion of part of the blastocoel roof does not prevent mesodermal cell migration except at the site of inversion where no FN matrix is available. Perturbation experiments using antibodies to FN demonstrate that the invagination of presumptive mesodermal cells does not occur when the monovalent antibodies are injected before or at the onset of gastrulation; on the other hand, the formation of a neural plate is not prevented when late gastrula stage embryos are treated with antibodies to FN. We conclude that the presence of FN is required for cell migration during gastrulation.

Antibodies directed against chick FN have been previously used to demonstrate the presence of FN on the inner surface of the ectoderm of gastrulating *Pleurodeles* embryos[7]. Specific antibodies to *Ambystoma* mexicanum plasma FN were prepared (Fig. 1) to improve the immunofluorescence staining. In whole-mount ectodermal explants, we could visualize a dense fibrillar FN-rich extracellular matrix on the inner surface of the blastocoel roof (Fig. 2*a–c*). In contrast, no FN could be detected (Fig. 2*c*) on the outer surface of the embryo.

To probe for a potential role of the extracellular matrix in gastrulation, part of the roof of the blastocoel was grafted inside-out near the site of invagination (Fig. 2*d*). In these embryos pioneer migrating mesodermal cells avoided the grafted region. At a later stage, the confluent mesodermal layer that formed uniformly beneath the ectoderm was found to overlap this region without adhering to it (Fig. 2*e*).

Injection of monovalent antibodies to FN into the blastocoel cavity just before gastrulation prevented the invagination of mesodermal cells. The gross appearance of the embryo (Fig. 2*f*) was strikingly different from that of the control (Fig. 2*g*). The blastocoel roof gave rise to a highly convoluted structure, possibly because it could not undergo normal epiboly. The lower surface corresponded to the non-invaginated presumptive mesodermal and endodermal cells. Only a very few pioneer migrating cells were found on the inner surface, which remained mostly naked (not shown).

Antibodies to FN were found to bind to the FN fibres at the surface of the blastocoel for at least 12 h after injection (not shown). Table 1 summarizes the results obtained at different stages before and during gastrulation. When monovalent antibodies to FN were injected in the blastocoel cavity at the blastula or early gastrula stages, a clear-cut inhibition of gastrulation was observed within 6 h. This phenomenon lasted for 24 h and led to abnormal development of the embryo as shown in Fig. 2*f*. Interestingly, when gastrulation was almost completed, injection of antibodies to FN did not affect neurulation. Some embryos did not survive 24 h; this was not due to a nonspecific toxic effect of the antibodies, since no cytolysis was observed

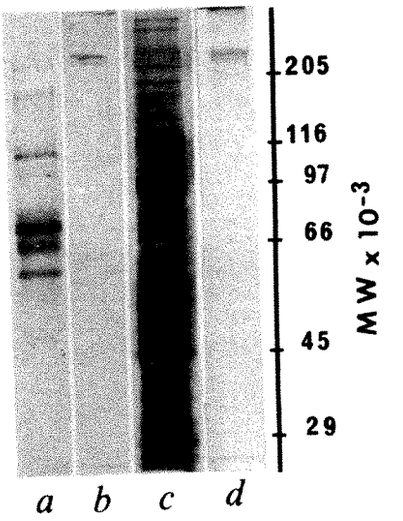


Fig. 1 Specificity of antibodies directed against *Ambystoma mexicanum* FN. *Ambystoma mexicanum* plasma FN was purified by affinity chromatography on a gelatin Sepharose column[20] and high titre (1:6,000 dilution for immunofluorescence labelling) antibodies were raised in rabbits. IgG and Fab' monovalent fragments were prepared according to Brackenbury *et al.*[21], 10% polyacrylamide gel electrophoresis[22] and immunoblotting[23]. *a*, *b*, Plasma from adult *Ambystoma mexicanum*. *a*, Several major bands are detectable after Coomassie blue staining. *b*, Only one close doublet of molecular weight (MW) 220,000 was revealed after transfer to nitrocellulose sheets, followed by incubations with rabbit IgG anti-FN (2 μg ml^{-1}) and Protein A coupled to peroxidase (5 μg ml^{-1}) and revealed with diaminobenzidine in the presence of H_2O_2 (standard MWs correspond to myosin (205,000), galactosidase (116,000), phosphorylase *b* (97,000), bovine serum albumin (66,000), ovalbumin (45,000) and carbonic anhydrase (29,000). *c*, *d*, Electrophoresis of *Pleurodeles waltlii* mid-gastrulae embryos solubilized in SDS sample buffer[22]. *c*, Coomassie blue staining shows more than 50 bands. *d*, Only one doublet of MW 220,000 reacted with anti-FN antibodies. The protein recognized both in the plasma and in the protein mixture extracted from embryos co-migrated with purified plasma FN. These antibodies were considered to be monospecific and were not used routinely as affinity purified because their titre decreased considerably with this procedure which prevented inhibition experiments.

Table 1 Effects of monovalent fibronectin antibodies on the gastrulation of *Pleurodeles waltlii* embryos

	Stages	6 h		24 h	
		Normal (%)	Blocked (%)	Normal (%)	Blocked (%)
Fab'anti FN	Blastula (20)	20	80	20	80 (25)
	Early gastrula (16)	—	100	—	100 (18)
	Late gastrula (15)	80	20	80	20
Preimmune Fab'	Blastula (20)	100	—	100	—
	Early gastrula (25)	100	—	100	—
Fab'anti FN+FN	Blastula (25)	90	10	90	10
	Early gastrula (30)	93	7	93	7
BSA	Blastula (30)	100	—	100	—
	Early gastrula (15)	100	—	100	—
Steinberg solution	Blastula (12)	100	—	100	—
	Early gastrula (15)	100	—	100	—

Embryos were injected at three stages of development as described in the legend to Fig. 2 (number of treated embryos for each stage is given in parentheses); in all cases, monovalent antibodies and bovine serum albumin (BSA) were used at 20 mg ml^{-1}; the final concentration (4–10 mg ml^{-1}) in the blastocoel cavity cannot be estimated rigorously. With antibodies to fibronectin, the blastocoel roof of most embryos treated before or at an early stage of gastrulation developed into convoluted structures as in Fig. 2*f*. Partial necrosis was observed after 24 h in some embryos (percent indicated in parentheses) in which mesodermal cell migration did not occur.

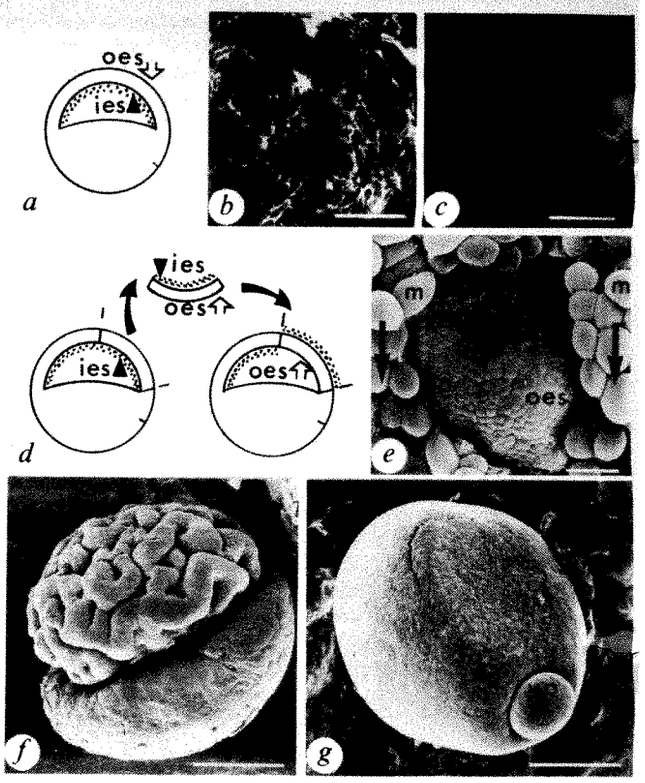


Fig. 2 Distribution and role of FN in *Pleurodeles waltlii* gastrulation. ***a–c***, Immunofluorescence labelling for FN: 4% formaldehyde-fixed or living ectoderm explants were incubated in anti-FN IgG (10 μg ml^{-1}) for 1 h followed by fluorescein isothiocyanate FITC-conjugated sheep anti-rabbit antibodies diluted 1:100 in 0.5% bovine serum albumin. Explants were whole-mounted in 90% glycerol, examined with epi-illumination through a Leitz Dialux 20 microscope and photographed with Kodak Tri-X films. *a*, Schematic representation of an early gastrula inner (filled triangle) and outer (open arrow) surface of the blastocoel roof. *b*, Staining for FN on the inner surface of the blastocoel roof. An extensive FN-rich fibrillar matrix covers the inner surface of the ectoderm layer. *c*, Unlike the inner surface, the outer surface of the blastocoel shows no fluorescence for FN. ***d, e***, Inverted explants were grafted to determine whether the FN-rich extracellular matrix mediates the migration of mesodermal cells. *d*, Schematic representation of the grafting procedure. An inverted ectodermal explant was grafted on the future dorsal side of the embryo above the blastoporal lip. The position of invaginated mesodermal cells was checked after gastrulation had progressed. *e*, Scanning electron micrographs of the blastocoel roof in a gastrula bisected 3 h after grafting. The mesodermal cells attach to the inner surface of ectodermal cells but do not adhere to the outer ectodermal surface of the graft. The overlapping cohesive sheet of mesodermal cells that follows the pioneer cells has been removed. It is clear that adhesion has not taken place between the migrating mesodermal cells and the outer surface of the ectodermal explant (arrows, direction of migration). ***f, g***, Injection of monovalent antibodies to FN into the blastocoel. 200 nl of a 20 mg ml^{-1} solution of Fab' anti-FN were injected into *Pleurodeles waltlii* blastulae or gastrulae. Similar amounts of preimmune Fab' or bovine serum albumin were injected as controls. Scanning electron micrographs were made 24 h after injection of blastulae. *f*, Gastrulation is almost completely inhibited when antibodies to FN are used; the blastocoel roof becomes folded extensively into disorganized convolutions. In contrast, the inner surface of the ectoderm remains smooth (not shown). The part of the embryo which should have invaginated is found as a cap. A very limited invagination can be observed at the boundary between the cap and the superficial ectoderm. This limited invagination can be visualized after removal of the ectoderm; however, mesodermal cells did not develop many filopodia and lamellipodia over the surface of the ectoderm and occupy only a very limited area as opposed to their homogeneous distribution over the entire surface of the ectoderm during normal development (not shown). *g*, Embryos injected with monovalent preimmune antibodies develop normally with only a small yolk plug (yp) at the site of invagination. For scanning electron microscopy, embryos or explants were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, *p*H 7.4–7.6, at room temperature and postfixed in 1% OsO_4 in the same buffer. Samples were dehydrated through a graded ethanol series, critical point dried, coated with gold, examined with a Jeol JSM-35 scanning electron microscope at 25 kV and photographed with Ilford FP4 film. Scale bars: *b, c*, 20 μm; *e*, 100 μm; *f, g*, 320 μm. ies, Inner ectodermal surface; m, mesodermal cell; oes, outer ectodermal surface; yp, yolk plug.

24 h after injection of the same amount at the late gastrula stage. In control experiments, all the embryos reached the neurula stage when injected with preimmune monovalent antibodies, with Steinberg solution or with bovine serum albumin; in addition embryos cultivated in the presence of antibodies to FN develop normally (not shown). Most importantly, when antibodies to FN were preincubated with *Ambystoma mexicanum* FN, gastrulation proceeded normally (Table 1).

In gastrulating amphibian embryos, the presence of an extracellular matrix was suggested only recently[8,9]. Its early appearance was documented *in vivo* using scanning electron microscopy and a possible function as a guiding structure for migrating mesodermal cells was suggested[10]. Migration of amphibian mesodermal cells was also observed *in vitro* on the extracellular matrix transferred from ectoderm explants[11]. However, although it has been reported that FN mediates adhesion of mesodermal cells *in vitro*, it has not been shown so far that these cells utilize FN *in vivo* as a substrate for migration[12]. We have previously shown that FN is present at the blastula stage[7] and we establish here that it is a component of the fibrillar extracellular matrix within which mesodermal cells migrate.

The presence of the extracellular matrix could be required for attachment in grafting experiments (Fig. 2*d*); however, this experiment could merely indicate an ability of mesodermal cells to adhere only to basal surfaces. The importance of FN was clearly assessed by using specific monovalent antibodies to FN. Nevertheless we cannot exclude that other ECM components may also be essential for attachment and migration of mesodermal cells. It should be stressed that in itself FN does not trigger the process of invagination since it is already incorporated into the extracellular matrix prior to gastrulation. The early deposition of FN was also observed in chick embryos before gastrulation[13–15] and before neural crest cell emigration[16]. It is likely that mesodermal cells have an intrinsic ability to respond to the FN-rich matrix with which they interact directly. However, it is not clear whether the matrix fibrils are oriented in the direction of movement. Rather, confluency at all stages of migration may allow the cells to migrate unidirectionally as a result of population pressure, a mechanism also suggested by Kubota and Durston[17] for amphibians and by Rovasio *et al.*[5] for avian neural crest cells.

Once migration of mesodermal cells was almost complete, antibodies to FN did not perturb the formation of a neural plate. However, we cannot exclude that antibodies to FN introduced at the late gastrula stage had no effect since interactions between mesodermal and ectodermal cells leading to neurulation may not last more than several hours[18]. Nevertheless, the presence of a FN-rich extracellular matrix does not seem to be required, as a direct association of mesodermal cells with the outer surface of the ectoderm leads to neurulation[19].

This work was supported by grants from the Ministère de l'Industrie et de la Recherche (82 E 1139, 81-E1082) and the Ministère de l'Education Nationale. Professors M. Jacobson and L. Saxen and Drs K. Yamada and J. Smith made a critical reading of the manuscript. We thank L. Addade and P. Grou for technical assistance.

Received 17 June; accepted 2 November 1983.

1. Holftreter, J. *J. exp. Zool.* **94**, 261–318 (1943).
2. Holftreter, J. *J. exp. Zool.* **95**, 171–212 (1944).
3. Vakaet, L. *Archs Biol.* **81**, 387–426 (1970).
4. Hynes, R. O. & Yamada, K. M. *J. Cell Biol.* **95**, 369–377 (1982).

5. Rovasio, R. A., Delouvee, A., Yamada, K. M., Timpl, R. & Thiery, J. P. *J. Cell Biol.* **96**, 462–473 (1983).
6. Wylie, C. C. & Heasman, *J. Phil. Trans. R. Soc.* **B299**, 177–183 (1982).
7. Boucaut, J. C. & Darribere, T. *Cell Tissue Res.* **234**, 135–145 (1983).
8. Tarin, D., *J. Embryol. exp. Morph.* **26**, 543–570 (1971).
9. Keller, R. E. & Schoenwolf, G. C. *Wilhelm Roux' Arch. dev. Biol.* **182**, 165–186 (1977).
10. Nakatsuji, N. & Johnson, K. E. *J. Cell Sci.* **59**, 61–70 (1983).
11. Nakatsuji, N. & Johnson, K. E. *J. Cell Sci.* **59**, 43–60 (1983).
12. Nakatsuji, N. & Johnson, K. E. *Cell Motil.* **2**, 149–161 (1982).
13. Critchley, D. R., England, M. A., Wakely, J. & Hynes, R. P. *Nature* **280**, 498–500 (1979).
14. Sanders, E. J. *J. Embryol. exp. Morph.* **71**, 155–170 (1982).
15. Duband, J. L. & Thiery, J. P. *Devl Biol.* **94**, 337–350 (1982).
16. Newgreen, D. & Thiery, J. P. *Cell Tissue Res.* **211**, 269–291 (1980).
17. Kubota, H. Y. & Durston, A. J. *J. Embryol. exp. Morph.* **44**, 71–80 (1980).
18. Duprat, A. M., Gualandris, L. & Rouge, P., *J. Embryol. exp. Morph.* **70**, 171–187 (1982).
19. Gualandris, L., Rouge, P. & Duprat, A. M., *J. Embryol. exp. Morph.* (in the press).
20. Engvall, E. & Ruoslahti, E. *Int. J. Cancer*, **20**, 1–5 (1977).
21. Brackenbury, R., Thiery, J. P., Rutishauser, U. & Edelman, G. M. *J. biol. Chem.* **252**, 6835–6840 (1977).
22. Laemmli, U. K. *Nature* **227**, 680–689 (1970).
23. Towbin, M., Staehelin, T. & Gurdon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354 (1979).

Cultivation of the liver forms of *Plasmodium vivax* in human hepatocytes

Dominique Mazier*, Irene Landau†, Pierre Druilhe*, François Miltgen†, Christiane Guguen-Guillouzo‡, Doanh Baccam†, Joyce Baxter†, Jean-Paul Chigot§ & Marc Gentilini*

* Département de Parasitologie et de Médecine Tropicale Groupe Hospitalier Pitié-Salpêtriere, 47 boulevard de l'Hôpital, 75013 Paris, France
† Laboratoire de Zoologie-Vers, Associé au CNRS, Muséum National d'Histoire Naturelle, 61 rue de Buffon, Paris Cédex 05, France
‡ Unité de Recherches Hépatologiques-U 49, INSERM, Hôpital Pontchaillou, 35011 Rennes, France
§ Service de Chirurgie, Groupe Hospitalier Pitié-Salpêtrière, 48 boulevard de l'Hôpital, 75013 Paris, France

The blood schizogonic cycle of human malaria parasites has thus far been the most exhaustively studied phase of parasite development. However, before entering red blood cells (RBCs), the parasite undergoes its first multiplication not in blood, but in hepatic cells. These hepatic stages were the last to be discovered[1] and only a few studies have been performed in humans and other primates[1–7]. Despite recent advances[8–11], *in vivo* studies have limitations and other approaches such as cultures of these liver forms may be necessary to investigate their chemosensitivity and their biochemical or immunological properties. Recently, sporozoites of species of rodent malaria have been made to infect cultured cell lines[12–15] or primary hepatocyte cultures[16–18]. We report here that the complete cycle of the human malaria parasite *Plasmodium vivax* can be obtained in primary cultures of human hepatocytes up to release of merozoites able to penetrate RBCs.

A new method for culturing metabolically active human hepatocytes using enzymatic perfusion has recently been described[19,20]. In the present experiment, we modified this method by canulating and perfusing a small blood vessel of a liver biopsy fragment obtained for diagnostic purposes. Preparation of hepatocyte monolayers and culture conditions are the same as previously described using rodent liver cells[17] except that the hepatocytes were seeded at a concentration of 5×10^5 cells per 35-mm Falcon Petri dish in 1.5 ml supplemented minimal essential medium. Such hepatocyte monolayers can easily survive for 1–2 weeks in conventional culture conditions or for several weeks with maintenance of high levels of specific functions when co-cultured with a rat liver epithelial cell line[20] (Fig. 1). In this experiment, since a liver biopsy fragment was obtained 2 days before availability of sporozoites, only four out of eight hepatocyte cultures were set up as co-cultures with epithelial cells (added at a concentration of 5×10^5 per dish) while the remaining four were set up as monocultures.

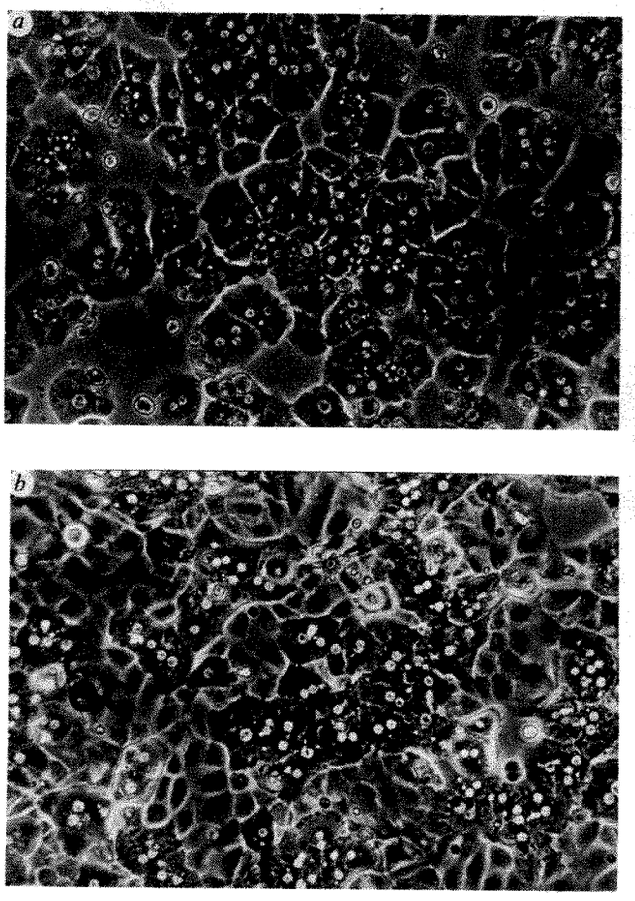


Fig. 1 *a*, Hepatocytes in monoculture (h). Note the flattened appearance of these cells. In monoculture, the hepatocytes will only remain fully functional for a short period (less than 2 weeks). *b*, Hepatocytes co-cultured with liver epithelial cells (ec). Hepatocytes appear more rounded and organized into cord-like groups. In these conditions the hepatocytes remain functional for up to 2 months. (×200.) Phase-contrast micrograph.

Sporozoites of *P. vivax* were obtained from *Anopheles stephensi* mosquitoes infected by membrane feeding with 10 ml of citrated blood containing gametocytes (5×10^7 ml^{-1}) collected from a patient who had been infected in India. Examination of mosquito midguts on day 11 following the blood meal showed that 15% of them were infected. On day 16, the mosquito salivary glands were aseptically dissected in culture medium. The culture supernatant from each Petri dish was decanted and groups of 10 pairs of salivary glands in 80 μl medium were added to each dish. The preparations were incubated for 45 min at 37 °C, with 5% CO_2 and 95% air after which 0.5 ml of medium was added, followed by an additional 1 ml 5 h later. Subsequently, the medium was changed daily. In one culture fixed on day 5 following sporozoite inoculation and stained with Giemsa, an exo-erythrocytic schizont of small size (10 μm) was identified. In two of three monocultures fixed on day 7 of culture, intracellular schizonts of various sizes were seen (Fig. 2*a–d*), but only a few were observed in the third culture. The larger ones measured 30 μm and contained up to 200–300 nuclei. Smaller schizonts ranged from 10 to 25 μm. The smallest was 5 μm and contained five nuclei. They were generally located close to the nucleus of the host cell which appeared unaltered by the presence of the schizont except for a condensed area surrounding a clear space around the schizont. Schizonts could be seen over the entire area of the culture but were more numerous in the vicinity of the introduced salivary glands. The number of schizonts seen was relatively high (about 30 in each of two dishes).

0028-0836/84/040367—03$01.00

© 1984 Macmillan Journals Ltd

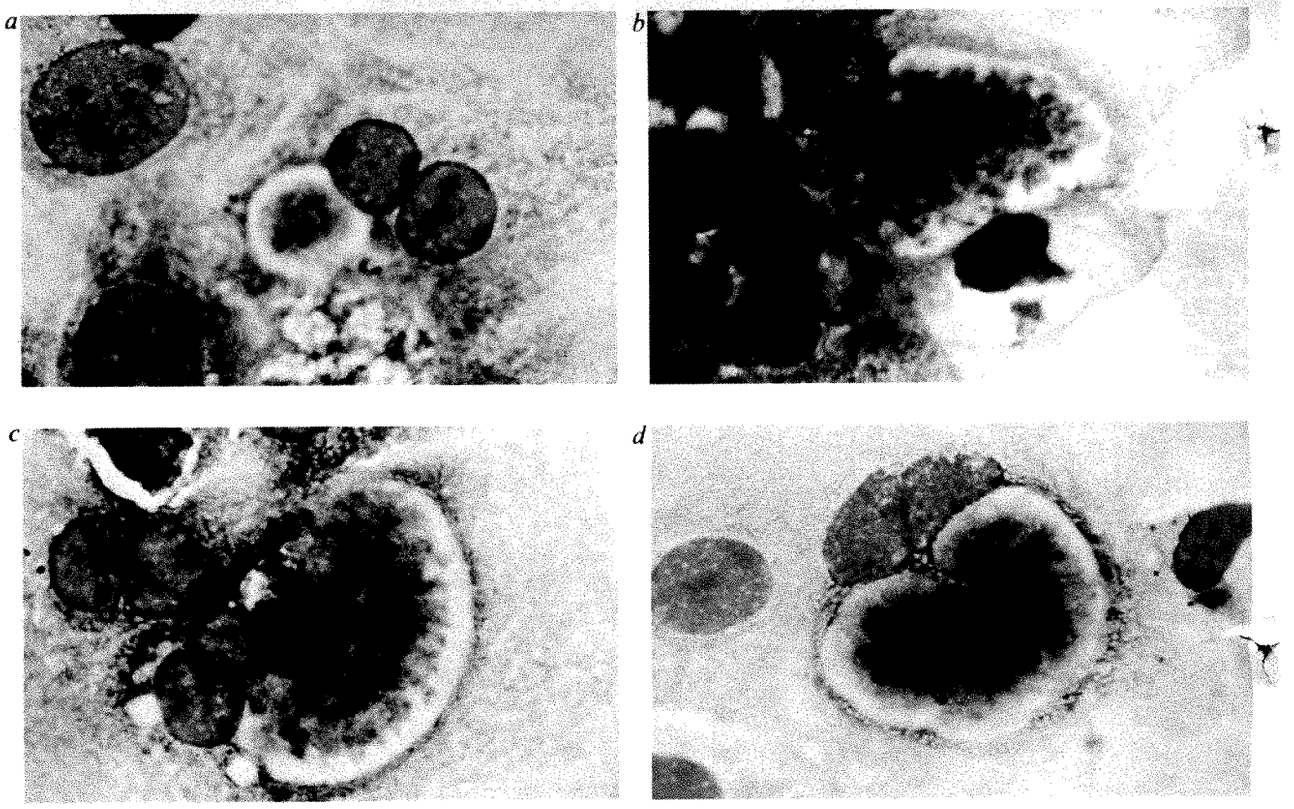


Fig. 2 Seven-day exo-erythrocytic parasites of *P. vivax* in cultural hepatocytes (×1,300). *a*, Early schizont with only a small number of nuclei. *b*, Maturing schizont with a greater number of nuclei. *c*, *d*, Late schizont undergoing segmentation. Giemsa staining.

To establish unequivocally whether the hepatic schizonts could achieve full maturity *in vitro*, we added RBCs to two hepatocyte co-culture dishes on day 10. Because *P. vivax* preferentially penetrates young RBCs, blood collected from a patient with a 30% reticulocytosis was used. Following two washes in culture medium, this blood was added at a concentration of 10^8 blood cells per culture dish. Thin blood films were made on days 12 and 14. Numerous intra-erythrocytic *P. vivax* parasites were identified on both preparations after staining with Giemsa. Most of the intra-erythrocytic forms were rings, although some were trophozoites containing malarial pigment, and one schizont with eight nuclei was seen. Schüffner's dots were present in many of the infected cells. In the hepatocytes of this latter culture dish fixed on day 14 no intra-hepatocytic parasite could be unequivocally identified.

No uninucleated body of recognizable parasite origin was seen in any of the dishes fixed on days 5, 7 and 14. However, we emphasized that the uninucleated liver forms of *P. vivax* known as hypnozoites, which have been described recently in the liver of infected chimpanzees[8], might be difficult to find on Giemsa-stained preparations because of their size. Unfortunately the small number of cultures infected in these experiments did not allow us to perform other more specific staining procedures, for example, using labelled antibodies.

It was further shown that the cultured hepatocytes had retained their differentiated functions throughout the experimental period by measuring their ability to produce albumin. Human albumin levels in culture supernatant, as measured by an immunonephelometric method, varied from 2.7 to 4 $\mu g\ ml^{-1}$ from day 1 to day 11.

These results demonstrate that the full pre-erythrocytic cycle of *P. vivax* can be completed *in vitro*, using the methods described. It is now important to determine, first, if a human cell is required and, second, if an hepatocyte must be fully differentiated and functional to support penetration of sporozoites and complete exo-erythrocytic development up to the point of infective merozoite release. Doby and Barker[21] observed a small number of intra-cytoplasmic bodies (11–21 μm) following inoculation of *P. vivax* sporozoites into a human liver cell culture which had been maintained *in vitro* for at least 1 month before the addition of the sporozoites. Beaudoin and Hollingdale recently reported that *Plasmodium falciparum* and *P. vivax* sporozoites entered cells of a human lung embryonic line (WI38) as well as a hepatoma line (Hep G_2-A_{16}), but that development was not observed[22]. It is noteworthy that in these previous studies the cells were never fully functional hepatocytes and in no case was complete exoerythrocytic development observed.

These results suggest that, although human malaria parasites may infect a variety of cell types, only the functionally intact hepatocyte may provide the proper conditions for complete exo-erythrocytic development of *P. vivax*. On the other hand, more extensive experiments performed using species of rodent malaria have clearly shown that the specificity for the host cell may not be exclusive[15–18]. Furthermore, indications gained from *in vivo* studies suggest that the species specificity of the liver forms of human plasmodium may not be as restrictive as for the erythrocytic stages[9].

One area of considerable interest associated with the cultivation of exo-erythrocytic stages of *P. vivax* could be to provide a new approach to evaluating the merit of the alternative hypothesis on the nature of the secondary exo-erythrocytic cycle, that is, a dormant uninucleated form called hypnozoite, as against a continuing intrahepatic schizogonic cycle.

As *in vitro* cultivation of the erythrocytic stages of malaria has led to major advances in our knowledge of these stages[23], the *in vitro* culture of the liver stages could also open up a wide range of applications. These are limited at present in part by the supply of sporozoites and of human hepatocytes. It is still necessary, therefore, to try to adapt the system to continuous cell lines. However, even if schizont development in other cell types is shown to be possible, it may well be found ultimately

that a homologous, functional hepatocyte is the cell of choice for certain applications, that is, chemotherapeutic studies which would be best performed using the cell type involved in the metabolism of the drugs being examined.

This work was supported by grants from the French Minister of Research and Industry (82.L.0760 and 82.L.0787) and from WHO/UNDP/World Bank/Special Programme for Research and training in Tropical Diseases. We thank Richard L. Beaudoin, Wallace Peters and Francie Tung for reviewing the manuscript and M. Danis, A. Datry, G. Lecso, and V. Soeun, for their helpful cooperation.

Received 1 August; accepted 28 October 1983.

1. Shortt, H. E. & Garnham, P. C. C. *Trans. R. Soc. trop. Med. Hyg.* **41**, 785–795 (1948).
2. Shortt, H. E., Fairley, N. H., Covell, G., Shute, P. G. & Garnham, P. C. C. *Trans. R. Soc. trop. Med. Hyg.* **44**, 405–419 (1951).
3. Rodhain, J. *Ann. Soc. belge Med. Trop.* **36**, 99–103 (1956).
4. Bray, R. S. *Am. J. trop. Med. Hyg.* **6**, 514–520 (1957).
5. Bray, R. S. *Am. J. trop. Med. Hyg.* **7**, 20–24 (1958).
6. Jeffery, G. M., Wolcott, G. B., Young, M. D. & Williams, D. Jr *Am. J. trop. med. Hyg.* **1**, 917–926 (1952).
7. Sodeman, T. M., Contacos, P. G., Smith, C. S., Jumper, J. R. & Collins, W. E. *J. Parasit.* **55**, 682–683 (1969).
8. Krotoski, W. A. *et al. Am. J. trop. Med. Hyg.* **31**, 1291–1293 (1982).
9. Druilhe, P., Miltgen, F., Landau, I., Rinjard, J. & Gentilini, M. *C. r. hebd. Seanc. Acad. Sci., Paris* **294**, 511–513 (1982).
10. Boulard, Y., Druilhe, P., Miltgen, F., Landau, I. & Rinjard, S. *Protistologica* **18**, 253–258 (1982).
11. Druilhe, P., Puebla, R. M., Miltgen, F., Perrin, L. & Gentilini, M. *Clin. exp. Immun.* (submitted).
12. Strome, C. P. A., de Santis, P. L. & Beaudoin, R. L. *in Vitro* **15**, 531–536 (1979).
13. Sinden, R. E. & Smith, J. *Trans. R. Soc. trop. Med. Hyg.* **74**, 134–136 (1980).
14. Hollingdale, M. R., Leef, J. L., McCullough, M. & Beaudoin, R. L. *Science* **213**, 1021–1022 (1981).
15. Hollingdale, M. R., Leland, P., Leef, J. L. & Beaudoin, R. L. *J. Parasit.* (in the press).
16. Lambiotte, M., Landau, I., Thierry, N. & Miltgen, F. *C. r. hebd. Séanc. Acad. Sci., Paris* **293**, 431–433 (1981).
17. Mazier, D. *et al. C. r. Hebd. Seanc. Acad. Sci., Paris* **294**, **111**, 963–965 (1982).
18. Pirson, P. *Trans. R. Soc. trop. Med. Hyg.* **76**, 422 (1982).
19. Gugen-Guillouzo, C. *et al. Cell biol. int. Rep.* **6**, 625–628 (1982).
20. GuGuen-Guillouzo, C. *et al.* in *Isolation, Characterization and Use of Hepatocytes*, 105–110 (eds Harris, R. A. & Cornell, W.) (Elsevier, Amsterdam, 1983).
21. Doby, J. M. & Barker, R. *C. r. Séanc. Soc. biol.* Rennes **170**, 661–665 (1976).
22. Beaudoin, R. L. & Hollingdale, M. R. in *Textbook of Malaria* (eds Wernsdorfer, W. & McGregor, I.) (Churchill Livingstone, Edinburgh, in the press).
23. Trager, W. & Jensen, J. B. *Science* **193**, 673–675 (1976).

Endogenous ouabain-like compound increases heart muscle contractility

Yakhin Shimoni, Mervyn Gotsman, Joseph Deutsch*, Silvia Kachalsky & David Lichtstein

Department of Physiology and *Department of Pharmaceutical Chemistry, Hebrew University Hadassah Medical School, Jerusalem, Israel

Cardiac glycosides such as digoxin or ouabain have long been known to influence the strength of contraction of cardiac muscle[1–3]. Although the mechanism of action of these compounds remains unknown[4,5], all the proposed modes of action are based on initial binding to specific membrane receptors which are part of the $(Na^+ + K^+)$ATPase complex. These receptors, well characterized and defined[6–8], suggest the existence of an endogenous substance capable of binding to them, in analogy with endogenous opiates, discovered long after morphine and its receptors[9]. As glycosides affect $(Na^+ + K^+)$ATPase activity, an endogenous substance may be a regulator of this important enzyme. Indeed, the search for endogenous regulators of the $(Na^+ + K^+)$ATPase or ouabain-like compounds (OLC) has recently intensified. These compounds, extracted and partially purified from mammalian brain[10–14], heart[15], blood[16–19] and urine[20,21], and from toad skin and plasma[22,23], have been shown to inhibit ^{3}H-ouabain binding and $(Na^+ + K^+)$ATPase activity. We report here that in addition to these effects, the OLC, highly purified from toad skin and sheep brain, increases the force of contraction of frog and guinea pig atrium.

The toad skin OLC was extracted from 50 g of back and abdominal toad skin (*Bufo viridis*) into 500 ml methanol. The methanol was filtered and evaporated and the residue redissolved in 20 ml distilled water. Material which was not dissolved was precipitated by high speed centrifugation (100,000*g*). The supernatant was separated and lyophilized. The residue was dissolved in a minimal volume of methanol (~1 ml) and 20–100-μl aliquots were injected on HPLC. The chromatographic pattern of the separation is shown in Fig. 1*a*. The main peak with biological activity (that is, inhibits ^{3}H-ouabain binding and $(Na^+ + K^+)$ATPase activity, see below) was obtained at a retention time of 28 min. This peak was collected and rechromatographed on the same system, and the separation is shown in Fig. 1*b*. The material from the peak at 28 min (second separation) was collected and used in the physiological experiments.

The sheep brain OLC was extracted from brain tissue by acidified acetone and separated on a Sephadex G-25 column as previously described[12,13]. The biologically active peak obtained from the Sephadex column was further separated on the HPLC column described above (for the toad skin OLC).

Figure 1*c* shows that the material separated at a retention time of 28 min from toad skin is capable of inhibiting ^{3}H-ouabain binding to synaptosomes prepared from rat brain. The inhibition is dose dependent with half-maximal inhibition at 100 μg of wet weight of skin per ml. By comparing this activity with ouabain (Fig. 1*c*), and assuming that ouabain and OLC have the same affinity towards their mutual binding site, the concentration of OLC in the skin was calculated to be approximately 0.6 nmol per g tissue, was used to calculate OLC concentrations in the following experiments. The OLC separated at 28 min also inhibited $(Na^+ + K^+)$ATPase activity in the rat brain microsomal fraction, but had no effect on Mg^{2+}-ATPase activity (data not shown). These findings confirm previous reports on the presence of ouabain-like compounds in this tissue[22,23]. Interestingly, the endogenous OLC compound is capable of inhibiting $(Na^+ + K^+)$ATPase activity only at concentrations 100-fold higher than those required to inhibit ouabain binding.

Tension recordings were done from frog atrial trabeculae, placed in a 2-ml chamber and perfused with a normal Ringer solution (for composition see Fig. 2 legend). One end was attached to an AKERS force transducer for measuring the strength of contraction. Constant stimulation was applied at a rate of 0.5 Hz and twitch magnitude was monitored on a pen recorder and oscilloscope and stored on magnetic tape.

Addition of the endogenous, partially purified OLC from toad skin resulted in a marked increase in the force of contraction. This increase was dose dependent and reversible on washout. A positive inotropic effect was sometimes seen at as low a dose as 5×10^{-8} M, but more commonly doses of 10^{-7} M and higher gave significant positive inotropy. An example is shown in Fig. 2*a*. This effect was seen in seven out of seven cases. The amount of positive inotropy, however, was quite variable in the individual experiments, as indeed is the case with the cardiac glycosides themselves[24]. A partial dose–response curve was obtained, limited by the quantity of purified compound. Figure 2*b* illustrates the larger effect produced by the higher doses. No saturation was observed in this limited range. It should be emphasized that, as mentioned above, the concentrations are all calculated from the binding experiments and the true concentrations may actually be different. In one case (Fig. 2*c*) a high dose of 6 μM produced toxic effects, similar to those obtained with high doses of ouabain[25]—an increase in the basal (tonic) tension and a reduction in the phasic (twitch) tension.

Several reports suggest that cardiac glycosides exert their inotropic effect by activating the release of endogenous stores of catecholamines[25–27]. Since our effect was obtained with incompletely identified compounds, we repeated the experiments in the presence of adrenergic blockers. As both α- and β-receptors have been found in several cardiac preparations, including the frog atrium[28–30], we used a combination of phentolamine and propranolol, respectively α- and β-blockers. In 10 out of 14 cases, the OLC caused a marked positive inotropic effect in the presence of concentrations between 10^{-6} and 5×10^{-6} g ml^{-1} of both blockers (Fig. 2*d*). As both blockers alone

0028–0836/84/040369—03$01.00

© 1984 Macmillan Journals Ltd

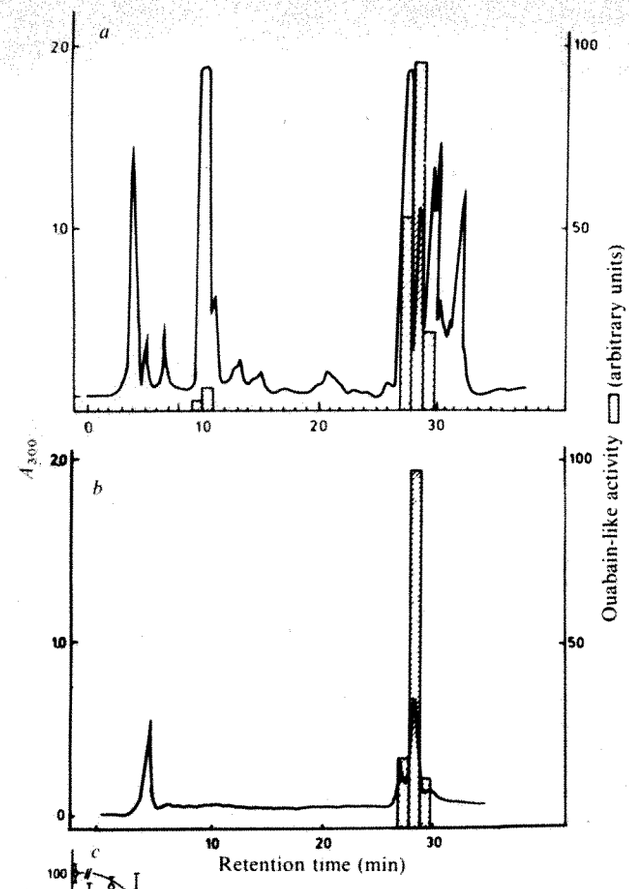


Fig. 1 *a*, *b*, HPLC of the toad skin OLC and its biological activity. The optical density is plotted (left ordinate) against retention time (abscissa). The right-hand ordinate is the relative biological activity, shown as hatched columns in the graph. This activity was measured by testing the capability of each fraction to inhibit both ^{3}H-ouabain binding and $(Na^+ + K^+)$ATPase activity. *c*, Inhibition of ^{3}H-ouabain binding to rat brain synaptosomes by toad skin extract. The inhibition of binding at different concentrations of ouabain (○) or OLC (●) are shown. The OLC was from samples taken at peak retention time of 28 min.

Methods: *a*, Methanol extract of toad skin (*Bufo viridis*) corresponding to 50 g of skin was dissolved in 1 ml methanol. A Tracor 985 HPLC instrument was used. Aliquots of 20–100 µl were applied to a Alltech amino column (4.6×250 mm, 10 µm particle size), equilibrated with 90% acetonitrile. The column was eluted with linear 90–70% acetonitrile gradient in 40 min, at a flow rate of 1 ml min^{-1}. The elution of the compounds was monitored at 300 nm, using a Tracor 970 A UV detector. Fractions of 1 ml were collected, evaporated and the residue was dissolved in 1 ml distilled water. 50-µl samples from this solution were used for measurements of biological activity (*c*). *b*, Fractions (1 ml) with a retention time of 28 min collected from the chromatography in *a* were evaporated to dryness and dissolved in 0.5 ml methanol. Aliquots of 20 µl were applied to the same HPLC system described above. The fractions with a retention time of 28 min were collected, evaporated to dryness and stored at −20 °C for 1–6 weeks. These samples were used in the physiological experiments described in Figs 2 and 3. *c*, The methods for synaptosome preparation have been described elsewhere[12]. ^{3}H-ouabain binding was determined by incubating about 0.2 mg synaptosomal protein in 0.5 ml medium, containing (in mM): Tris-HCl buffer (*p*H 7.4) 50, EDTA 0.5, NaCl 80, $MgSO_4$ 4, ATP 2 (Tris, vanadium-free), 32 nM ^{3}H-ouabain (NEN, 19.5 Ci mmol^{-1}), with or without toad skin extract. Following incubation for 60 min at 37 °C, at which time a binding equilibrium is achieved, the reactions were terminated by the addition of 3 ml of cold 50 mM Tris-HCl (*p*H 7.4), and filtration over Whatman GF/B filters. Filters were washed twice with 3 ml of the same Tris buffer, dried and assayed for radioactivity in a Packard liquid scintillation counter at counting efficiency of ~35%. Specific binding was calculated by subtracting the binding observed in the presence of 100 µM unlabelled ouabain. Specific binding represented 93% of the total binding in control conditions. The control binding in the absence of extract or unlabelled ouabain was 12.5 pmol ^{3}H-ouabain per mg protein, and was taken as 100%.

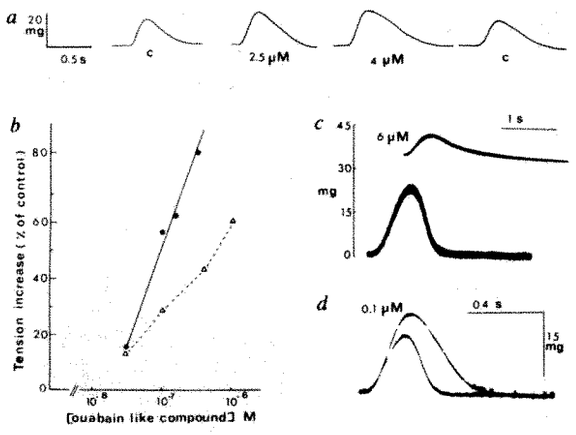


Fig. 2 *a*, The positive inotropic effect of the toad skin OLC on frog atrium. Pen recorder tracings, obtained from frog atrial trabeculae (4–6 mm long, 200–400 µm diameter), during constant 0.5-Hz stimulation. From left to right are the responses in control(c) Ringer, following addition of 2.5 µM OLC, 4 µM OLC and following 20 min washout (c). Control Ringer solution contained (in mM): NaCl 90, $NaHCO_3$ 20, KCl 2, $CaCl_2$ 1.1, $MgCl_2$ 1.0, Na_2HPO_4 0.4, glucose 5.5, bubbled with a 95–5% O_2–CO_2 mixture. The *p*H was 7.6–7.7 and the temperature 21–24 °C. Longer washouts gave more complete reversal of the positive inotropic effect. *b*, A partial dose–response curve. In two cases, four concentrations of OLC were tested. The plot gives the tension increase versus dose (see text for discussion of concentrations of OLC and their estimation). The doses were given cumulatively, with steady state being attained at each dose, before addition of the higher dose. In other experiments two or three doses were used, giving larger effects at higher doses. *c*, A toxic effect of OLC. At a higher concentration (6 µM in this single case) toxic manifestations developed, similar to those obtained with high ouabain levels. The plot shows the elevation in baseline tension as well as the reduced twitch, in the presence of 6 µM OLC, as compared with the control twitch. *d*, The effect of OLC in the presence of α- and β-blockers. Two superimposed oscilloscope records of tension: in a 'control'Ringer solution, containing also 5×10^{-6} g ml^{-1} phentolamine and propranolol (smaller twitch), and after the addition of 0.1 µM OLC(larger twitch).

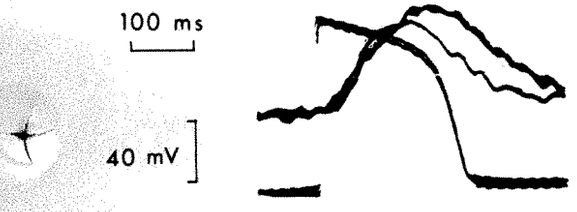


Fig. 3 The positive inotropic effect of sheep brain OLC on sheep heart. OLC was extracted from seven sheep brains by acidified acetone, and partially separated by Sephadex G-25 chromatography, as previously described[12,13]. The active material obtained from this separation was further purified using the HPLC system described in Fig. 1*a* legend. The OLC concentration was estimated by comparing its ability to displace ^{3}H-ouabain binding to rat synaptosomal membranes. The upper tracings show the superposition of tension traces obtained from a sheep ventricular trabeculum. The smaller tension is in a control solution (containing, in mM: NaCl 140, KCl 5.4, $NaHCO_3$ 12, CaCl 2, $MgCl_2$ 1, Na_2HPO_4 0.4, glucose 5, continuously bubbled with 95–5% O_2–CO_2, with a *p*H of 7.4 at 36 °C). The larger tension response was obtained following the addition of 5×10^{-8} M sheep brain OLC. The positive inotropy was fully reversed on washout. The vertical bar equals 20 mg, as well as 40 mV. The lower traces show the superimposed action potentials, recorded with an intracellular, 3 M KCl microelectrode. There was no significant change in resting potential or in the action potential configuration, aside from a possible small elevation in the plateau phase.

have negative inotropic effects, the two cases without effect and the two in which a negative inotropic effect was produced were attributed to persistent, delayed blocker action.

We next tested this compound on a mammalian preparation to investigate a possible more general applicability. In three experiments on atrial trabeculae from guinea pig hearts, the OLC gave a large positive inotropic response (data not shown). In one case, the same effect was produced in the presence of 5×10^{-6} g ml^{-1} α- and β-blockers.

Brain OLC was previously shown to bind to ouabain receptors and to inhibit $(Na^+ + K^+)$ATPase activity[10–14]. In a single trial (Fig. 3), it produced a reversible positive inotropic effect on a trabeculum from sheep ventricle. This 22% increase in contractile force was accompanied by very little change in the action potential configuration, as measured by an intracellular microelectrode.

The chemical nature of the OLC is obviously of major importance. Partial characterization using Sephadex columns and sensitivity to proteolytic enzymes (data not shown) reveals that the toad skin and brain OLCs are of low molecular weight (<500) and are not peptides. It is well known that the toad skin contains toxins which are capable of inhibiting $(Na^+ + K^+)$ATPase, the bufodienolide toxins[31]. It is therefore possible that the OLC separated by us are these steroidal toxins. Experimental results, however, do not favour this possibility: (1) The retention time on the HPLC system described above is in the range of 5–10 min for steroidal compounds (such as ouabain itself), which does not correspond to the main peak of activity found (retention time of 28 min). (2) Only ~5% of the biological activity present in the 28-min peak (aqueous solution) partitioned into 10 volumes of chloroform or ether, indicating that the active principle is relatively polar, unlike the bufodienolides. (3) The UV spectrum of bufodienolides shows a peak at 300 nm, while the spectrum of the compound separated by us shows a maximum at 278 nm and a shoulder at 300 nm (data not shown).

In conclusion, we have shown that the endogenous OLCs extracted from toad skin and mammalian brain are capable of increasing the force of cardiac muscle contraction. This raises the possibility that they serve as physiological regulators of cardiac function. Clearly, further investigation is needed.

This study was supported by the Israel National Academy of Sciences and the Israel Ministry of Health.

Received 7 June; accepted 7 September 1983.

1. Cattel, M. & Gold, H. *J. Pharmac. exp. Ther.* **62,** 116–125 (1938).
2. Cotten, M. & Stopp, P. E. *Am. J. Physiol.* **192,** 114–120 (1958).
3. Langer, G. A. & Serena, D. A. *J. molec. cell. Cardiol.* **1,** 65–70 (1970).
4. Lee, K. S. & Klaus, W. *Pharmec. Rev.* **23,** 194–250 (1971).
5. Noble, D. *Cardiovasc. Res.* **14,** 495–514 (1980).
6. Baker, P. F. & Willis, J. P. *J. Physiol., Lond.* **224,** 441–462 (1972).
7. Akera, T. & Brody, T. M. *Pharmac. Rev.* **29,** 187–220 (1978).
8. Wallick, E. T., Lane, L. K. & Scwartz, A. *A. Rev. Physiol.* **41,** 397–411 (1979).
9. Hughes, J. *et al. Nature* **258,** 577–579 (1975).
10. Haupert, G. T. Jr & Sancho, J. M. *Proc. natn. Acad. Sci. U.S.A.* **76,** 4658–4660 (1979).
11. Fishman, M. *Proc. natn. Acad. Sci. U.S.A.* **76,** 4661–4663 (1979).
12. Lichtstein, D. & Samuelov, S. *Biochem. biophys. Res. Commun.* **96,** 1518–1523 (1980).
13. Lichtstein, D. & Samuelov, S. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1453–1456 (1982).
14. Schwartz, A. *et al. Ann. N. Y. Acad. Sci.* **402,** 253–271 (1982).
15. De Pover, A., Castaneda-Hernandez, G. O. & Godfraind, T. *Biochem. Pharmac.* **31,** 267–271 (1982).
16. Poston, L. *et al. Br. med. J.* **232,** 847–379 (1981).
17. Hamlyn, J. M. *et al. Nature* **300,** 650–652 (1982).
18. Gruber, K. A., Whiteker, J. M. & Buckalew, V. M. *Nature* **287,** 743–745 (1980).
19. Cloix, J. F., Miller, E. D., Pernollet, M. G., Devynck, M. A. & Meyer, P. *C. r. hebd. Séanc. Acad. Sci., Paris* **296,** 213–216 (1983).
20. de Wardener, H. E. & Clarkson, E. M. *Clin. Sci.* **63,** 415–420 (1982).
21. Clarkson, E. M., Raw, S. M. & de Wardener, H. E. *Kidney Int.* **16,** 710–421 (1979).
22. Flier, J. S., Maratos-Flier, E., Pallotta, J. A. & McIsaac, D. *Nature* **279,** 341–343 (1979).
23. Flier, J. S., Edwards, M. W., Daly, J. J. & Myers, C. W. *Science* **208,** 503–505 (1980).
24. Hart, G., Noble, D. & Shimoni, Y. *J. Physiol., Lond.* **334,** 103–131 (1982).
25. Deslauriers, Y., Ruiz-Ceretti, E., Schanne, O. F. & Payet, M. D. *Can. J. Physiol. Pharmac.* **60,** 1153–1159 (1982).
26. Tanz, R. *J. Pharmac. exp. Ther.* **144,** 205–213 (1964).
27. Seifen, E. *Br. J. Pharmac.* **51,** 481–490 (1974).
28. Koch-Weser, J. *Circulation Res.* **28,** 109–118 (1971).
29. Niedergerke, R. & Page, S. *Proc. R. Soc.* **B213,** 325–344 (1981).
30. Hordof, A. J., Rose, E., Danilo, P. & Rosen, M. R. *Am. J. Physiol.* **242,** H677–H682 (1982).
31. Meyer, K. & Linde, H. in *Venomous Animals and their Venoms* Vol. 2 (eds Bucherl, W. & Buckley, E.) 521–552 (Academic, London, 1971).

β-Adrenergic modulation of calcium channels in frog ventricular heart cells

Bruce P. Bean*, Martha C. Nowycky & Richard W. Tsien

Departments of Physiology and Neuroanatomy, Yale University School of Medicine, New Haven, Connecticut 06510, USA

Adrenergic modulation of calcium channels profoundly influences cardiac function[1,2], and has served as a prime example of neurohormonal regulation of voltage-gated ion channels[1–7]. Channel modulation and increased Ca influx[2,8,9] are mediated by elevation of intracellular cyclic AMP[10–17] and protein phosphorylation[18,19]. The molecular mechanism of the augmented membrane Ca conductance has attracted considerable interest. An increase in the density of functional channels has often been proposed[20–22], but there has previously been no direct evidence. Single-channel recordings show that isoprenaline or 8-bromocyclic AMP increase the proportion of time individual channels spend open by prolonging openings and shortening the closed periods between openings[2,23–25]. To look for an additional contribution of changes in the number of functional channels, we applied ensemble fluctuation analysis[26] to whole-cell recordings[27,28] of cardiac Ca channel activity. Here we present evidence that in frog ventricular heart cells β-adrenergic stimulation increases N_F, the average number of functional Ca channels per cell. We also find that isoprenaline slows the time course of both activation and inactivation, and that the enhancement of peak current decreases gradually with greater membrane depolarization.

Frog ventricular cells were chosen because they have a robust β-adrenergic response[8,22]. We used barium as the current carrier instead of Ca to avoid possible contamination from Ca-activated potassium channels, to block currents through background K channels[29] and to improve resolution of the fluctuation analysis by increasing the single-channel current. Series-resistance compensation was used (Fig. 1 legend) and linear leak and capacity

* To whom reprint requests should be addressed at: Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52242, USA.

0028-0836/84/040371—05$01.00

© 1984 Macmillan Journals Ltd

currents were subtracted to give the traces shown. All experiments were carried out at room temperature (22 °C).

The β-adrenergic agonist isoprenaline strongly enhanced Ca channel current carried by Ba ions (Fig. 1). Figure 1*a* shows whole-cell currents recorded during depolarizing voltage-clamp pulses from −60 mV to +10 mV. Ca channel current increased from about 0.7 nA in the absence of drug, to about 3.5 nA in the presence of isoprenaline: the effect was fully reversed following washout of the drug. Figure 1*b* illustrates the time course of the response and the stability of the current amplitude. The increase of Ca channel current was fairly typical; in 25 cells exposed to 0.5 μM isoprenaline, the whole-cell current increased by a factor of 6.2 ± 0.6 (mean ± s.e.m.), with individual increases as large as 15-fold.

The current carried by Ca channels (I) depends on three factors: the number of functional channels (N_F), the probability that an individual channel is open (p), and the current carried by an open channel (i). Which of these factors changes with β-adrenergic modulation? To answer this question, we applied ensemble fluctuation analysis as developed by Sigworth[26]. Ensembles of successively evoked current transients were collected, aligned in time and analysed for the time course of the mean current and the time course of the variance associated with differences in channel activity from one record to the next. Mean and variance records from control and isoprenaline runs are illustrated in Fig. 2*a,b*. Both the mean current and the variance rose to maximum levels at the end of the depolarizing pulse; like the mean current, the variance increased about fivefold after exposure to isoprenaline. To estimate N_F, p and i for each run, plots of the variance versus mean current (Fig. 2*b,c*) were fitted by parabolic curves given by $\text{var} = iI - I^2/N_F$. This is the relationship expected for N_F identical, independent, functional channels having one non-zero current level i (ref. 25). The values for N_F and i were obtained by a least-squares curve-fitting procedure. The maximal probability p_{max} was then calculated as $p_{max} = I_{max}/(N_F i)$.

In the experiment shown in Fig. 2, N_F increased from ~11,000 to ~37,000 with isoprenaline. The maximal probability increased from 0.40 to 0.58 while the unitary current i remained unchanged at about 0.15 pA. These findings were representative of collected results from a total of eight frog ventricular cells (Table 1). The average value of N_F increased about threefold, while p_{max} increased by about 50%. After recovery from the isoprenaline effect, fluctuation analysis was more difficult because of Ca channel rundown and sweep-to-sweep shifts in baseline. Nevertheless, results from three cells suggested that N_F returned to its pre-isoprenaline level.

One of the most important assumptions of the fluctuation analysis is that the functional channels behave as a single, homogeneous population. Although this is untested, all of our observations seem consistent with this view. When frog ventricular cells are studied with long depolarizing pulses, the Ba current decays as a single exponential over a broad range of potentials. When fluctuation analysis is done on the falling phase of current in these ionic conditions, variance-mean plots are well fitted by a single parabola, with p_{max} near 0.4, just as in analysis of the rising phase in Fig. 2*b*.

This is the first evidence that the number of functional calcium channels is enhanced by β-adrenergic stimulation, as proposed by Reuter and Scholz[21] and by others[20,22]. At the membrane potential level studied here (+10 mV), the overall enhancement of Ca channel current is dominated by the increase in N_F. At other potentials other factors may have a more important role, as discussed below.

Is the Ca channel current simply scaled by β-adrenergic stimulation, or are there changes in time or voltage dependence? Whole-cell recordings allow the kinetics to be studied with good time resolution over a broad range of potentials. Figure 3*a* shows that the enhancement of inward Ba current in frog ventricular cells is accompanied by a clear slowing in the time course of activation (see also Figs 2*a*, 4*a*). The slowing is obvious when the control record is scaled up to match the isoprenaline-enhanced trace (lower part of Fig. 3*a*). The isoprenaline-induced slowing seemed little changed when $[Ba]_0$ was varied between 1 and 30 mM or when membrane potential was varied between −35 and +25 mV. Most of the analysis was done at +10 mV in 10 mM $[Ba]_0$. When the onset of inward current was fitted by a single exponential with a delay (a good approximation for all but the first millisecond after the step), the exponential time constant increased with isoprenaline in 13 out of 13 frog ventricular cells. The time constant was 1.58 ± 0.08 ms (mean ± s.e.m.) in control preparations and 2.47 ± 0.08 ms in isoprenaline-exposed preparations. Neonatal rat ventricular cells show a similar slowing during exposure to isoprenaline (Fig. 4*b*), although the enhancement of peak inward current was at most twofold, and typically much less.

Figure 4*a* compares the peak current–voltage relationship before and after β-adrenergic stimulation, and Fig. 4*b* plots the

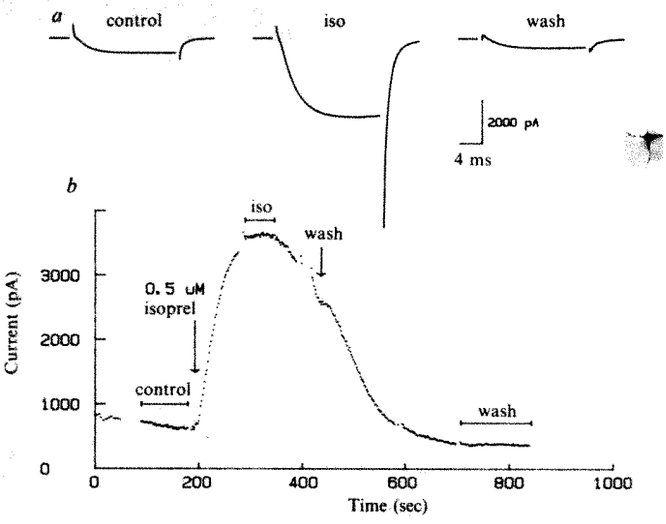


Fig. 1 Increase of Ba currents by isoprenaline. *a*, Currents elicited by a voltage step to +10 mV from a holding potential of −60 mV. Tail currents evoked by a repolarization to −40 mV. Leak and capacity currents (obtained from mirror-image hyperpolarizing pulses, 10 times smaller) have been subtracted, and a 750-μs period is blanked due to amplifier saturation during capacity transients. Each current shown is the mean current of the series of pulses indicated in *b*. *b*, Time course of isoprenaline response. Current measured at the end of the 20-ms test pulse. 200 μl of isoprenaline stock solution was squirted into the 1.5-ml chamber to give a final concentration of 0.5 μM. During the wash, the chamber volume was replaced 20 times with agonist-free solution within the first minute. Note that the current amplitude began to fall before the drug was washed out; in other experiments, current fell back to a stable value near its original level in 5–10 min even though the drug was not washed out, suggesting desensitization or depletion of some intracellular constituent. Cell F04B. Total capacity 75 pF. Series-resistance compensation for 2.2 MΩ out of a measured series resistance of 3.5–3.8 MΩ.

Methods: Single ventricular cells were isolated from bullfrog (*Rana catesbiana*) hearts following the procedure described by Hume and Giles[34] for atrial cells (see also ref. 35). Our procedure included some modifications. After 2–3 h in the final 0.05% collagenase solution, the pieces of ventricle were triturated using a Pasteur pipette. After another 2–3 h of digestion, the cell-containing collagenase solution was diluted 1:4 with (in mM) 1 $CaCl_2$, 160 NaCl, 5 KCl, 1 $MgCl_2$, 10 glucose, 5 HEPES *p*H 7.6. Cells were used 2–24 h after being isolated. For recording whole-cell voltage-clamp currents[7,28], cells were placed in solution containing (in mM) 10 $CaCl_2$, 130 NaCl, 5 KCl, 1 $MgCl_2$, 10 glucose, 5 HEPES *p*H 7.6. Electrodes (1–3 MΩ) contained (in mM) 120 CsCl, 10 Cs-EGTA, 5 $MgCl_2$, 10 HEPES *p*H 7.5. After formation of a gigaseal and rupture of the cell mumbrane, the external solution was changed to (in mM) 10 $BaCl_2$, 135 TEA-Cl, 1 $MgCl_2$, 10 glucose, 10 HEPES *p*H 7.65. Pipette series-resistance (measured as the time constant of the uncompensated capacity transient divided by the cell capacity) was 2–8 MΩ, of which 60–80% was compensated.

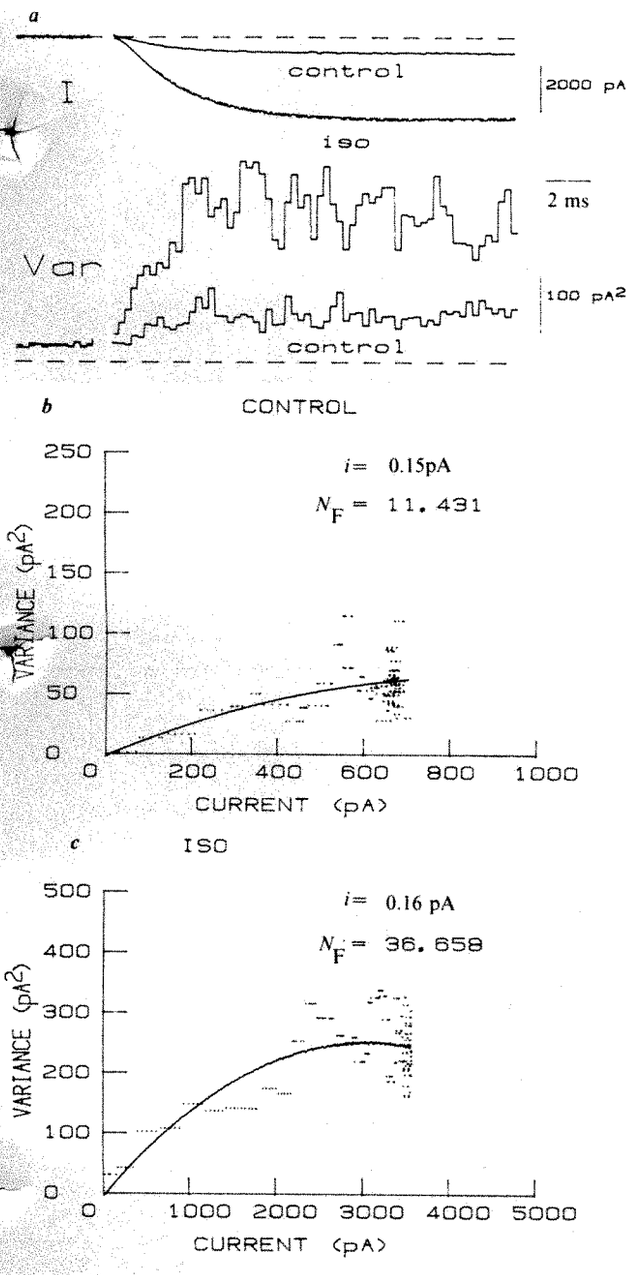


Fig. 2 Fluctuation analysis of Ca channel currents. Same experiment as Fig. 1. Ensembles of currents were elicited by a series of identical pulses to +10 mV (46 in the control and 31 with isoprenaline). Currents were sampled at 30-μs intervals after being filtered at 3 kHz (4-pole Bessel). Mean current and variance were computed for each time point. To minimize errors from slow drifts in current size, variance was calculated from successive pairs of records and averaged over the pairs; also, the first record of each pair was first scaled by a constant factor to allow for very slow rundown. The first millisecond of the test pulse was omitted from the analysis because the current showed an outward bump during this time; the variance was approximately at its background level. *a*, Mean and variance as a function of time. Mean currents corrected for capacity and leak current. The variance was averaged over 10 successive time points. Dotted lines indicate zero levels. *b*, *c*, Variance plotted against mean current. The background variance (computed from the current at the holding potential) was first subtracted from the variance during the test current.

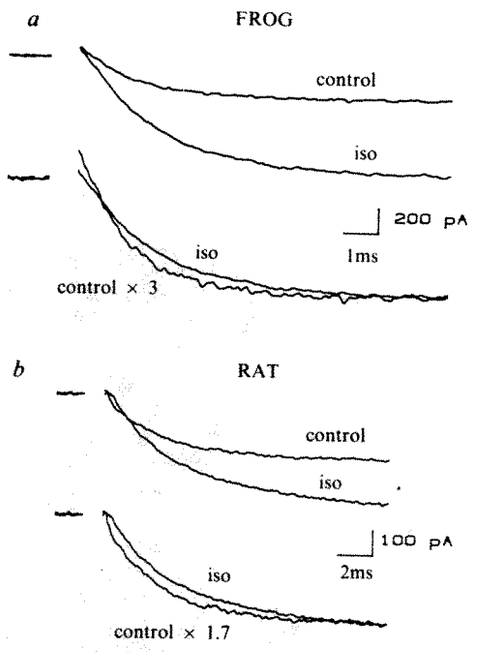


Fig. 3 Effect of isoprenaline on the time course of Ca channel activation. Recordings are shown at orginal amplification (upper traces in each panel), and with control record scaled up to the same magnitude as the isoprenaline trace (lower traces in each panel). *a*, Frog ventricular cell, 10 mM Ba external solution. Currents evoked by a step depolarization from −60 to +10 mV. Cell F06I. *b*, Cultured rat ventricular cell, 10 mM Ba external solution as in *a*. Currents elicited by a depolarizing step from −60 to +30 mV. Cells were cultured from 1–3-day-old rat hearts[36]. The recordings shown here were made on day 3 after culture. Cell M80I.

ratio I(iso)/I(control) as a function of potential. The enhancement of Ca channel current falls from about 11-fold at −40 mV to slightly more than 4-fold at +10 mV (close to the 6-fold average value mentioned earlier). The ratio continues to decrease as the test potential approaches and exceeds the reversal potential for Ca channel current (near +50 mV in the control run). At strongly positive potentials, isoprenaline increases the outward current very little.

The voltage dependence cannot be accounted for by errors due to the series resistance of the pipette or the longitudinal resistance of the cell (see Fig. 4 legend). Interference from other ionic channels would also appear unlikely, in view of the ionic conditions (external Ba and tetraethylammonium (TEA), internal Cs and EGTA), and experiments using the Ca channel blocking drug verapamil (Fig. 4*c–e*). Verapamil totally abolishes the isoprenaline-enhanced current, whether it is inward (0 mV, Fig. 4*c*) or outward (+120 mV, Fig. 4*e*). At the reversal potential in the control run (+50 mV, Fig. 4*d*), the net driving force for Ca channel current is zero and as expected, neither isoprenaline[21] nor verapamil[30] has any effect. This experiment leaves little doubt that the outward current reflects Ca channel activity alone. Thus, it is notable that β-stimulation hardly increases the peak outward current at all (Fig. 4*a*,*e*).

The main effect of isoprenaline in Fig. 4*e* is a marked slowing of the decay of the outward current. Slowing of inactivation is also manifested by the decay of inward currents accompanying less positive test depolarizations (data not shown), and by the behaviour of inward tail currents evoked by sudden repolarization after test pulses of varying duration, studied in another cell (Fig. 4*f*,*g*). In a control run (Fig. 4*f*), the amplitude of tail currents decreases gradually along with outward current inactivation; after exposure to isoprenaline (Fig. 4*g*), the amplitude of tails is maintained, like the outward current.

Outward current and tail current appear not to vary concomitantly in their response to isoprenaline: inward tail currents seem to be strongly enhanced at a time (vertical arrow) when

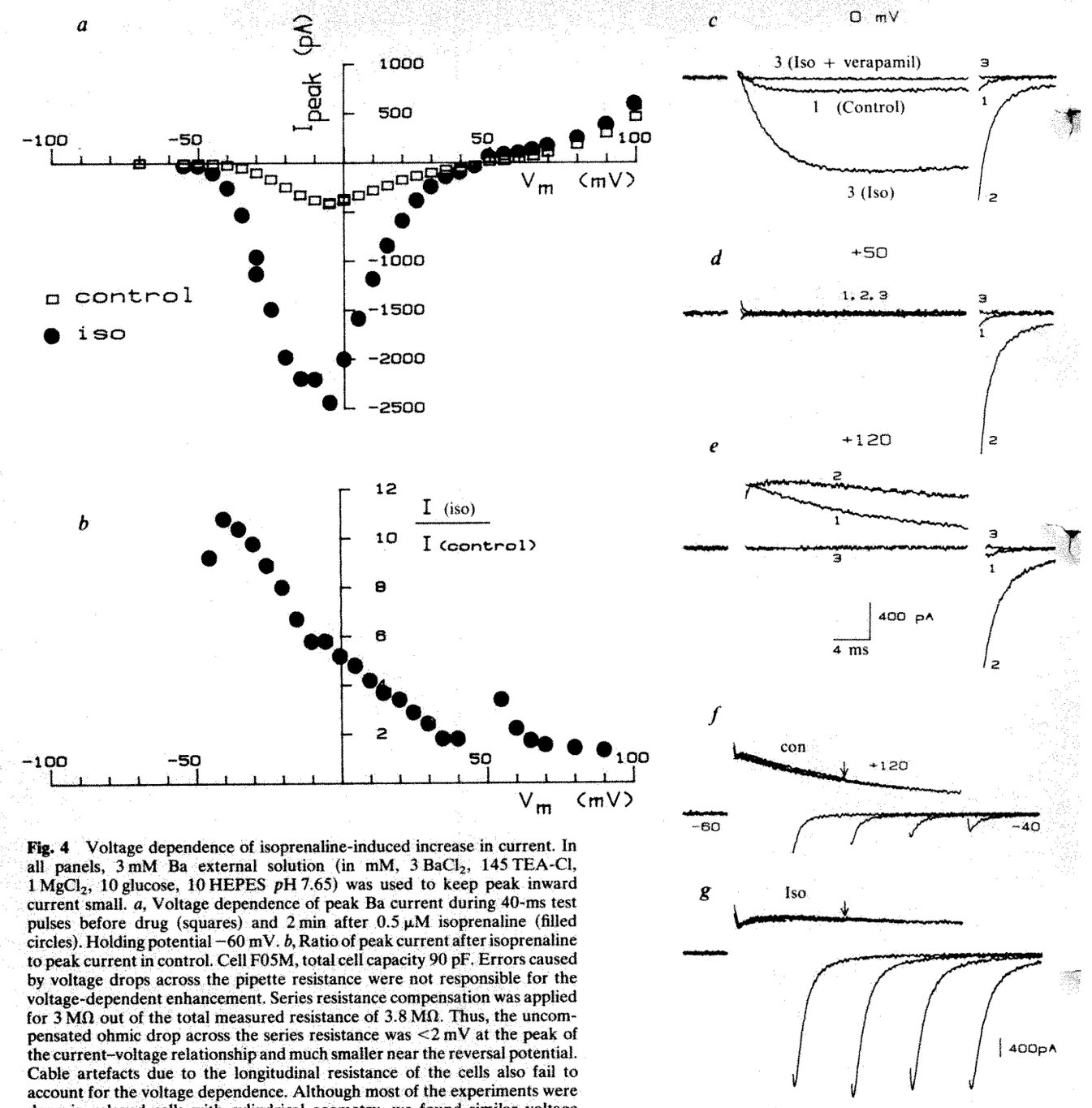


Fig. 4 Voltage dependence of isoprenaline-induced increase in current. In all panels, 3 mM Ba external solution (in mM, 3 $BaCl_2$, 145 TEA-Cl, 1 $MgCl_2$, 10 glucose, 10 HEPES *p*H 7.65) was used to keep peak inward current small. *a*, Voltage dependence of peak Ba current during 40-ms test pulses before drug (squares) and 2 min after 0.5 μM isoprenaline (filled circles). Holding potential −60 mV. *b*, Ratio of peak current after isoprenaline to peak current in control. Cell F05M, total cell capacity 90 pF. Errors caused by voltage drops across the pipette resistance were not responsible for the voltage-dependent enhancement. Series resistance compensation was applied for 3 MΩ out of the total measured resistance of 3.8 MΩ. Thus, the uncompensated ohmic drop across the series resistance was <2 mV at the peak of the current–voltage relationship and much smaller near the reversal potential. Cable artefacts due to the longitudinal resistance of the cells also fail to account for the voltage dependence. Although most of the experiments were done in relaxed cells with cylindrical geometry, we found similar voltage dependence in cells that were deliberately allowed to round up in Ba–TEA solution, even when large, low-resistance pipettes were used and series-resistance voltage drops were <1 mV. *c–e*, Verapamil block of isoprenaline-enhanced currents. Consecutive runs in the absence of drug (trace 1), after exposure to 0.5 μM isoprenaline (trace 2), and after exposure to 50 μM verapamil in the continued presence of isoprenaline (trace 3). Step depolarizations from a holding potential of −60 mV to the test potentials indicated, followed by step repolarization to −40 mV. The capacity transients are blanked for 1.2 ms (*a*), 1.4 ms (*b*) and 2.0 ms (*c*). Noise on records is due to digitization of current traces used for subtraction of linear leak current. Cell F09E. *f*, *g*, Comparison between behaviour of outward currents during depolarization to +120 mV and inward tail currents following repolarization to −40 mV, before (*f*) and after 0.5 μM isoprenaline (*g*). Inward current evoked by depolarization to 0 mV was increased sevenfold (record not shown). Arrow marks time when outward currents in control and isoprenaline runs are approximately equal. Records are blanked for 720 μs following the onset of the repolarizing step to allow for complete settling of capacity transient. Cell F10G.

the outward current remains essentially unchanged. One possibility is that at strongly positive potentials some or all of the channels show a lower conductance after isoprenaline. We cannot rule out an alternative possibility, however, that the amplitude of the tail is severely underestimated in control because it decays within the settling time of the capacity transient.

The present study shows that β-adrenergic stimulation increases the number of functional channels seen with ensemble fluctuation analysis. Isoprenaline slows the settling time constants for activation and inactivation. The enhancement of Ca channel current is largest at weak depolarizations and smallest at strongly positive potentials. These findings with whole-cell recordings may be compared with studies using cell-attached patch recordings.

The large increase in N_F in frog myocytes seems at variance with unitary recordings in mammalian cells, where changes in the apparent number of channels are seen only rarely[24,25] or not at all. It is important to realize, however, that fluctuation

Table 1 Fluctuation analysis of the effect of isoprenaline on the factors that determine Ca channel current

	I_{max}(pA)			i(pA)			p_{max}			N_F		
Cell	Con	Iso	Wash	Con	Iso	Wash	Con	Iso	Wash	Con	Iso	Wash
1P	313	1,508	249	0.09	0.12	0.05	0.41	0.66	0.21	8,664	18,295	22,975
1Q	650	2,672	—	0.12	0.18	—	0.48	0.59	—	11,316	24,794	—
1W	538	4,034	253	0.11	0.14	0.14	0.01	0.58	0.20	—	47,650	8,574
1Z	502	3,325	348	0.09	0.18	0.15	0.10	0.40	0.06	—	45,918	—
3J	496	4,771	—	0.25	0.14	—	0.50	0.10	—	3,961	—	—
4B	708	3,648	419	0.15	0.16	0.08	0.40	0.58	0.11	11,431	36,658	—
4C	335	1,614	207	0.16	0.14	0.05	0.52	0.55	0.07	4,000	20,974	—
5F	792	4,376	220	0.12	0.14	0.15	0.26	0.19	0.43	23,652	—	4,791
6H	683	3,993	—	0.10	0.20	—	0.41	0.72	—	16,791	27,261	—
Mean	557	3,327	299	0.13	0.15	0.10	0.34	0.49	0.18	11,402	31,650	12,113
s.e.m.	±52	±367	±29	0.017	0.008	0.020	0.06	0.07	0.05	2,658	4,480	4,523

Experimental conditions as in Figs 1, 2. N_F was considered indeterminate if the apparent value of p_{max} fell below 0.20. Average cell capacitance was 57 ± 3.5 pF (mean ± s.e.m.). Con, control; Iso, isoprenaline.

analysis and unitary current analysis can show different biases in the way they assign changes to N and p. This distinction depends on convention and on the time resolution of the measurements[1,7,31]. In our fluctuation analysis, N_F refers to the average number of functional channels readily available to open in response to the test depolarization. Since the analysis is based on differences between adjacent records, a channel is not counted toward N_F during any silent periods much longer than the inter-pulse interval. This definition is consistent with the idea that Ca channels continually undergo transitions between functional (phosphorylated) and nonfunctional (dephosphorylated) states[18], and that the number of channels increases during the β-adrenergic response[2,16,20–22]. N_F is to be distinguished from N_T, the total number of channels in an area of membrane. In a membrane patch, N_T can in principle be determined by counting the maximal number of simultaneous openings during a very long period of recording. We have used N_F to express the idea that channels undergo occasional transitions between functional and nonfunctional conditions, distinct from rapid (millisecond) opening reactions. In single channel recordings, such transitions would presumably give rise to groups of sweeps with no openings.

The slowing of the activation time course was seen in both frog and rat ventricular cells. This might appear surprising, as single-channel analysis in cultured rat ventricular cells has provided convincing evidence that isoprenaline or 8-bromo-cyclic AMP increase the rate constants leading to Ca channel opening[2,23–25]. In fact, the data of Cachelin *et al.*[24] also show a decrease in backward rate constants leading away from the open state. According to our calculations, the net effect predicted from their data is a slowing of activation time course, very much like that seen experimentally (Fig. 3). The agreement leaves open the possibility that after isoprenaline, all channels share the same kinetic properties. This is one of the more critical assumptions of the fluctuation analysis.

The pronounced voltage dependence of the increase in Ca channel current was unexpected because previous studies in multicellular preparations have usually shown equal increases at all potentials (for example, refs 21, 32, 33, but see ref. 8). Differences in the composition of internal or external media are possible factors that cannot be ruled out. The voltage dependence of the β-adrenergic response at relatively weak depolarizations in our experiments seems similar to that seen in single channel recordings (see ref. 2, Fig. 2 and ref. 25, Fig. 5).

The almost complete lack of effect of isoprenaline at strong depolarizations was perhaps our most surprising result. We have considered two alternative explanations. (1) The lack of increase in outward current could be attributed, as already mentioned, to properties of open Ca channels: for example, isoprenaline might recruit channels which show little or no conductance at strongly positive potentials. (2) The large number of channels that are not readily available for activation at +10 mV might become activated at strongly positive potentials even in the absence of β-stimulation. Distinguishing between these hypotheses would be possible if the approach illustrated in Fig. 4*f,g* could be extended to the point of resolving the earliest phase of the tail current.

We thank P. Hess, A. P. Fox and J. B. Lansman for helpful discussion and the USPHS, the American Heart Association and the Miles Institute for support.

Received 1 July; accepted 3 November 1983.

1. Tsien, R. W. & Siegelbaum, S. A. in *Physiology of Membrane Disorders* (eds Andreoli, T. E., Hoffman, J. F. & Fanestil, D. D.) 517–538 (Plenum, New York, 1978).
2. Reuter, H. *A. Rev. Physiol.* **41,** 413–424 (1979).
3. Kehoe, J. & Marty, A. *A. Rev. Biophys. Bioengng* **9,** 437–465 (1980).
4. Nicoll, R. A. *Trends Neurosci.* **5,** 369–374 (1983).
5. Reuter, H. *Nature* **301,** 569–574 (1983).
6. Tsien, R. W. *A. Rev. Physiol.* **45,** 341–358 (1983).
7. Siegelbaum, S. A. & Tsien, R. W. *Trends Neurosci.* **6,** 307–313 (1983).
8. Vassort, G. *et al. Pflügers Arch. ges. Physiol.* **309,** 70–81 (1969).
9. Brown, H. F., McNaughton, P. A., Noble, D. & Noble, S. J. *Phil. Trans. R. Soc.* B**270,** 527–537 (1975).
10. Tsien, R. W., Giles, W. & Greengard, P. *Nature new Biol.* **240,** 181–183 (1972).
11. Meinertz, T., Nawrath, H. & Scholz, H. *Naunyn-Schmiedebergs Archs Pharmak.* **279,** 327–338 (1973).
12. Watanabe, A. M. & Besch, H. R. *Circulation Res.* **35,** 316–324 (1974).
13. Reuter, H. *J. Physiol., Lond.* **242,** 429–451 (1974).
14. Vogel, S. & Sperelakis, N. *J. molec. cell. Cardiol.* **13,** 51–64 (1981).
15. Trautwein, W., Taniguchi, J. & Noma, A. *Pflügers Arch. ges. Physiol.* **392,** 307–314 (1982).
16. Nargeot, J., Nerbonne, J. M., Engels, J. & Lester, H. A. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
17. Tsien, R. W. *Adv. Cyclic Nucleotide Res.* **8,** 363–420 (1977).
18. Osterrieder, W. *et al. Nature* **298,** 576–578 (1977).
19. Rinaldi, M. L., Capony, J.-P. & Demaille, J. G. *J. molec. cell. Cardiol.* **14,** 279–289 (1982).
20. Sperelakis, N. & Schneider, J. *Am. J. Cardiol.* **37,** 1079–1085 (1976).
21. Reuter, H. & Scholz, H. *J. Physiol., Lond.* **264,** 49–62 (1977).
22. Niedergerke, R. & Page, S. *Proc. R. Soc.* B**197,** 333–367 (1977).
23. Reuter, H., Stevens, C. F., Tsien, R. W. & Yellen, G. *Nature* **297,** 501–504 (1982).
24. Cachelin, A. B., de Peyer, J. E., Kokubun, S. & Reuter, H. *Nature* **304,** 462–464 (1983).
25. Reuter, H., Cachelin, A. B., de Peyer, J. E. & Kokubun, S. *Cold Spring Harb. Symp. quant. Biol.* **48** (in the press).
26. Sigworth, F. J. *J. Physiol., Lond.* **307,** 97–129 (1980).
27. Hamill, O., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391,** 85–100 (1981).
28. Fenwick, E. M., Marty, A. & Neher, E. *J. Physiol., Lond.* **331,** 599–635 (1982).
29. Cranefield, P. F. & Gadsby, D. C. *J. Physiol., Lond.* **318,** 34–35P (1981).
30. Lee, K. S. & Tsien, R. W. *Nature* **302,** 790–794 (1983).
31. Sigworth, F. J. *J. Physiol., Lond.* **307,** 131–142 (1980).
32. Noma, A., Kotake, H. & Irisawa, H. *Pflügers Arch. ges. Physiol.* **388,** 1–9 (1980).
33. Kass, R. A., Wiegers, S. E. *J. Physiol., Lond.* **322,** 541–558 (1982).
34. Hume, J. R. & Giles, W. *J. gen. Physiol.* **78,** 19–42 (1981).
35. Tarr, M., Trank, J. W. *Experientia* **32,** 338–339 (1976).
36. Harary, I., Hoover, F. & Farley, B. *Meth. Enzym.* **32,** 740–745 (1974).

Reversible shape change of Triton-treated erythrocyte ghosts induced by Ca^{2+} and Mg-ATP

Yoshinosri Jinbu, Shingo Sato & Makoto Nakao

Department of Biochemistry, Tokyo Medical and Dental University School of Medicine, Yushima, Bunkyo-ku, Tokyo, Japan

The shape of the human erythrocyte depends on intracellular ATP content[1,2] and echinocytic erythrocyte ghosts obtained in the presence of Mg-ATP acquire a diskocytic shape[3–5] after incubation at 37 °C; however, agreement is lacking about the molecular basis of the shape changes. The suggestion that phosphorylation of cytoskeletal structures underlying the membrane is involved[5,6] has been disputed and alternative explanations based on lipid bilayer theory[7,8] and metabolism of phospholipid[9,10] have been proposed. Recently, we re-examined the effect of ATP on the shape of ghosts and found that it consists of two distinct steps[11]. We have, therefore, now examined the effect of physiological concentrations of Ca^{2+} and ATP on the cytoskeleton of the erythrocyte membrane directly using Triton-treated ghosts which had lost this permeability barrier. Our findings suggest that a noncovalent effect of ATP on the cytoskeleton is a prerequisite for shape change.

Untreated ghosts were spherical in 10-mM Tris-Cl buffer. On addition of 10^{-7}–10^{-6} M Ca^{2+} buffer *p*H 7.4, they crenated immediately, but rapidly changed into diskocytes on further addition of 1 mM Mg-ATP (ref. 11). A similar shape change was also observed when Triton-treated ghosts were used. It was clear that these Triton-treated ghosts were not intact for the following reasons: first, the membrane preparation showed decreased amounts of band 3 and 4.2 in SDS-polyacrylamide gel electrophoresis (PAGE) patterns[12] and these bands were recovered in the supernatant instead of the pellet (Fig. 1). Second, Triton-treated ghosts did not shrink in hypertonic sucrose solutions (0.6 M, 0.8 M), demonstrating the absence of an osmotic barrier. Third, in dark-field microscopy, the surface of untreated ghost membranes was smooth, while that of Triton-treated ghosts membranes was rough (Fig. 2); the rough surface seemed to consist of a large number of small particles, as opposed to spicules or small projections. Electron microscopy after negative staining showed a number of small vesicles (diameter 0.08–0.22 μm) and a filamentous network (Tsukita *et al.*, unpublished observation). Fourth, 1% Triton could be used instead of 0.5% Triton, although 1% Triton-treated ghosts were less readily visible.

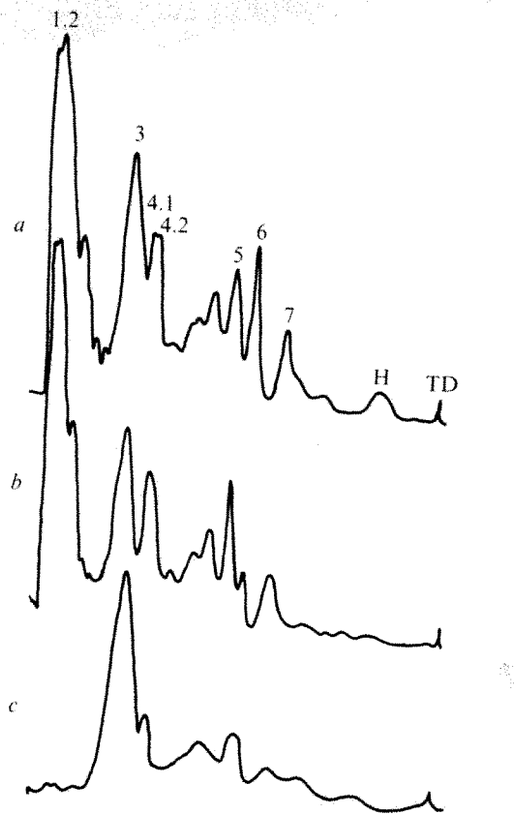


Fig. 1 Densitometric scan of Coomassie blue-stained SDS polyacrylamide gels (5.6%). 10 mM Tris ghosts (*a*), pellet (*b*), supernatant fractions (*c*) derived from 10 mM Tris ghosts after 0.5% Triton treatment. Gels were run and bands numbered accordingly to Fairbanks *et al.*[19]. In each case, 40 μg was applied.
Methods: Human erythrocytes obtained from ACD blood (fresh or preserved for about 4–10 weeks) were washed with physiological saline and haemolysed by 1:50 dilution with 10-mM Tris-HCl buffer, *p*H 7.4, or with 20-mM Tris-maleate buffer containing 1×10^{-3} M $CaCl_2$, and 1×10^{-3} M EGTA, *p*H 7.4 (10^{-6} M Ca^{2+} buffer), at 0 °C, and centrifuged at 18,000 r.p.m. for 20 min at 4 °C. When long-preserved blood was used, the membranes were preincubated for 20 min at 37 °C in 2-mM Mg-ATP solution (*p*H 7.4) (or with 2-mM Mg-ATP in 10^{-6} M Ca^{2+}-buffer). Membranes (converted to diskocytic form by preincubation) were washed with 1 mM $MgCl_2$ at 4 °C (18,000 r.p.m., 20 min). This transformed them to spheres as previously described[11]. Ghosts from fresh cells or pretreated ghosts from preserved blood were mixed with 10 volumes of 1-mM $MgCl_2$ (*p*H 7.4) containing 0.5% Triton X-100. After 5 min at 0 °C, the suspensions were centrifuged at 6,000 r.p.m. for 20 min.

Table 1 Effect of nucleotides and EGTA on the diameters of Triton-treated erythrocyte ghosts shrunk by 10^{-6} M Ca^{2+}

Addition	Diameter μm, $n=30$
None	4.29 ± 0.47
1 mM $MgCl_2$ + 1 mM EGTA	7.53 ± 0.59
1 mM Mg-ATP	6.37 ± 0.63
1 mM Mg-ADP	4.52 ± 0.60
1 mM Mg-GTP	4.51 ± 0.72
1 mM Mg-UTP	4.85 ± 0.55
1 mM Mg-ITP	4.50 ± 0.34

The diameters of Triton-treated ghosts were measured in dark-field light micrographs. Average diameter of ghosts was determined in randomly selected fields and 30 Triton-treated ghosts were measured for each suspension. The preparation of 10^{-6} M Ca^{2+} buffer is described in Fig. 3 legend. Triton-treated ghosts in 10^{-6} M Ca^{2+} buffer were mixed with the indicated solutions at *p*H 7.4, 0 °C and photographs were taken after 10 min at room temperature. When these Triton-treated ghosts were fixed with 1% glutaraldehyde and then 2% osmium tetroxide the results obtained were essentially the same. Both fixatives were dissolved in 10 mM imidazole-HCl and Ca^{2+} EGTA buffer instead of 20 mM Tris-maleate and Ca^{2+} EGTA buffer.

Triton-treated ghosts were larger when prepared with 10-mM Tris-Cl buffer instead of 10^{-6}-M Ca^{2+}-buffer, but shrank for a period of several minutes when suspended in Ca^{2+} buffer (10^{-7}–10^{-6} M) (Figs 2, 3) and became the same size as the Triton-treated ghosts prepared in 10^{-6}-M Ca^{2+} buffer. In fact, shrunken ghosts were spherical and swollen ghosts were diskocytic. These shrunken Triton-treated ghosts swelled on addition of 1 mM Mg-ATP at both 6 °C and room temperature within several minutes (Figs 2, 3) and shrank again after being washed with 1 mM $MgCl_2$ (*p*H 7.4) containing 10^{-6} M Ca^{2+} at both 6 °C and room temperature in the *p*H range, 6.8–7.8. The same shape change was also observed when the incubated Triton-treated ghosts were fixed for 30 min with 1% glutaraldehyde buffered with 10 mM imidazole-HCl (*p*H 7.4) at 0 °C or at room tem-

0028–0836/84/040376—03$01.00

© 1984 Macmillan Journals Ltd

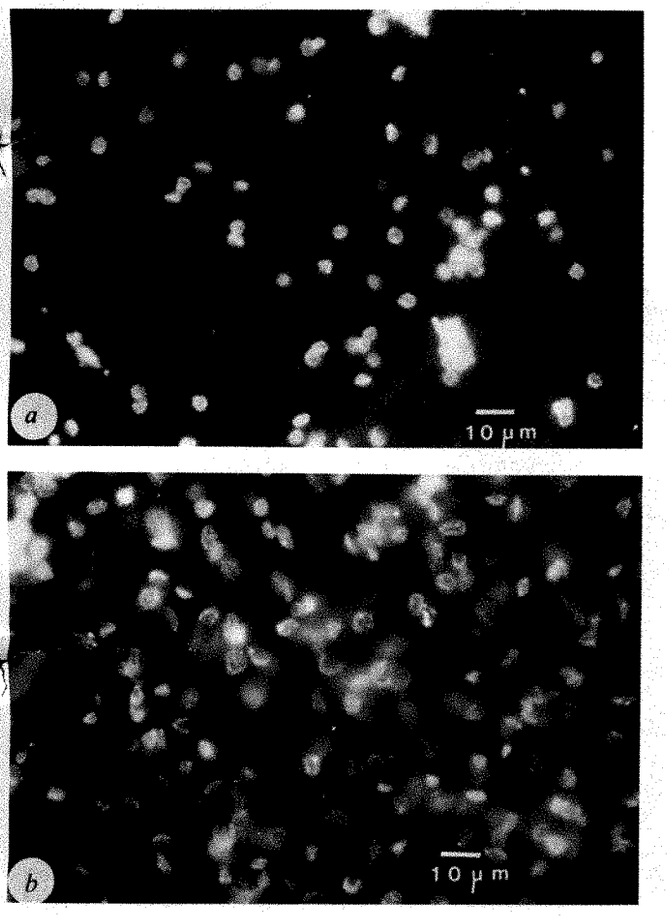


Fig. 2 Dark-field light micrographs of Triton-treated ghosts. The ghosts were prepared with 10 mM Tris-Cl buffer (*p*H 7.4), incubated in 2 mM Mg-ATP (*p*H 7.4) at 37 °C for 30 min, washed with 1 mM $MgCl_2$ (*p*H 7.4) and treated with 0.5% Triton at 0 °C for 5 min. 10 μl of Triton-treated ghosts was dropped into 170 μl of 10^{-6} M Ca^{2+}-buffer at 0 °C (*a*) and 20 μl 10-mM Mg-ATP in 20 mM Tris-maleate buffer (*p*H 7.4) was added at 0 °C (*b*). The shapes of ghosts and Triton-treated ghosts were observed under a dark-field light microscope (Nikon Model S). In 10^{-6} M Ca^{2+}-buffer, Triton-treated ghosts appear to be rough-surfaced small spheres, but on addition of Mg-ATP, they appear to be rough-surfaced disks. These rough-surfaced ghosts seem to be quite different from intact ghosts. The Ca^{2+}-buffer used is described in Fig. 3.

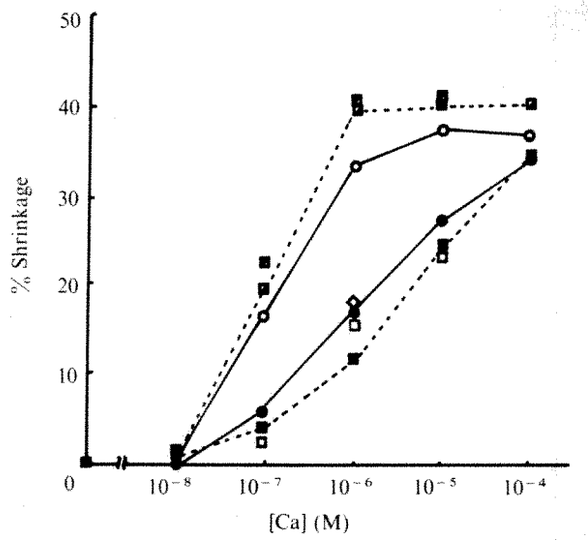


Fig. 3 Effect of Mg-ATP on the shrinkage of Triton-treated erythrocyte ghosts A 10-μl aliquot of Triton-treated ghosts prepared with 10-mM Tris-Cl buffer and 1-mM $MgCl_2$ was mixed with 170-μl Ca^{2+} buffer containing the indicated concentration of Ca^{2+}. ○, Triton-treated ghosts from fresh cells; ●, Triton-treated ghosts from fresh cells on addition of 20 μl 10-mM Mg-ATP; □, Triton-treated ghosts from preserved cells (10 weeks); ◩, Triton-treated ghosts from preserved cells preincubated with adenine and inosine[3]; ◪, Triton-treated ghosts from preserved cells preincubated with 2-mM Mg-ATP after haemolysing and washing, Triton-treated ghosts from preserved cells preincubated with 2 mM Mg-ATP in the presence of 1 mM Mg-ATP; ■, Triton-treated ghosts from preserved cells in the presence of 1 mM Mg-ATP.
Methods: Thirty Triton-treated ghosts were measured as described in Table 1 legend. Shrinkage (%) = $(c-a/c)\times 100$, where c = diameter of Triton-treated ghosts in 1 mM Mg-EGTA solution (*p*H 7.4) a = diameter of Triton-treated ghosts in the indicated Ca^{2+} buffer. Ca^{2+} EGTA buffer was prepared with 20 mM Tris-maleate buffer (*p*H 7.4) containing 0.3 ml 1 mM $CaCl_2$ and various amounts of EGTA as follows in a total volume of 3 ml: Ca^{2+} 1×10^{-8} M, 10 mM EGTA 0.633 ml; Ca^{2+} 1×10^{-7} M, 1 mM EGTA 0.957 ml; Ca^{2+} 1×10^{-6} M, 1 mM EGTA 0.414 ml; Ca^{2+} 1×10^{-5} M, 1 mM EGTA 0.366 ml; Ca^{2+} 1×10^{-4} M, 1 mM EGTA 0.60 ml (ref. 20).

perature and then post-fixed with 2% osmium tetroxide for 30 min at the same temperature. In these experiments, 10 mM imidazole-HCl, Ca^{2+} EGTA buffer was used instead of 20 mM Tris-maleate buffer[13]. Since fixation by 0.5–2% glutaradehyde was not completed in 30 min at 0 °C, we could not determine the exact time required for the shape change. Mg-pyrophosphate, Mg-UTP, Mg-GTP, Mg-ITP, Mg-adenylyl-imidodiphosphate(AMPPMP) and Mg-adenylyl-(β-γ)methylene diphosphate(AMPPCP) were not effective (Table 1) in reversing Ca^{2+}-mediated shrinkage. In the absence of ATP, low concentrations of Ca^{2+} induced shrinkage of the Triton-treated ghosts: the $K_{0.5}$ for Ca^{2+} was about 10^{-7} M, in contrast with the relatively high value of 3×10^{-6} M in the presence of ATP (Fig. 3). Triton-treated ghosts, like untreated ghosts, could change shape reversibly depending on Mg-ATP in the presence of 10^{-6} M Ca^{2+}.

The results described above showed that crenation or disk formation of ghosts occurred in hypotonic solutions with low concentrations of Ca^{2+} or Mg-ATP, respectively, and the shrinking or swelling, respectively, of Triton-treated ghosts occurred in the same conditions. Therefore, crenation of ghosts corresponds to the shrinkage of Triton-treated ghosts, and disk formation of ghosts, to the swelling of Triton-treated ghosts. When isotonicity was restored by adding KCl or NaCl, Triton-treated ghosts shrank immediately and did not respond to either Ca^{2+} or Mg-ATP, agreeing with results reported by Johnson *et al.*[14]. Since shrinking and swelling of Triton-treated ghosts were fast, even at 6 °C, and Triton-treated ghosts lost the permeability barrier, Mg-ATP must act on the cytoskeleton directly and the effect is essentially different from phosphorylation of lipid and protein. The effect of Mg-ATP might not be due to removal of Ca^{2+}, because the shape change occurred even in the presence of 3×10^{-3} M EGTA + 3×10^{-3} M Ca^{2+}, although a nominally equimolar mixture of Ca^{2+} and EGTA will have an indeterminate Ca^{2+} concentration. The sensitivity to Ca^{2+} presumably does not depend on calmodulin because most of calmodulin is lost in the membranes during preparation of ghosts[15] and calmodulin inhibitors[16] (chlorpromazine, trifluoperazine and W-7) did not inhibit this Ca^{2+}-induced shrinking (not shown).

Figure 3 shows that phosphorylation of the Triton-treated ghosts seems to be correlated with the Ca^{2+}-induced shrinking and ATP-induced fast swelling. Both Triton-treated ghosts from fresh cell ghosts without preincubation and from preserved cell ghosts preincubated with Mg-ATP (Fig. 3) were highly sensitive to Ca^{2+} in the absence of Mg-ATP. On addition of Mg-ATP, both of them became relatively insensitive to Ca^{2+} (Fig. 3). However, Triton-treated ghosts from preserved cells without preincubation were not highly sensitive to Ca^{2+} with or without the addition of Mg-ATP (Fig. 3). After alkaline phosphatase treatment of fresh cell ghosts[6], their Triton-treated ghosts became less sensitive to Ca^{2+} ions, and then they recovered Ca^{2+} sensitivity by re-incubation with 2-mM Mg-ATP for 20 min at 37 °C (Table 2).

Table 2 Ca^{2+} sensitivity of Triton-treated erythrocyte ghosts

Source	Pretreatment of intact ghosts	Addition of Mg-ATP	Shape	Ca^{2+} sensitivity
Fresh	None	+	Swell	Low
		−	Shrink	High
	Phosphatase*, wash	+	Swell	Low
		−	Swell	Low
	Phosphatase + P_i*, wash	+	Swell	Low
		−	Shrink	High
	Phosphatase, wash then reincubated with Mg-ATP	+	Swell	Low
		−	Shrink	High
	Heat-denatured phosphatase	+	Swell	Low
		−	Shrink	High
Preserved	None	+	Swell	Low
		−	Swell	Low
	Preincubation† with Mg-ATP	+	Swell	Low
		−	Shrink	High

* Ghosts in a haematocrit of 50% in 10-mM Tris-Cl buffer (*p*H 7.4) incubated with 0.2 mg per ml alkaline phosphatase (*Escherichia coli*, Sigma III R) with and without 10-mM P_i at 4 °C for 20 min as described Birchmeier and Singer[6].

† Preincubation was carried out with 2 mM Mg-ATP at 37 °C for 20 min.

‡ K_m values for Ca^{2+} were roughly 10^{-7} M (High) and 10^{-6} M (Low).

We expected that preserved cell ghosts were dephosphorylated, but that fresh cell ghosts, as well as old cell ghosts treated with Mg-ATP, could be phosphorylated. Direct measurement supported this. The amounts of phsophate in lipid and protein of Triton-treated ghosts were determined after extracting extensively with chloroform, methanol and 1.2 M HCl (10:10:1). Although the phosphate content of lipid was almost constant (about 17 μg per mg protein), the phosphate content of protein was gradually decreased from 0.63 μg per mg protein to 0.25 μg per mg protein during preservation (1–13 weeks). Dephosphorylation of membrane protein seemed to occur during storage. Phosphorylation of membrane protein with 1 mM Mg-ATP^{32} was reported by various investigators[17,18]. In this experiment, preincubation with 1-mM Mg-[γ-^{32}P]ATP (specific radioactivity 1.3×10^7 c.p.m. per μmol of ATP) at 37 °C for 30 min resulted in labelling of the ghosts with ^{32}P ($\sim2.6\times10^4$ c.p.m.), and about 85% of this ^{32}P (about 2.3×10^4 c.p.m.) was removed by Triton treatment. The remaining 15% of the label was present in the Triton-treated ghosts (122 pmol per mg protein), although 50% of phospholipids was removed after Triton treatment. After extensive extraction with acid/chloroform/methanol the ^{32}P content of the membrane was 36 pmol per mg protein. Since the value, 122 pmol per mg, is so small compared with the total ghost phospholipid, 1×10^6 pmol per mg protein, it is not likely that the phosphorylation of lipid is more important than phosphorylation of protein.

We consider that this shrinking and swelling of Triton-treated ghosts involve effects produced on the cytoskeleton at physiological concentrations of Ca^{2+} and ATP and that phosphorylation of cytoskeleton and a noncovalent effect of ATP on cytoskeleton are the basis of this phenomenon.

Received 21 June; accepted 3 November 1983.

1. Nakao, M., Nakao, T. & Yamazoe, S. *Nature* **187**, 945–946 (1969).
2. Nakao, M., Nakao, T., Yamazoe, S. & Yoshikawa, H. *J. Biochem.* **49**, 487–492 (1961).
3. Nakao, M., Nakao, T., Tatibana, M. & Yoshikawa, H. *J. Biochem.* **47**, 694–695 (1960).
4. Palek, J., Stewart, G. & Lionetti, F. *Blood* **44**, 483–597 (1974).
5. Sheetz, M. P. & Singer, S. J. *Cell Biol.* **73**, 638–646 (1977).
6. Birchmeier, W. & Singer, S. J. *J. Cell Biol.* **73**, 647–659 (1977).
7. Sheetz, M. P. & Singer, S. J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4457–4461 (1974).
8. Sheetz, M. P., Painter, R. G. & Singer, S. J. *J. Cell. Biol.* **70**, 193–203 (1976).
9. Quist, E. E. & Reece, K. L. *Biochem. biophys. Res. Commun.* **95**, 1023–1030 (1980).
10. Fairbanks, G., Patel, V. & Dino, J. *Scand. J. clin. lab. Invest.* **41**, 139–144 (1981).
11. Jinbu, Y., Nakao, M., Otsuka, M. & Sato, S. *Biochem. biophys. Res. Commun.* **112**, 384–390 (1983).
12. Yu, J., Fischman, D. A. & Steck, T. L. *J. supramolec. Struct.* **1**, 233–248 (1973).
13. Hellewell, S. B. & Taylor, D. L. *J. Cell. Biol.* **83**, 633–648 (1979).
14. Johnson, R. M., Taylor, G. & Meyer, D. M. *J. Cell Biol.* **86**, 371–376 (1980).
15. Jarrett, H. W. & Penniston, J. T. *J. biol. Chem.* **253**, 4683 (1978).
16. Nelson, G. S., Andrews, M. L. & Karnovsky, M. J. *J. Cell Biol.* **96**, 730–735 (1983).
17. Harris, H. W. Jr & Lux, S. E. *J. biol. Chem.* **255**, 11512–11520 (1980).
18. Imhof, B. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 3264–3268 (1980).
19. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. *Biochemistry* **10**, 2606–2617 (1971).
20. Ogawa, Y. *J. Biochem.* **64**, 255 (1968).

Viscoelastic properties of erythrocyte membranes in high-frequency electric fields

H. Engelhardt, H. Gaub & E. Sackmann

Physik Department, E22, Technische Universität München, D-8046 Garching/München, FRG

The high deformability of erythrocytes which is essential for their transport through the capillaries depends critically on their discoid shape and on the elasticity of the plasma membrane, which may be determined by interactions of the cytoskeleton, the lipid/protein leaflet and the glycocalyx. Although techniques exist for measurement of the static elastic properties of erythrocytes, the cells are continuously deformed *in vivo*, the stress varying within periods of a few seconds. Thus dynamic elastic behaviour is essential for their physiological function. We present here a novel means of measuring the dynamic elastic constants of the red cell based on the transient deformation of individual cells in an inhomogeneous high-frequency (HF) electric field. By microscopy it is possible to record cellular elongations as small as 200 nm occurring within time scales of 1 ms. A main advantage is that the cellular response is linear and thus can be more readily interpreted theoretically. We have observed a creep function consisting of two exponentials with response times of 0.1 s and 1 s, which can be described in terms of a simple viscoelastic model. A remarkable temperature dependence of the membrane elasticity between 25 °C and 15 °C is observed for freshly drawn cells but not for trypsinized ones.

Blood was freshly drawn from the fingertip and diluted in 1 mM phosphate buffer (*p*H 7.5), containing 300 mM D-mannitol and 5 mM glucose. For the trypsin treatment the cells were incubated at 37 °C for 30 min after addition of 10 p.p.m. trypsin and 4 p.p.m. EDTA to the solution. The cell suspension was placed between two electrodes with sharp edges (Fig. 1*a*). Due to the different conductivities of the cytoplasm ($\sigma_i = 1\Omega^{-1}\,m^{-1}$) and the external medium ($\sigma_a = 10^{-2}\,\Omega^{-1}\,m^{-1}$) the behaviour of the cells in the inhomogeneous field depends on the frequency, *f*, in the following way[1]. At $f > f_2(\sim10^7\,Hz)$ both media behave like insulators and the cells swim freely. At $f_1(\sim10^5\,Hz) < f < f_2(\sim10^7\,Hz)$ the conductivity of the cytoplasm can no longer be neglected. This leads to the accumulation of opposite charges at the two ends of the cell opposing the electrodes as indicated in Fig. 1*b*. Due to this so-called Maxwell–Wagner polarization[1] the cell behaves as an electric dipole and couples to the electric field. At $f < f_1$ the cells start to rotate about an axis parallel to the edges of the electrodes. In contrast to Holzapfel *et al.*[2] we observe the rotation of individual cells[3].

For the experiments the frequency is adjusted to a certain value within the second frequency regime. The cells are attracted to one of the electrodes by a bias field of $10^4\,V\,m^{-1}$. For the deformation the amplitude is suddenly increased to 2×10^4–$10^5\,V\,m^{-1}$ for 2.5 s and is then reset to the original value. The time-dependent elongation of the cell—the so-called creep function, $C(t)$—as well as the decay in the original shape, are measured microscopically as shown in Fig. 1.

0028–0836/84/040378—03$01.00

© 1984 Macmillan Journals Ltd

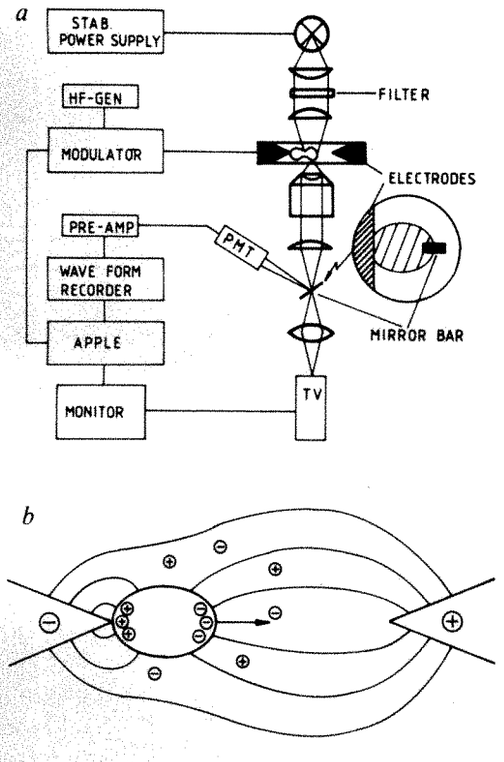


Fig. 1 *a*, Schematic view of an experimental device used to measure the creep ($C(t)$) and decay ($R(t)$) functions after application of a high-frequency field jump. The electrodes are made of commercial razor blades. The curvature of their edges is about 0.5 μm and the electrode distance is 50 μm. *b*, Schematic view of electric field lines and charge accumulation within the cell. This is due to the Maxwell–Wagner polarization which arises as a consequence of the different conductivities of the cytoplasm ($\sigma_i = 1\Omega^{-1}\,m^{-1}$) and that of the outside medium ($\sigma_0 = 10^{-3}\Omega^{-1}\,m^{-1}$). **Methods:** Deformation was achieved in the frequency range 5×10^5–2×10^6 Hz if the conductivity of the outside medium was adjusted to $\sigma_0 = 10^{-2}\Omega^{-1}\,m^{-1}$. The cells were fixed to one of the electrodes by a bias voltage of 1 V. For the transient deformation, field jumps of 2–5 V amplitude and ~2.5 s duration were applied. The HF field was generated by a sine generator equipped with a computer-controlled amplitude modulation. A modified Zeiss Axiomat microscope and a Zeiss oil immersion objective (type Planapo 1.3; oil Pol∞/017) (×100) were used. Bright-field illumination at 410 nm was supplied by a halogen lamp driven by a stabilized power supply. An image of the object was projected onto a TV camera, which allowed certain cells to be selected and observed during measurement. The deformation was measured with a bar-like mirror, deposited on a glass plate, positioned in the focal plane of the objective. The light reflected by this mirror was focused on a photomultiplier (RCA 931 b). If the cell is elongated, the light intensity at the photomultiplier is decreased, the decrease in intensity being proportional to the elongation of the cell. The photomultiplier output is first amplified and then fed into a waveform recorder (Biomation model 1015). From there, the signal is transferred to an Apple microcomputer for signal averaging up to 256 events.

Figure 2 shows typical response curves for a freshly drawn cell (Fig. 2*a*) and for a cell treated with trypsin. Both the creep ($C(t)$) and the decay ($R(t)$) functions exhibit a fast and a slow component. $C(t)$ can be well represented by

$$C(t) = A_0 - A_1 e^{-t/\tau_1} - (A_0 - A_1) e^{-t/\tau_2} \qquad (1)$$

A similar equation holds for $R(t)$. A quantitative evaluation of the experimental curves shows that the time dependencies of $C(t)$ and $R(t)$ are slightly different. This is due to the electric field inhomogeneity through which the force decreases with the degree of elongation (Fig. 3).

As shown in Fig. 2*a*, the maximum amplitude A_0 at 13 °C is smaller by a factor of 5 than at 30 °C. This effect has been observed for 9 out of 10 cells. It is reversible if the temperature is increased and decreased several times. Obviously, this remarkable temperature dependence is not observed for trypsinized cells (Fig. 2*b*). For a first quantitative evaluation of the viscoelastic response curves, it is helpful to introduce a viscoelastic model. As $C(t)$ and $R(t)$ consist of a fast and a slow process, the model of Fig. 3*b* is used[4]. A different model proposed by Rand[5] is adapted to the behaviour of cells at large deformations and long time scales. Our model is characterized by four parameters: two spring constants (μ_1, μ_2) and two viscosities (γ_1, γ_2) which can be determined as follows.

The amplitudes and relaxation times of equation (1) are expressed in terms of the four parameters of the model and the force F_0 by solving the differential equations of the model (Fig. 3) using standard procedures. The parameters are then determined by fitting the theoretical functions $C(t)$ (and $R(t)$) to the experimental response curves. This was done using an Apple computer by the Newton procedure. Finally, μ_1, μ_2, γ_1 and γ_2 are determined in units of the force F_0. Since F_0 cannot be measured yet, it has been estimated in the following way. For $f_1 < f < f_2$ the electric field, E_i, inside the cell is small compared with that at the outside, E_0, since $\sigma_i \gg \sigma_0$. F_0 is obtained by integrating the Maxwell tension over the surface area A of the cell and by time averaging. It is given approximately by: $F_0 \sim 1/4\,\varepsilon_0\varepsilon \times E^2 A$. For $\varepsilon = 80$, $A = 10\,\mu m^2$ and $E_a \sim E_0 = 10^5\,V m^{-1}$ one obtains $F_0 \sim 2\times10^{-11}$ N. The absolute values of the parameters in Table 1 hold for this value of F_0. The spring constant μ_1 is directly given by the maximum amplitude (which was adjusted to ~1 μm) according to

$$\mu_1 = A_0 F_0 = 2\times10^{-5}\,N\,m^{-1} \qquad (2)$$

μ_1. A measure of the elastic modulus of the cell, is of the same order of magnitude as the elastic shear modulus ($7\times10^{-6}\,N\,m^{-1}$ at 25 °C) obtained with the micropipette method[6–8].

To account for the two exponentials of $C(t)$, the model must contain two dash pots and two springs. A manifold of other arrangements would account for the response function. The model was chosen for its simplicity. To describe the membrane viscoelasticity in terms of extensive elastic constants it is necessary to determine the change of the cellular shape quantitatively. Following Hochmuth *et al.*[7] and Waugh[9], an upper limit on the surface viscosity of the plasma membrane may be estimated according to $\eta = \mu \times \tau$. The value of $\gamma = 3\times10^{-6}\,N\,s\,m^{-1}$ is again of the same order of magnitude as that obtained with the micropipette technique: $7\times10^{-7}\,N\,s\,m^{-1}$ (refs 7, 8). It is, however, two orders of magnitude higher than the surface viscosity of the lipid protein bilayer of the red cell as obtained from lateral diffusion studies[9,10]. This shows that the membrane

Table 1 Amplitudes (A_0, A_1), relaxation times (τ_1, τ_2), spring constants (μ_1, μ_2) and viscosities (γ_1, γ_2) for normal and trypsinized cells.

Type of cell	Temperature (°C)	A_0 (μm)	A_1 (μm)	τ_1 (s)	τ_2 (s)	μ_1 (10^{-5} N m^{-1})	μ_2 (10^{-5} N m^{-1})	γ_1 (10^{-6} N m^{-1})	γ_2 (10^{-6} N m^{-1})
Normal	30	1	0.3	0.16	0.9	1.0	0.74	3.7	2.9
	16	0.2	—	—	—	—	—	—	—
Trypsinized	30	1	0.64	0.1	0.9	1.0	0.48	1.7	2.4
	14	1.1	0.45	0.1	0.9	1.1	1.25	2.6	3.8

The absolute values hold for an external force of $F_0 = 2\times10^{-11}$ N.

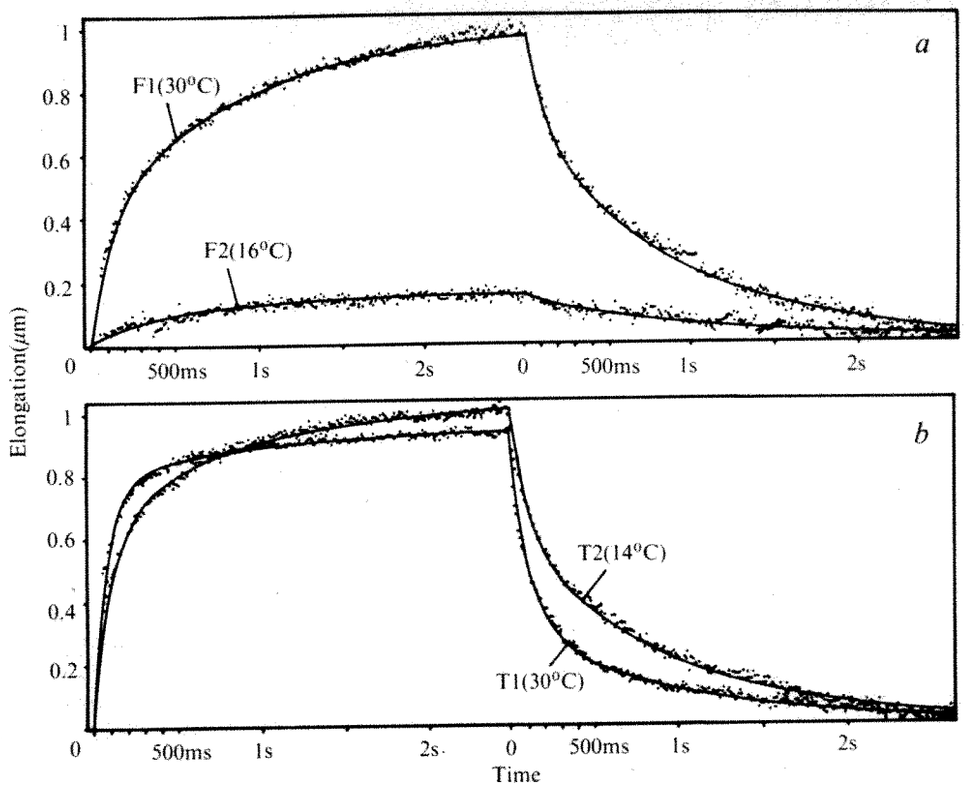


Fig. 2 *a*, Response curves of freshly drawn erythrocytes obtained after application of field jumps of 5 V, $f = 6 \times 10^5$ Hz. The maximum elongation is $A_0 = 1$ μm at 30 °C and $A_0 = 0.21$ μm at 16 °C. The number of repetitions $n = 10$. *b*, Comparison of response curves of trypsinized cells obtained at 30 °C and 14 °C. The frequency is the same as in *a*, voltage 6 V, repetition number $n = 10$, maximum amplitude $A_0 = 1$ μm at both temperatures.

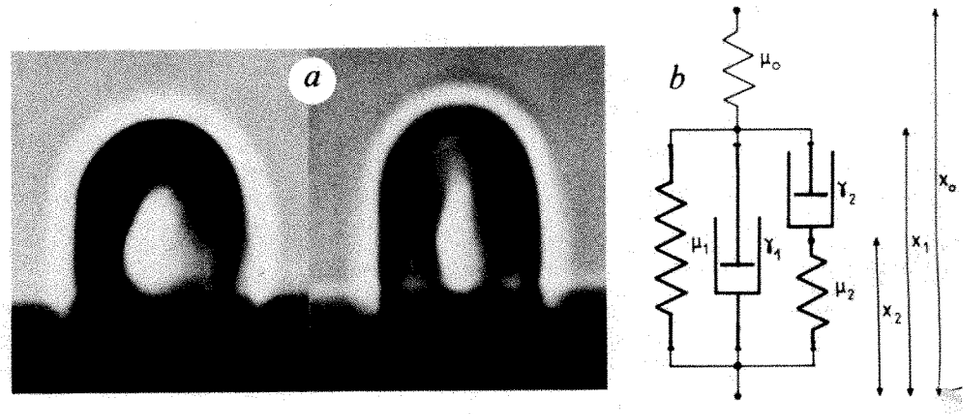


Fig. 3 *a*, Photomicrograph of a cell in the presence of a small bias high-frequency field (left) and after increasing the field strength to 10^5 V m^{-1} which leads to a reversible deformation of the cell (right). Note that the cells have a discoid shape in both cases. *b*, Viscoelastic model of a red blood cell membrane consisting of a parallel arrangement of a Maxwell body (right) and a Voigt body (left). The following notations are used: μ_1, μ_2, stiffness constants of springs; γ_1, γ_2, viscosities of the dash pots; x_1, x_2, elongations of springs of Voigt body and Maxwell body, respectively. The spring having the stiffness constant μ_0 has been introduced to account for the fact that the force F_0 is proportional to the elongation x_1 of the blood cell due to the inhomogeneity of the electric field. The model obeys the following differential equations: $\gamma_1 \dot{x}_1 = F_0 - \mu_1 x_1 - \mu_2 x_2$ and $\gamma_2 \dot{x}_2 = \gamma_2 \dot{x}_1 - \mu_2 x_2$.

viscoelasticity is largely determined by the glycocalyx and the spectrin–actin cytoskeleton which are both coupled to the bilayer[11,12].

The technique described here enables time-resolved measurements in the linear range of deformation and the application of simple equations of viscoelasticity[4]. This is a major advantage compared with the micropipette method[5,6,7] and tether formation[9] or the techniques based on the cellular deformation in viscometric flows[12,13], where non-linear effects have a dominant role. The electric fields are well below the limit of dielectric breakthrough[15,16] and the forces involved ($F_0 \sim 2 \times 10^{-11}$ N) are very weak. The field jump technique thus bridges the gap between the non-disturbing methods, based on the frequency analysis of thermally excited membrane undulations[17–19], and the strongly disturbing techniques mentioned above.

Received 24 June; accepted 7 November 1983.

1. Pohl, H. A. *Dielectrophoresis* (Cambridge University Press, 1978).
2. Holzapfel, C., Vienken, J. & Zimmermann, U. *J. Membrane Biol.* **67,** 13–26 (1982).
3. Engelhardt, H. thesis, Munich (1983).
4. Fung, Y. C. *Biomechanics* (Springer, New York, 1981).
5. Rand, R. P. *Biophys. J.* **4,** 303–316 (1964).
6. Evans, E. A. & Skalak, R. *Mechanics and Thermodynamics of Biomembranes* (CRC, Boca Raton, Florida, 1980).
7. Hochmuth, R. M., Buxbaum, K. L. & Evans, E. A. *Biophys. J.* **29,** 177–182 (1980).
8. Waugh, R. & Evans, E. A. *Biophys. J.* **26,** 115–120 (1979).
9. Waugh, R. *Biophys. J.* **38,** 19–27 (1982).
10. Kapitza, H. G. & Sackmann, E. *Biochim. biophys. Acta* **595,** 56–64 (1980).
11. Heast, C. W. M. *Biochim. biophys. Acta* **694,** 331–352 (1982).
12. Branton, D., Cohen, C. M. & Tyler, I. *Cell* **24,** 24–32 (1981).
13. Bessis, M. & Mohandas, M. *Blood Cells* **1,** 307–313 (1975).
14. Schmid-Schönbein, H., von Gosen, J., Heinrich, L., Klose, H. J. & Volger, E. *Microvasc. Res.* **6,** 366–376 (1973).
15. Zimmermann, U., Scheurich, P., Piwlat, G. & Benz, R. *Angew. Chem. (Engl. Edn)* **20,** 325–344 (1981).
16. Neumann, E., Gerisch, G. & Opatz, K. *Naturwissenschaften,* **67,** 414–415 (1980).
17. Servuss, R. M., Harich, W. & Helfrich, W. *Biochim. biophys. Acta* **436,** 900–903 (1976).
18. Brochard, F. & Lennon, J. F. *J. de Phys.* **36,** 1035–1047 (1975).
19. Fricke, K. & Sackmann, E. *Biochim. biophys. Acta* (in the press).

Effect of IFN-γ on the immune response *in vivo* and on gene expression *in vitro*

Masataka Nakamura, Tim Manser, Gregory D. N. Pearson, Michael J. Daley & Malcolm L. Gefter

Department of Biology, Building 56–701, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

T lymphocytes produce a variety of immunoregulatory molecules including gamma interferon (IFN-γ)[1–3] and antigen-specific suppressor and enhancer factors[4–7]. During our studies of active substances obtained from cloned T-cell lines, we observed that certain fractions administered to mice resulted in enhancement of immune responses. Preliminary characterization of the substance suggested that it could be IFN-γ and we therefore undertook a study of the action of IFN-γ produced by recombinant DNA methodology on immune responses. We found that for several antigens, administration of IFN-γ to mice leads to two- to five-fold enhancement of antibody formation provided that the IFN-γ and antigen are administered together. The effect was dose dependent, giving a maximal response at 500–600 anti-viral units per mouse. Preliminary studies suggest that the macrophage may be the target of IFN-γ action. Addition of IFN-γ to cultures of a macrophage cell line leads to a greater than 10-fold increase in the level of RNA coding for *I*-region-encoded cell surface molecules.

Various doses of murine IFN-γ, produced by monkey cells transfected with a simian virus 40 (SV40)-based expression vector containing the murine IFN-γ gene, were injected into BDF_1(B6XDBA/2) mice along with the antigen hen egg lysozyme (HEL) or the antigen dinitrophenol-conjugated bovine γ-globulin (DNP-BGG). Administration of IFN-γ affected indirect plaque-forming cell (PFC) responses to both HEL and DNP in a similar dose-dependent manner. A two- to five-fold enhancement of PFC was observed when 500–2,000 units of IFN-γ were administered (Fig. 1*a* and *b*). Administration of doses as high as 6,700 or as low as 100 anti-viral units per mouse did not significantly alter the PFC response. Stimulation of the PFC response (direct plaques) using 1,000 units per mouse was also observed with the antigen sheep red blood cells (data not shown). Preparations of IFN-γ derived from independently transfected monkey cells were equally active and showed the same dose–response curve.

The enhancement of the PFC response observed might have been due to recruitment of responding cells to draining lymph nodes, rather than to an actual increase in the number of reactive cells[8]. If the total number of PFC per animal remained unchanged then one would expect that the total antibody secreted per animal would remain constant. Accordingly, serum titres of treated mice were examined. Five mice were injected with 250 units of IFN-γ or phosphate-buffered saline (PBS) on both the day of immunization with the antigen HEL and again on the following day. Individual mice were bled 12 days after immunization and the amount of antibody specific for HEL was determined by radioimmunoassay (monoclonal antibody to HEL was used as a standard). The control group had an average of 1.0 mg ml^{-1} of anti-HEL antibody whereas the IFN-γ-treated group had an average of 3.4 mg ml^{-1}. The stimulation index of 3.4 agrees with that observed in PFC responses. The range of stimulation indices for individual mice was 1.5–9.2.

The IFN-γ preparations used above contained components of fetal calf serum as well as cellular components secreted by monkey cells. To examine whether the enhancement of the PFC response by IFN-γ preparations was mediated by IFN-γ itself, the preparations were passed over a column containing anti-IFN-γ immunoglobulin. As seen in Table 1, the stimulating activity was removed by such a treatment but could be subsequently recovered from the column. Similarly, the stimulating activity was sensitive to *p*H 2.0, a property of IFN-γ (see Table 1). Direct evidence that the enhancement activity was exclusively due to INF-γ was obtained by using murine IFN-γ isolated from *Escherichia coli* cells expressing a mouse IFN-γ gene. These data are shown in Fig. 1*c* and *d*. Two- to four-fold enhancement of PFC responses to HEL and DNP was observed upon administration of IFN-γ at the same doses used earlier. These results strongly indicated that the increase in the PFC response was due to the IFN-γ molecule itself.

To gain some insight into the mechanism of enhancement of antibody formation, we studied the effect of altering the time of administration of IFN-γ relative to antigen challenge. Administration of IFN-γ at the same time as immunization was the most effective (Table 2). Administration of IFN-γ 1 and 2 days before immunization resulted in some stimulation of the anti-HEL response whereas no enhancement was observed if IFN-γ was given on both day 3 and 4 after immunization. This result suggests that perhaps some early step of the immune response such as antigen processing by macrophages could be affected by IFN-γ. IFN-γ, produced by recombinant DNA technology as well as T cells stimulated with mitogens, has been shown to have profound effects on macrophage-dependent cell killing[9–11] and on expression of cell surface molecules associated

Table 1 Neutralization of enhancing activity of IFN-γ on the PFC response by anti-IFN-γ globulin or treatment at *p*H 2

Treatment	IFN(U)	PFC per 10^6 cells ± s.e.	SI	*P*
	Control	1,453 ± 26	1.00	
None	400	4,448 ± 214	3.06	<0.001
None	800	7,786 ± 162	5.36	<0.001
Anti-IFN-γ effluent	(400)	2,325 ± 114	1.60	NS
Anti-IFN-γ effluent	(800)	1,658 ± 199	1.15	NS
Anti-IFN-γ eluate	400	5,768 ± 570	3.97	<0.001
Anti-IFN-γ eluate	800	5,198 ± 380	3.58	<0.001
	Control	666 ± 21	1.00	
*p*H 7.3	500	1,761 ± 179	2.64	<0.005
*p*H 7.3	1,000	2,535 ± 209	3.81	<0.001
*p*H 2.0	(500)	685 ± 63	1.03	NS
*p*H 2.0	(1,000)	925 ± 84	1.39	NS

The monkey cell-derived IFN-γ was passed through a column of Sepharose-4B (Pharmacia) conjugated with anti-IFN-γ globulin (Enzo Biochemical, New York). The material absorbed to the column was eluted with 4 M NaCl. Effluent and eluate were dialysed against 100 volumes of PBS for 24 h. For acid treatment of IFN-γ, the samples were dialysed against either sodium acetate buffer (*p*H 2.0) or PBS (*p*H 7.3) for 16 h and were then dialysed against PBS for 24 h. Procedures for immunization, IFN-γ injection and PFC assay were as described in the legend to Fig. 1. HEL was used as an antigen. Units in parentheses indicate the amount of IFN-γ equivalent to those contained in a given volume before treatment. SI, stimulation index; *P*, probability; NS, not significant.

Table 2 Time dependency of enhancement activity

Injection day	PFC per 10^6 cells ± s.e.	SI	*P*
Control	1,348 ± 158	1.00	
−2, −1	3,693 ± 466	2.74	<0.01
0, 1	5,946 ± 767	4.41	<0.005
3, 4	1,949 ± 456	1.45	NS

Mice were immunized with HEL on day 0 as in Fig. 1 and injected with 250 U of IFN-γ in 0.5 ml of PBS on both days indicated above. The control group was injected with 0.5 ml of PBS on day 0 and day 1. PFC responses were determined as in Fig. 1.

0028-0836/84/040381—02$01.00

© 1984 Macmillan Journals Ltd

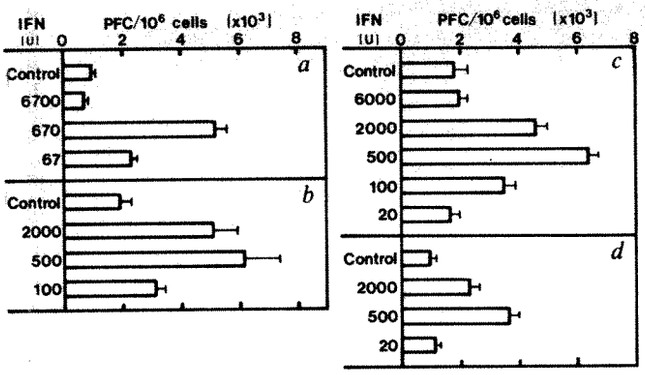


Fig. 1 Effect of IFN-γ on the plaque-forming cell response of mice. Eight- to 12-week-old BDF_1 mice (Jackson Laboratories, Bar Harbor) were immunized with either 100 μg of HEL (graphs *a* and *c*) or DNP-BGG (graphs *b* and *d*) in complete Freund's adjuvant intraperitoneally. The INF-γ preparations, which were supplied by Genentech Inc. (South San Francisco), were culture supernatants (20,000 U ml^{-1}) of the monkey cells transfected with an SV40-based expression vector containing a mouse IFN-γ gene (the approach to its production was basically that which has been reported for the production of cloned human IFN-γ)[16] and a product purified from *E. coli* harboring an expression vector that contained murine IFN-γ cDNA (specific anti-viral activity of 8×10^6 U per mg protein)[17]. Left (*a* and *b*) and right (*c* and *d*) graphs show the results obtained by using the monkey cell-derived and the *E. coli*-derived IFN-γ, respectively. The mice were injected intravenously with various doses of IFN-γ diluted in 0.5 ml of PBS. Injections were on both the day of immunization and the following day. Doses reported are the total amount administered. The control group received 0.5 ml of PBS intravenously. Eight days after immunization a PFC assay was carried out using cells of parathymic lymph nodes for the HEL response or spleen for the DNP response according to a modified method of Jerne[18]. HEL- or trinitrophenol-conjugated sheep red blood cells (SRBC) (Scott Laboratories, Fiskeville, Rhode Island) were used as indicator cells. Lyophilized guinea pig serum (Colorado Serum Co.), absorbed on SRBC, was used as a source of complement at a final dilution of 1:40. Rabbit anti-mouse immunoglobulin serum was used at a final dilution of 1:400 to detect IgG PFC. Each bar represents the average PFC response of five mice plus the standard error (s.e.).

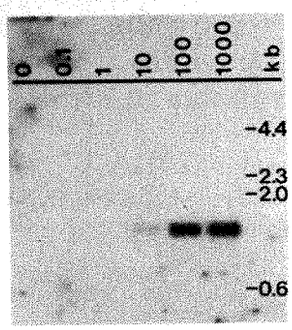


Fig. 2 Increase in the level of *I*-region-encoded RNA due to the addition of IFN-γ to the culture medium of the mouse macrophage cell line P388D1. The DBA/2-derived macrophage cell line P388D1 (ref. 19) was grown in monolayer culture to approximately 30% confluence, whereupon various amounts of *E. coli*-derived murine IFN-γ were added directly to the medium. The cells were then incubated at 37 °C for 48 h and collected. Total cellular RNA was prepared from each cell sample as previously described[20,21]. 15 μg of each RNA sample were glyoxalated, electrophoresed in a 1.2% agarose gel and transferred to nitrocellulose as described[22]. The nitrocellulose filter was then baked for 2 h at 80 °C under vacuum and hybridized (in a buffer containing 50% formamide, 5×SSC and 10% dextran sulphate) at 42 °C for 20 h with an *I*-region (A^d_α) cDNA clone probe (labelled with ^{32}P via nick-translation) which was kindly provided by Dr Mark Davis, NIH. After multiple washings in 2×SSC at 65 °C the filter was exposed to X-ray film for 48 h using intensifier screens. The resulting autoradiogram is shown. The positions of molecular weight standards run in an adjacent lane (not shown) are indicated. Lane 1, no IFN-γ added; lane 2, IFN-γ added to a final concentration of 0.1 anti-viral units per ml culture medium; lane 3, 1 unit per ml; lane 4, 10 units per ml; lane 5, 100 units per ml; lane 6, 1,000 units per ml. The size of the hybridizing band (1.2 kb) is that expected for $I\text{-}A_\alpha$ mRNA.

with antigen presentation[12–14]. Also it has been reported that recombinant human IFN-γ increases synthesis of HLA-DR molecules on human melanoma cell lines[15]. It may be that one of the biological functions of IFN-γ is to convert macrophages from an inactive to an active state, thereby promoting antigen presentation and hence immune responses.

We tested the effect of the bacteria-derived IFN-γ on the expression of *I*-region-encoded antigens in a macrophage cell line, P388D1. As seen in Fig. 2, treatment of cultured cells with doses of IFN-γ of 10–1,000 units ml^{-1} leads to a profound increase in the level of expression of RNA derived from the I-region. The effect was dose dependent and suggests that IFN-γ may act by inducing synthesis of mRNAs that encode proteins involved in antigen processing and presentation.

We have shown that IFN-γ is an immunoregulatory agent as assayed *in vivo* using recombinant DNA-generated preparations. The ability of IFN-γ to enhance the level of induced antibodies was seen with several antigens. The time-dependent nature of the phenomenon suggests that an early step in the immune response is affected. The induction of *I*-region-specific RNA seen in a macrophage cell line suggests that this early step may be antigen processing and/or presentation. Interferon may be a general enhancer of other differentiated functions within the immune system. We are currently investigating whether IFN-γ has demonstrable physiological effects on T and B cells. The basis of stimulation of *I*-region-derived mRNA is also being studied.

We acknowledge Genentech Inc. for providing the recombinant IFN-γ. We also thank Dr Daniel Capon of Genentech for his help and Audrey Childs for the preparation of this manuscript. This work was supported by NIH grants 1 P01 CA28900 and 5-R01-AI13357 and American Cancer Society grant NP-6M. M.D. is supported by NIH grant 5-F32-CA06873. T.M. is supported by ACS grant PF-2240.

Received 25 July; accepted 28 October 1983.

1. Marcucci, F., Waller, M., Kirchner, H. & Krammer, P. *Nature* **291,** 79–81 (1981).
2. Benjamin, W. R., Steeg, P. S. & Farrar, J. J. *Proc. natn. Acad. Sci. U.S.A.* **79,** 5379–5383 (1982).
3. Le, J., Vilcek, J., Saxinger, C. & Prensky, W. *Proc. natn. Acad. Sci. U.S.A.* **79,** 7857–7861 (1982).
4. Minami, M., Okuda, K., Furusawa, S., Benacerraf, B. & Dorf, M. E. *J. exp. Med.* **154,** 1390–1402 (1981).
5. Takabayashi, K. *et al. J. Immun.* **130,** 2552–2556 (1983).
6. Lonai, P. *et al. J. exp. Med.* **154,** 942–951 (1981).
7. Apte, R. N., Eshhar, Z., Löwy, I., Zinger, H. & Mozes, E. *Eur. J. Immun.* **11,** 931–936 (1981).
8. Korngold, R., Blank, K. J. & Murasko, D. M. *J. Immun.* **130,** 2236–2240 (1983).
9. Meltzer, M. S., Benjamin, W. R. & Farrar, J. J. *J. Immun.* **129,** 2802–2807 (1982).
10. Pace, J. L., Russell, S. W., Torres, B. A., Johnson, H. M. & Gray, P. W. *J. Immun.* **130,** 2011–2013 (1983).
11. Steeg, P. S., Johnson, H. M. & Oppenheim, J. J. *J. Immun.* **129,** 2402–2406 (1982).
12. McNicholas, J. M., King, D. P. & Jones, P. P. *J. Immun.* **130,** 449–456 (1983).
13. King, D. P. & Jones, P. P. *J. Immun.* **131,** 315–318 (1983).
14. Wong, G. H., Clark-Lewis, L., McKimm-Breschkin, J. L., Harris, A. W. & Schrader, J. W. *J. Immun.* **131,** 788–792 (1983).
15. Basham, T. Y. & Merigan, T. C. *J. Immun.* **130,** 1492–1494 (1983).
16. Gray, P. W. *et al. Nature* **295,** 503–508 (1982).
17. Gray, P. W. & Goeddel, D. V. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5842–5846 (1983).
18. Mishell, B. B., Shiigi, S. M. & Mishell, R. I. in *Selected Methods in Cellular Immunology* (eds Mishell, B. B. & Shiigi, S. M.) 72–77 (Freeman, San Francisco, 1980).
19. Koren, H. S., Handwerger, B. S. & Wunderlich, J. R. *J. Immun.* **114,** 894–897 (1975).
20. Glisin, V., Crkvenjakov, R. & Byus, C. *Biochemistry* **13,** 2633–2637 (1974).
21. Ullrich, A. *et al. Science* **196,** 1313–1319 (1977).
22. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5201–5205 (1980).

Surface dynamics of the integral membrane protein bacteriorhodopsin

Max A. Keniry, H. S. Gutowsky & Eric Oldfield

School of Chemical Sciences, University of Illinios at Urbana-Champaign, 505 South Mathews Avenue, Urbana, Illinois 61801, USA

Recently, there has been great interest in determining the three-dimensional structures of membrane proteins, particularly bacteriorhodopsin, for which a variety of possible folding arrangements have been suggested[1–4]. In this paper we present nuclear magnetic resonance (NMR) spectra[5–8] of deuterated bacteriorhodopsin, and use the data to help interpret the various suggested bacteriorhodopsin folding patterns. The results strongly indicate that (1) a membrane surface (±1 residue) may be defined by NMR in bacteriorhodopsin; (2) all amino acids inside the surface are essentially crystalline; (3) all amino acids outside the surface (surface residues) in bacteriorhodopsin are highly mobile on the time scale of the ^{2}H NMR experiments; (4) NMR data may be used to help evaluate the various structural models that have been proposed; (5) aggregation of purple membrane sheets may lead to an immobilization of the surface residues.

In Fig. 1 *A–I*, we show ^{2}H NMR spectra of a variety of ^{2}H-labelled bacteriorhodopsin samples. In all cases, the spectrum observed consists of a sharp central component and a broad powder pattern. The central component arises, we believe, from surface residues in bacteriorhodopsin. Observation of this component requires acquisition of at least 2,000–4,000 data points, Fig. 1, *J–M*, to avoid truncation effects and its 'disappearance'. The component can then be quantified by means of computer simulation.

The central components arise from deuterons that reorient over a wide angular range in a time several-fold less than the inverse of the frequency of the rigid lattice quadrupolar splitting, that is, $\tau_c \ll 10^{-5}$ s. In principle, the deuterium could be in very mobile surface residues, in isotropically tumbling membrane fragments, or perhaps in residual HO^{2}H in our sample. We rule out the possibility that residual HO^{2}H is a significant contributor to the central component because additional exchanges of the samples with ^{2}H-depleted water do not reduce the intensity of the central component, and the absolute intensity of a corresponding unlabelled membrane sample, exchanged with ^{2}H-depleted water, is negligible. All samples (with different labelling of the amino acids by ^{2}H) contain essentially the same protein/water ratios, having been exchanged three times with deuterium-depleted water by centrifugation (for 14 h at 130,000*g*) followed by resuspension in deuterium-depleted water (200 mg membrane per 5 ml water), but the intensity of the central component varied widely. Even lyophilization and resuspension in ^{2}H-depleted water does not change the intensity of the isotropic components (data not shown).

We rule out the possibility that the central components arise from isotropically tumbling membrane fragments since then the isotropic/rigid ratios should be the same for each labelled amino acid, which is not the case. Instead, there is a strong correlation between the position of the residue within the purple membrane postulated by the various experimental and theoretical models[1–4], and the intensity of the central component resonances of Fig. 1 *A–I*. We thus ascribe at least 90–95% of the intensity of the central component to surface residues undergoing fast isotropic motion.

In Fig. 2 we show the folding pattern of bacteriorhodopsin in the purple membrane of *Halobacterium halobium* postulated recently be Engelman *et al.*[4]. To interpret our ^{2}H NMR results, we define a membrane surface, designated B in Fig. 2. Residues to the outside of line B are surface residues, which we assert will be mobile on the ^{2}H NMR timescale (giving the central 'isotropic' components, Fig. 1, *A–I*), while residues inside of line B will be rigid, or crystalline, and lead to the broad powder-pattern line shapes of Fig. 1. Similarly, we define two alternative membrane surfaces, A and C, which are two residues outside or inside of line B respectively. The earlier models of Engelman *et al.*[1], Ovchinnikov[2] and Khorana[3] may be treated similarly, to define various possible membrane surface planes.

We show in Fig. 3 *A–D*, the correlations we have obtained, for a selection of deuterium-labelled amino-acids, between the percentage of the residues located on the surface of bacteriorhodopsin (for a particular model, refs 1–4), and the percentage of the total ^{2}H signal intensity of the isotropic component (mobile, surface residues) obtained from simulations of the spectra of Fig. 1 (and data at 37 °C for Phe, Tyr and Arg, not shown). There is a significant correlation for all models, Fig. 3 and Table 1. The earliest model of Engelman *et al.* (ref. 1, Fig. 3*A*) has the poorest agreement with the ^{2}H NMR results. We predict a theoretical slope (m) of 1.0, for a 1:1 correspondence between percent surface residues and percent isotropic component, while the model gives $m = 1.12$ with a correlation coefficient $\rho = 0.80$. The later models of Ovchinnikov (Fig. 3*B*), Khorana (Fig. 3*C*) and Engelman (Fig. 3*D*) all give considerably better agreement (Table 1). Each part of Fig. 3 includes the perfect agreement, theoretical line ($m = 1$, $c = 0$).

We have also examined each of the published bacteriorhodopsin models using changes in surface definition of ±1, and ±2 residues. The results indicate that the originally proposed surfaces gave the best fit (Table 1). As an example, we show in Fig. 3*E* typical results for the Engelman/82 model[4]. For a +2 and a −2 residue change in surface definition the correlation with the NMR results is much poorer (+2, $m = 0.69$, $c = -2.1$, $\rho = 0.69$; −2, $m = 1.25$, $c = 10.8$, $\rho = 0.68$).

Table 1 Correlation between the number of surface residues predicted for various theoretical models of bacteriorhodopsin structure and the experimental ^{2}H NMR results

Model	Slope (m)	Intercept (c)	Correlation coefficient (ρ)
Ideal*	1.00	0.00	1.00
Wertz-Scheragar†	0.98	17.5	0.61
Engelman, 1980‡	1.12	−3.1	0.80
+1§	0.89	−3.3	0.81
+2	0.56	−1.2	0.69
−1	1.24	1.7	0.86
−2	1.12	12.1	0.70
Ovchinnikov, 1982‖	1.08	2.0	0.87
+1	0.90	−1.1	0.79
+2	0.64	−1.6	0.64
−1	1.15	5.3	0.85
−2	1.09	15.2	0.68
Khorana, 1982¶	1.10	−0.2	0.85
+1	0.76	0.7	0.77
+2	0.60	0.2	0.64
−1	1.29	3.3	0.85
−2	1.15	13.9	0.63
Engelman, 1982#	0.93	3.0	0.90
+1	0.77	1.4	0.76
+2**	0.69	−2.1	0.69
−1	1.20	4.6	0.82
−2††	1.25	10.8	0.68

Results were calculated assuming a 1:1 correspondence between the % surface residues for a given model (S, %) and the intensity (I, %) of the central, isotropic ^{2}H NMR component as $S(\%) = m(I, \%) + c$.
* For perfect agreement, $m = 1.00$, $c = 0.00$ and $\rho = 1.00$, where ρ is the correlation coefficient, m is the slope and c is the intercept, as defined above. † Ref. 9. ‡ Ref. 1. § Surface defined as 1 and 2 residues outside (+) and 1 and 2 residues inside (−) from published model, respectively. ‖ Ref. 2. ¶ Ref. 3. # Ref. 4, line B in Fig. 2. ** Line A in Fig. 2. †† Line C in Fig. 2.

0028-0836/84/040383—04$01.00

© 1984 Macmillan Journals Ltd

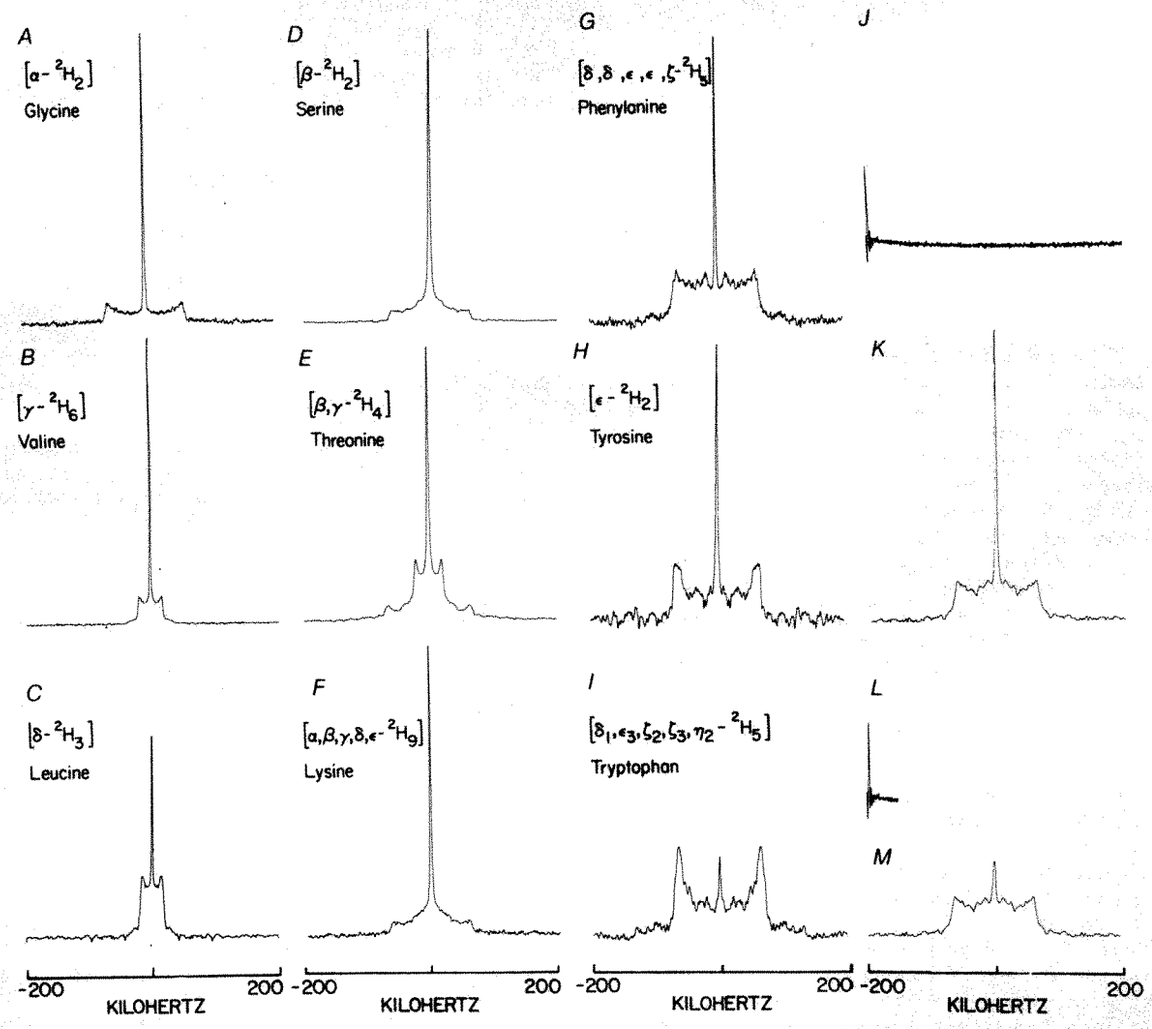


Fig. 1 Experimental ^2H NMR spectra (at 55.3 MHz and 4 °C) of selectively ^2H-labelled membranes, showing intensity of the central (surface) component, and truncation effects. *A–I*, ^2H-labelled amino acid, as indicated. *J–M*, [$^2H_5$]phenylalanine-labelled bacteriorhodopsin in the time and frequency domains. *J*, *K*, 4,096 data points, *L*, *M*, 512 data points. Note that inadequate digitization causes a loss of central-component intensity. The spectra were obtained on a home-built instrument which consists of a 3.5-inch bore 8.45 Tesla superconducting solenoid (Oxford Instruments), a Nicolet 1180 computer interfaced to a Nicolet NIC-2090 50-ns transient recorder system (Nicolet), and a variety of digital and radiofrequency electronices[10]. Spectra were recorded for a 800-μl sample, with a quadrupole-echo pulse sequence[11] using a 90° pulse width of ~3 μs. The spectrometer was prepared for data acquisition as described previously[12]. DL[β-$^2H_4$]serine was synthesized from ethyl acetamidocyano-acetate by reduction with sodium borodeuteride, followed by hydrolysis with strong acid[13] and recrystallization from aqueous ethanol. DL[β, γ-$^2H_4$]threonine (~75% in the *threo* form) was synthesized by condensing [$^2H_4$]acetaldehyde (Merck, Sharpe and Dohme) with *bis*-glycine copper (II)[14]. Copper was removed on a column of Dowex 50W-X8 (Bio-Rad) with 5M NH_4OH as the eluant. After removal of the solvent under reduced pressure, the DL[β, γ-$^2H_4$]threonine was recrystallized from methanol/water (4: 1, v/v). [α-$^2H_2$]glycine, L[δ-$^2H_3$]leucine, and DL[α, β, γ, δ, ε-$^2H_9$]lysine were purchased from Merck, Sharpe and Dohme, and L[α, β, γ, δ, ε-$^2H_{10}$] isoleucine was from Cambridge Isotopes. DL[γ-$^2H_6$]valine, L[δ_1, δ_2, ε_1, ε_2, ζ-$^2H_5$]phenylalanine, L[ε_1, ε_2-$^2H_2$]tyrosine and L[δ_1, ε_3, ζ_2, ζ_3, η_2-$^2H_5$]tryptophan were prepared as described previously (refs 15, 16 and references cited therein). *Halobacterium halobium* R_1 (the kind gift of Professor T. Ebrey) was grown and harvested as described previously[15,16]. Deuterated amino acids were incorporated into a defined medium at the following concentrations: [$^2H_2$]Gly, 0.6 g per 10l; DL[γ-$^2H_6$]Val, 10.0 g per 10l; L[δ-$^2H_3$]Leu, 0.2 g per 5l; L[α, β, γ, δ, ε-$^2H_{10}$]Ileu, 0.2 g per 5l; DL[β-$^2H_2$] Ser, 3.0 g per 10l; DL[β, γ-$^2H_4$]Thr, 5.0 g per 10l; L[α, β, γ, δ, ε-$^2H_9$]Lys, 0.2 g per l; L[δ_1, δ_2, ε_1, ε_2, ζ-$^2H_5$]Phe, 1.3 g per 10l; L[ε_1, ε_2-$^2H_2$]Tyr, 2.5 g per 10l; L[δ_1, ε_2, ζ_2, ζ_3, η_2-$^2H_5$]Trp, 5.0 g per 10l. We have assumed that only L-amino acids were incorporated when DL mixtures were added to the growth medium. Purple membranes were isolated essentially by the method of Becher and Cassim[17], as described previously[15,16]. Membranes were exchanged three times with ^2H-depleted water (Aldrich) prior to NMR spectroscopy, by equilibrating in the H_2O (unbuffered and salt free) for 1 h at 23 °C, followed by centrifugation at 130,000*g* for ~14 h. Amino acid incorporation was checked in radiotracer experiments with 50 μCi of uniformly [^{14}C]-labelled amino acids per 500ml growth medium as described previously[15,16]. Val, Leu, Ileu, Thr, Lys, Phe, Tyr and Trp showed essentially no (≤5%) breakdown and reincorporation into other amino acids. However, only 52% of the ^{14}C-Ser counts in the purple membrane hydrolysate were found to be associated with that amino acid, the other radioactivity being associated with glycine (16%), alanine (12%) and tryptophan (9%). Similarly, only 81% of the ^{14}C-Gly counts were associated with glycine, the other radioactivity being incorporated into tyrosine (12%) and serine (6%). The amounts of endogenous amino-acid synthesis were not determined.

We also show in Fig. 3*F* the correlation between the per cent residues on the surface of a typical globular protein (the Wertz-Scheraga scale)[9], and the per cent isotropic component. This shows an even poorer agreement ($m = 0.98$, $c = 17.5$, $\rho = 0.61$), illustrating we believe the large differences between the membrane protein bacteriorhodopsin and a typical globular protein.

Finally, we note that the ^2H NMR spectra of highly aggregated purple membrane sheets (obtained by mild tryptic cleavage of the C-terminal residues) do not exhibit the intense central components characteristic of mobile surface residues. For example, in the case of [γ-$^2H_6$]valine, the central component contributes 35% to the intensity of the untreated membrane

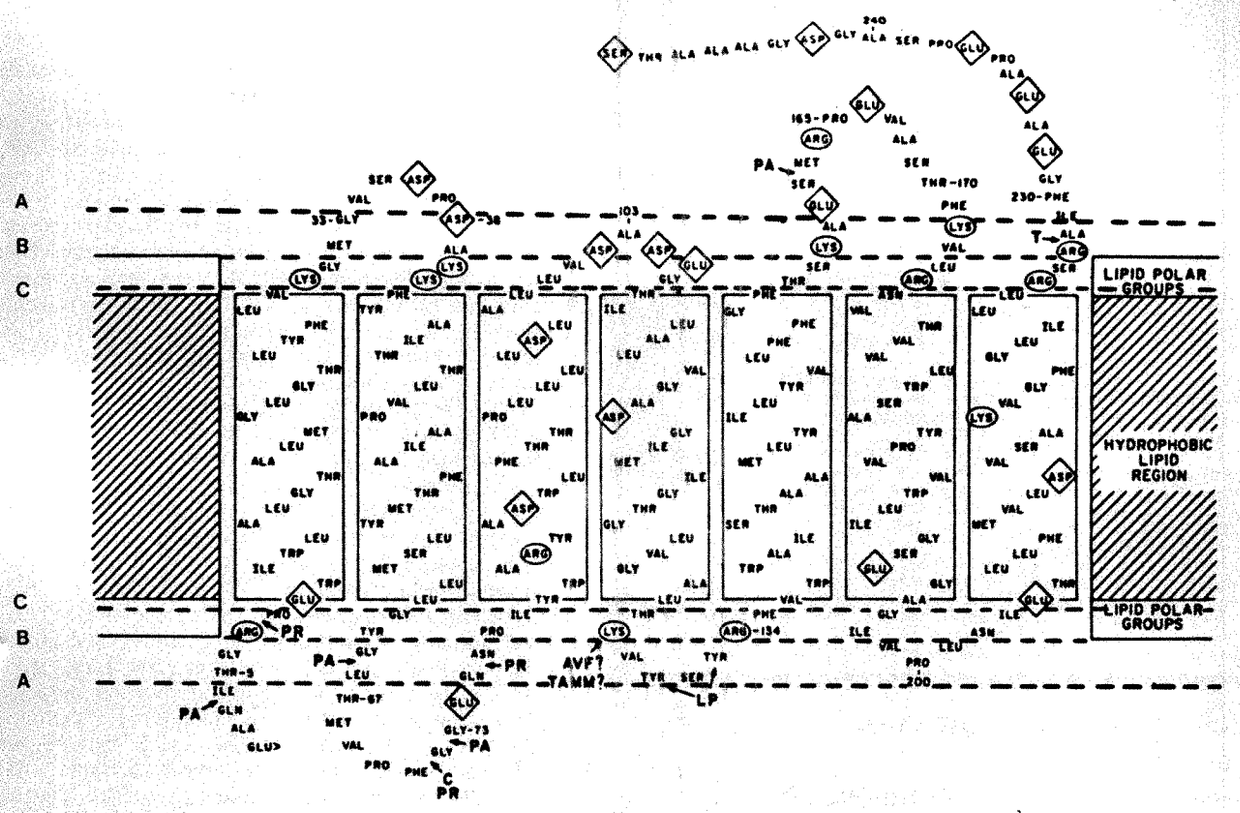


Fig. 2 Proposed model of secondary structure of bacteriorhodopsin (ref. 4) showing various definitions of the membrane surfaces (A, B and C).

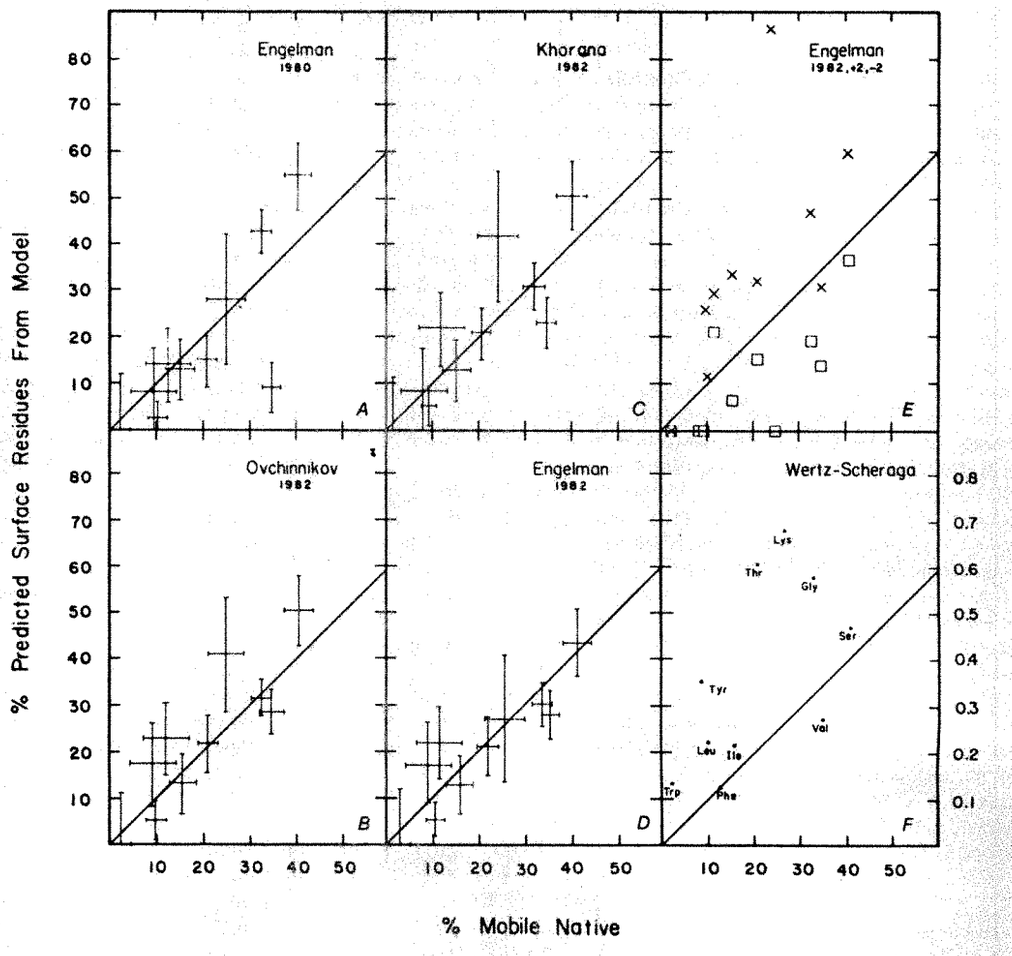


Fig. 3 Correlations between per cent surface residues for various theoretical models of bacteriorhodopsin structure, and per cent isotropic component in the ^2H NMR spectrum (Fig. 2). *A*, Engelman, 1980 model. *B*, Ovchinnikov, 1982 model[2]. *C*, Khorana, 1982 model[3]. *D*, Engelman, 1982 model[4]. *E*, Engelman, 1982 model using as surface definitions lines *A* (+2, □) and *C* (−2, ×) from Fig. 2. *F*, Correlations with the Wertz-Scheraga index[9]. The vertical error bars are percentage errors for an error of ± one residue. The horizontal error bars represent an estimate of the experimental error in the central component intensity determination. The straight lines in each part are the theoretical relationship ($m=1$, $c=0$).

spectrum but only 7% to the intensity of the spectrum of the membrane after digestion, even though no valines are removed (unpublished results). This suggests that the motional states of membrane surface residues are highly sensitive to the nature of the surroundings, and such dynamic interactions could have a role in processes such as cell–cell recognition.

This work was supported in part by the US NIH and the US NSF. E.O. is a PHS Research Career Development Awardee.

Received 21 June; accepted 25 October 1983.

1. Engelman, D. M., Henderson, R., McLachlan, A. D. & Wallace, B. A. *Proc. natn. Acad. Sci. U.S.A.* **77,** 2023–2027 (1980).
2. Ovchinnikov, Y. A. *FEBS Lett.* **148,** 179–191 (1982).
3. Huang, K.-S., Radhakrishnan, R., Bayley, H. & Khorana, H. G. *J. biol. Chem.* **257,** 13616–13623 (1982).
4. Engelman, D. M., Goldman, A. & Steitz, T. A. *Meth. Enzym.* **88,** 81–88 (1982).
5. Kang, S. Y., Gutowsky, H. S. & Oldfield, E. *Biochemistry* **18,** 3268–3271 (1971).
6. Barnes, R. G. in *Advances in Nuclear Quadrupole Resonance* (ed. Smith, J. A. S.) Vol 1, 335–355 (Pergamon, New York, 1974).
7. Oldfield, E., Meadows, M., Rice, D. & Jacobs, R. *Biochemistry* **17,** 2727–2740 (1978).
8. Oldfield, E. *et al. Biochem. Soc. Symp.* **46,** 155–181 (1980).
9. Wertz, D. H. & Scheraga, H. A. *Macromolecules* **11,** 9–15 (1978).
10. Oldfield, E. & Meadows, M. *J. mag. Res.* **31,** 327–335 (1978).
11. Davis, J. H., Jeffrey, K. R., Bloom, M., Valic, M. I. & Higgs, T. P. *Chem. phys. Lett.* **42,** 390–394 (1976).
12. Rothgeb, T. M. & Oldfield, E. *J. biol. Chem.* **256,** 1432–1446 (1981).
13. Berlinquet, L. *Can. J. Chem.* **33,** 1119–1129 (1955).
14. Sato, M., Okawa, K. & Akabori, S. *Bull. Chem. Soc. Japan* **30,** 937–938 (1957).
15. Kinsey, R. A., Kintanar, A. & Oldfield, E. *J. biol. Chem.* **256,** 9028–9036 (1981).
16. Kinsey, R. A. *et al. J. biol. Chem.* **256,** 4146–4149 (1981).
17. Becher, R. M. & Cassim, J. Y. *Prep. Biochem.* **5,** 161–178 (1975).

Preferential integration of yeast transposable element Ty into a promoter region

Hermann Eibel* & Peter Philippsen†

Microbiology Department, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

Mobile genetic elements have been identified in several eukaryotic organisms and some classes have been found to share common structural features with the proviral forms of animal retroviruses[1–7]. The representatives of this class of mobile elements in the yeast *Saccharomyces cerevisiae* are called Ty elements[8], which could be a useful model system for studying the transposition of retrovirus-like elements. Here we have attempted to answer two questions often raised in discussions of the biological importance of transposition: what is the frequency of spontaneous Ty transposition, and are there certain chromosomal regions into which Ty elements preferentially integrate? We chose the *LYS2* gene to investigate these questions because it allows direct selection of both mutants and revertants[9]. We have found that 2% of spontaneous lys2 mutants are caused by Ty transposition with a preferential integration into the transcription initiation region.

303 mutant strains with no (lys2) or significantly reduced (lys2aau) expression of the *LYS2* gene were isolated from two haploid *S. cerevisiae* strains as described in Fig. 1 legend. Both strains carried about 35 Ty elements[2,8]. Total DNA from groups of five or six of these mutants was cleaved with the restriction nucleases *Xho*I or *Xho*I plus *Bgl*II. These minipools were screened by DNA hybridizations[10] for size alterations in the wild-type *LYS2* restriction fragments using the cloned *LYS2* gene[11] as probe (Fig. 1*a*). Non-wild-type hybridization signals were observed within five minipools, and examples are documented in Fig. 1*b*. Rescreening of the individual strains in the five minipools identified in each case one mutant strain with altered *LYS2*-specific restriction fragments; three lys2 mutants (lys2–4, lys2–8, lys2–19) and two lys2aau mutants (lys2–17, lys2–655). Further analysis with *Bam*HI cleaved DNAs revealed in all cases an approximately 6-kilobase(kb) insertion as documented in Fig. 1*c*.

To identify the inserted sequences, we cloned and analysed at first the lys2–655 allele. After determining the sizes of restriction fragments which would cover part of the unaltered *LYS2* gene and part of the inserted DNA, we chose a 5.6-kb *Bam*HI–*Sal*I fragment for cloning into pBR322. The restriction map of this clone (pCH655) is shown in Fig. 2*a*, together with the restriction maps of Ty*1* and Ty*2*, the two known types of Ty elements[12,13]. A comparison clearly identifies the insertion in lys2–655 as a Ty*1* element. Furthermore, hybridization experiments against *Eco*RI-cleaved S288C DNA using pCH655 as probe revealed a typical Ty*1* pattern[8] (data not shown). Since the lys2–655 mutant could grow without lysine (lys2aau phenotype) it is not surprising that the Ty*1* had inserted in this mutant into the promoter region, as indicated in Fig. 2*a*, and not in the transcribed portion of the *LYS2* gene. The actual distance between the Ty*1* integration site and the start of the *LYS2* coding region is 107 base pairs (bp) as determined by DNA sequence analysis (D. Pridmore and P.P., unpublished).

The expression of the lys2–655 allele is only 20% of the wild-type level (H.E., M. Winston, J. K. Bhattacharjee and P.P., manuscript in preparation). We assume that the reduced transcription is still directed by *LYS2* promoter elements and not by δ promoter sequences in the Ty ends[3], as δ promoted transcription would proceed away from the *LYS2* gene as judged from the Ty*1* orientation in this insertion mutant.

The insertions in the other four mutants were identified by an extensive hybridization analysis using five restriction nucleases as Ty*1* elements in the three lys2 mutants lys2–4, lys2–8, lys2–19 and as a Ty*2* element in lys2–17, the second lys2aau mutant. Examples are documented in Fig. 2*b* and *c* for the *Xho*I and *Xho*I–*Bgl*II hybridization spectra, respectively. These five cases of Ty insertions were found among 303 spontaneous lys2 and lys2aau mutants. Such mutants occur with a frequency of 5×10^{-6} in haploid *S. cerevisiae* (ref. 9 and Fig. 1 legend). The spontaneous frequency of Ty transposition in these strains, which carry around 35 Ty elements[2,8], is therefore approximately 10^{-7}.

*Xho*I cleaves most Ty elements in their terminal δ sequences[12,13] and cleaves once within the *LYS2* gene[11]. *Xho*I-cleaved DNA from all five mutant strains showed a strong signal at around 3 kb (Fig. 2*b*). This indicates a clustering of the Ty insertion sites 3 kb upstream from the *LYS2 Xho*I site, a region which coincides with the *LYS2* promoter. A more precise localization of the Ty insertion sites was obtained with *Xho*I–*Bgl*II-co-digested DNA using the 650-bp *Bgl*II–*Eco*RI fragment as probe (see Fig. 1*a*), which contains the *LYS2* promoter. This probe detects only short restriction fragments (Fig. 2*c*). The sizes of these fragments minus the known distance between the Ty end and the δ *Xho*I site revealed the integration sites of the respective Ty elements. The result is presented in Fig. 3. Four of the five Ty insertions map within 40–110 bp in front of the *LYS2* translation start and one Ty maps 220 bp after this start.

This clustering around the start of the 4,000-bp long *LYS2* gene is remarkable, since any Ty insertion within the coding part of the gene would have been detected as a lys2 mutant in the selection procedure. As the α-aminoadipate-based selection for lys2 mutants allows the isolation of a great variety of lys2 mutants, including nonsense and missense mutations[14], its influence on the nonrandom distribution of Ty elements is highly improbable. In addition, insertion of DNA into the *LYS2* gene is not *a priori* impossible; for example, when *S. cerevisiae* cells are transformed with linear DNA harbouring *LYS2* sequences at both ends, an efficient insertion into the *LYS2* coding region is observed[11] resulting in a lys2$^-$ phenotype.

The target preference of Ty elements is therefore best explained as an intrinsic property of the elements, which enables Ty elements to discriminate between favourable targets and other less 'attractive' DNA sequences. This could be based on the ability of the Ty transposition machinery to recognize differ-

* Present address: Institut für Biologie III, Universität Freiburg, Schänzlestrasse 1, D-7800 Freiburg i.B., FRG.
† To whom reprint requests should be addressed.

0028–0836/84/040386—03$01.00

© 1984 Macmillan Journals Ltd

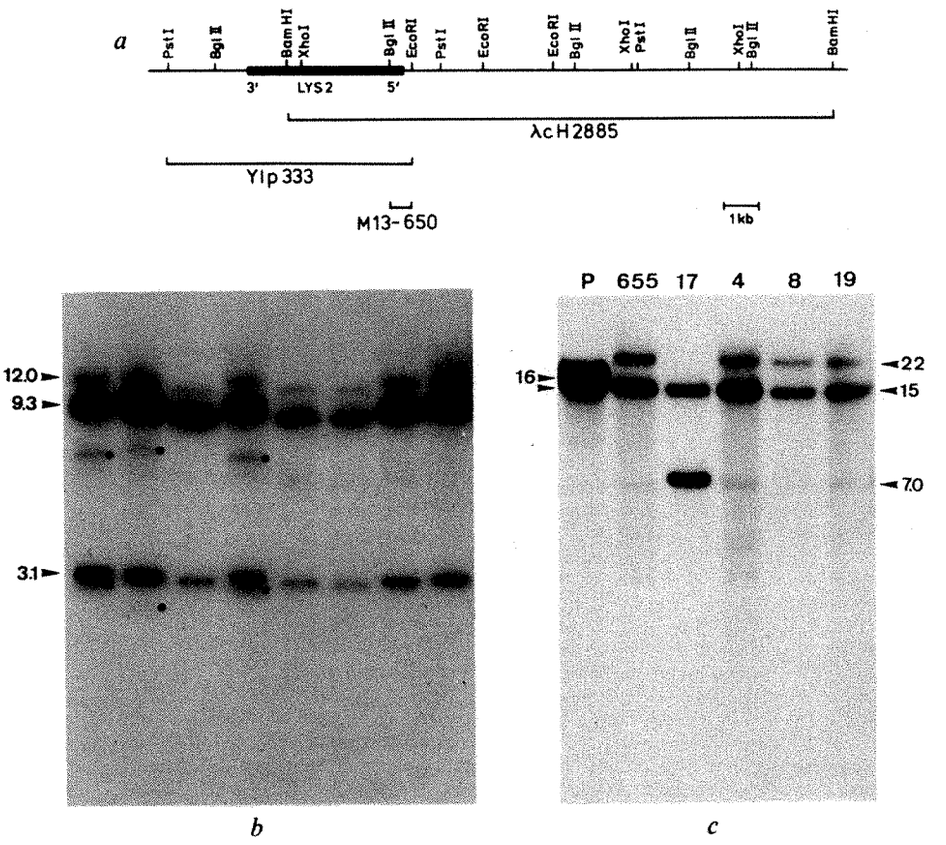


Fig. 1 Screening of lys2 mutants for DNA rearrangements. ***a***, Restriction map of the *LYS2* gene region in chromosome II from *S. cerevisiae*[11] and origin of *LYS2* probes. λCH2885 carries the 16-kb *Bam*HI fragment cloned into λ1059 and YIp333 carries the 6.9-kb *Pst*I–*Eco*RI fragment cloned into pBR322[11]. M13–650 carries the 650-bp *Bgl*II–*Eco*RI fragment of the *LYS2* promoter region cloned into the M13 vector mp8[33]. ***b***, Hybridization signals of *Xho*I restriction digests obtained with pools of mutant DNAs. Cleaved DNA was separated in 0.5% agarose and probed with λCH2885. The 9.3- and 3.1-kb signals correspond to internal *Xho*I fragments of the probe (see *a*); the 12-kb signal represents the *Xho*I fragment extending to the right of the probe. The *Xho*I fragment extending to the left of the probe is ~20 kb and was not transferred in most cases. New signals pointing to DNA rearrangements are marked in three of the eight lanes. Each lane contained a DNA mixture from six spontaneous lys2 mutants obtained from strain V2. ***c***, *LYS2*-specific *Bam*HI restriction fragments in parental and mutant strains. DNA of the parental strain S288C and the mutants lys2–655, lys2–17, lys2–4, lys2–8 and lys2–19 (from left to right) was cleaved with *Bam*HI, separated in 0.5% agarose and hybridized with YIp333. The parental DNA (lane P) exhibits the two expected signals of 15 and 16 kb, which correspond to the sequences extending to the left (3′) and right (5′) of the cleaved *Bam*HI site in the *LYS2* gene, respectively (see *a*). All mutant DNAs have lost the 5′-specific 16-kb signal and four show a 22-kb signal instead, indicating a 6-kb insertion. DNA from the mutant lys2–17 also carries a 6-kb insertion in the 16-kb fragment but one with a *Bam*HI site, leading to a 7- and a 15-kb signal. The latter one co-migrates in this case with the unchanged 15-kb fragment from the 3′ side, but its existence could be clearly demonstrated using λCH2885 as probe (not shown).

Methods: The selection for lys2 mutants is based on the use of α-aminoadipate as sole nitrogen source as described by Chattoo *et al.*[9]. 95% or more of *S. cerevisiae* cells which grow on α-aminoadipate plus lysine are lys2 mutants; among them are some with residual activity, called lys2aau mutants. We used the two haploid strains V2 (a trp1 his4) and S288C(α gal2 mal) as source for mutants. The frequency of spontaneous lys2 plus lys2aau mutants was in both cases 5×10^{-6} per cell, which is in agreement with previous work[9]. Cells from 60 single colonies of strain V2 were grown to stationary phase in full medium and 5×10^{7} cells each were plated on α-aminoadipate plus lysine. From each plate we collected only one lys2 and one lys2aau mutant in order to be sure to analyse independent mutation events. In total we collected 54 lys2 and 58 lys2aau mutants. A second set of mutants was derived from strain S288C. Cells were grown this time in minimal medium (lacking lysine) to the end of the logarithmic phase and plated at high densities on selective medium. After an incubation at 30 °C for 5 days, 196 mutant colonies were obtained. 176 of these mutants complemented a lys5 tester strain but not a lys2 tester strain and were classified as lys2. 15 of the mutants grew on selective medium but also on medium lacking lysine and complemented lys2 and lys5 mutations; they were classified as lys2aau. The remaining 5 mutants were lys5. DNAs were isolated as described previously[11]. Hybridization of the cleaved DNA after transfer to nitrocellulose was performed according to Southern[10] with the modifications introduced by Smith and Summers[34]. Nick translation[35] was used to label the hybridization probes. The lengths of the restriction fragments were determined from a comparison with co-migrating λ*Hin*dIII fragments[36].

ences in the chromatin structure of regulatory and coding sequences resulting in a higher affinity for opened regions. DNA sequence characteristics which would lead to such opened regions have not yet been elucidated. It has been noted previously that target regions for Ty transposition contain 60–80% A·T base pairs[15,16,20] in a genome with an average A·T content of 60%. The DNA sequence at the beginning of the *LYS2* gene shows one A+T-rich region (72%) centred around 70 bp in front of the translation start and another A+T-rich region (80%) centred around 240 bp after the start (D. Pridmore and P.P., unpublished). These locations coincide with the Ty integration sites.

Our data do not allow any conclusion as to whether the 3′-flanking regions of genes are also Ty landing sites, as such insertions would probably not be detected in the selection system used. In this context it is also important whether our observations made with Ty insertions at the *LYS2* locus are specific for that gene, or if similar events also occur at other loci in *S. cerevisiae*. Ty insertions have been observed in front of the *HIS4* (refs 16–18), *ADH2* (refs 19, 20), *CYC7* (refs 21, 22), *CAR1* (ref. 23) and *HIS3* (ref. 24) genes. The selection system used to recover these mutants would not have allowed the detection of Ty insertions into coding regions, except for *HIS4*, where two Ty insertions in front of the gene were characterized[16–18]. A conclusion as to the target specificity would have been difficult, if not impossible, from these studies. The nonbiased lys2 selection system with its potential for isolating many Ty insertion mutants therefore allows a thorough analysis of Ty transposition in yeast.

Several laboratories have recently found Ty elements or δ sequences close to the 5′ end of several tRNA genes (summarized in ref. 25). If δ sequences and tRNA genes were randomly distributed, they should be about 20–30 kb apart in the yeast genome. The close association found is therefore remarkable and may again reflect the attractiveness of regions 5′ to transcribed sequences as Ty landing sites.

Retrovirus-like mobile elements in higher eukaryotes were also frequently detected around promoter regions[26–32] and further studies may confirm a preference for such regions as integration sites. Thus insertions of these elements would frequently

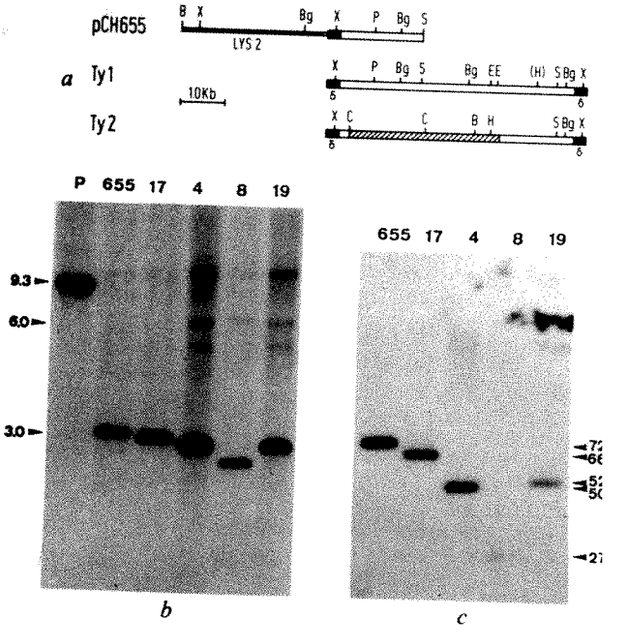


Fig. 2 Identification and mapping of Ty elements at the *LYS2* locus. *a*, Restriction map around the *LYS2*–Ty junction of the mutant lys2–655 as determined from the cloned 5.6-kb *Bam*HI–*Sal*I segment. The known restriction maps of Ty*1* and Ty*2*[12,13], which differ by a 3.5-kb substitution, are also shown. B, *Bam*HI; X, *Xho*I; Bg, *Bgl*II; P, *Pst*I; S, *Sal*I; E, *Eco*RI; H, *Hin*dIII; C, *Cla*I. The thin black line represents transcribed *LYS2* sequences[11]. Black boxes represent δ sequences, which are the 335-bp long terminal repeats in Ty elements[8,15,16]. *b*, Autoradiogram of *Xho*I restriction spectra from DNA of strain S288C and the five insertion mutants. The hybridization probe was nick-translated YIp333 which hybridizes in both parent strains to a 9.3-kb and a 20-kb *Xho*I fragment[11] (the latter did not transfer well). DNA of all mutant strains showed a new signal at around 3 kb. The corresponding new *Xho*I sites reside within the left δ sequences of the inserted Ty elements[15]. The *Xho*I fragment extending from the right δ sequences of the inserted Ty elements to the *Xho*I site upstream of the *LYS2* promoter can only be seen as weak signals in the 6–7-kb range due to the short homology with the probe. For orientation see Fig. 1*a*. The weak signals at around 5 and 10 kb are probably due to cross-hybridizing sequences elsewhere in the genome[11]. *c*, Autoradiogram of *Xho*I–*Bgl*II restriction spectra in 1.2% agarose from DNA of the five insertion mutants. The probe was M13–650, which carries the *LYS2* promoter (see Fig. 1*a*). The numbers at the arrows designate fragment sizes in base pairs which were determined from co-migrating pBR322 *Hpa*II fragments[37]. The size of 720 bp for the signal in lys2–655 DNA was independently determined from the pCH655 clone (see *a*) and was confirmed by DNA sequence analysis (D. Pridmore, personal communication).

Methods: The 5.6-kb *Bam*HI–*Sal*I fragment harbouring the *LYS2*–Ty*1* junction of the mutant lys2–655 was cloned into the plasmid vector pBR322[38] after an enrichment step. 10 μg of *Sal*I + *Bam*HI-cleaved lys2–655 DNA were electrophoresed on a 0.5% agarose gel and fragments of 5–7 kb electroeluted as described by Yang *et al.*[39]. The yeast DNA was ligated with *Sal*I + *Bam*HI-cleaved pBR322 DNA which had also been purified by electroelution. Ampicillin-resistant transformants in *Escherichia coli* were screened for the presence of the 5.6-kb *Sal*I–*Bam*HI fragment by colony hybridization[40] using YIp333 as a probe. Sheared and denatured unlabelled pBR322 was added in excess to suppress hybridization between the labelled pBR322 sequences in the YIp333 probe and pBR322 DNA present in the *E. coli* transformants. One of the positive clones was picked and shown to contain the right plasmid, which was named pCH655.

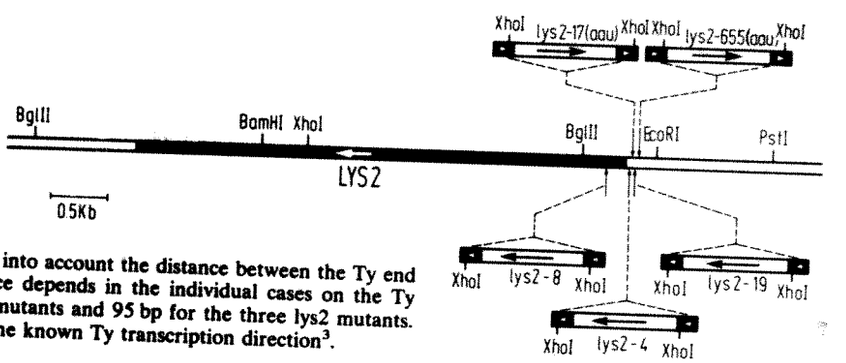


Fig. 3 Map of Ty insertion sites. The insertion sites of the five Ty elements in the transcription start region of *LYS2* are indicated by arrows. The 4-kb long *LYS2* gene is drawn as black bar. The 5′ end of this gene was determined from a DNA sequence analysis which identified the translation start (D. Pridmore and P.P., unpublished). The 3′ end was determined by several independent S_1 mapping experiments[11]. The 6-kb long Ty elements are not drawn to scale. Their insertion sites were calculated from the *Bgl*II–*Xho*I fragment sizes measured in Fig. 2*c* by taking into account the distance between the Ty end and the *Xho*I site in the δ sequence. This distance depends in the individual cases on the Ty orientation[15,16] and is 240 bp for the two lys2aau mutants and 95 bp for the three lys2 mutants. The arrows within the Ty elements correspond to the known Ty transcription direction[3].

lead to a modulation of gene expression rather than to gene inactivation. This could be a molecular basis for the often postulated role of mobile DNA elements in evolutionary processes.

We thank Ursula Fleig for the m13-650 clone, Agathe Stotz for technical assistance, also Lucia Panzeri, Jürg Gafner and David Pridmore for many stimulating discussions, Tom Bickle for critically reading the manuscript and Elvira Amstutz for typing it. This work was supported by Swiss National Fund grant 3.644.80 and by a fellowship to H.E. from the Deutsche Forschungsgemeinschaft.

Received 17 August; accepted 22 November 1983.

1. Temin, H. M. *Cell* **21**, 599–600 (1980).
2. Eibel, H., Gafner, J., Stotz, A. & Philippsen, P. *Cold Spring Harb. Symp. quant. Biol.* **45**, 581–591 (1981).
3. Elder, R. T., Loh, E. T. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2432–2436 (1983).
4. Rubin, G. M. *et al. Cold Spring Harb. Symp. quant. Biol.* **45**, 619–628 (1981).
5. Flavell, A. J. & Ish-Horowicz, D. *Nature* **292**, 591–595 (1981).
6. Shiba, T. & Saigo, K. *Nature* **302**, 119–124 (1983).
7. Varmus, H. E. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 411–503 (Academic, New York, 1983).
8. Cameron, J. R., Loh, E. T. & Davis, R. W. *Cell* **16**, 739–751 (1979).
9. Chattoo, B. B. *et al. Genetics* **93**, 651–665 (1979).
10. Southern, E. *J. molec. Biol.* **98**, 503 (1975).
11. Eibel, H. & Philippsen, P. *Molec. gen. Genet.* **191**, 66–73 (1983).
12. Roeder, G. S. & Fink, G. R. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 299–328 (Academic, New York, 1983).
13. Williamson, V. M. *Int. Rev. Cytol.* (in the press).
14. Chattoo, B. B., Palmer, E., Bun-Ichiro, O. & Sherman, F. *Genetics* **93**, 67–79 (1979).
15. Gafner, J. & Philippsen, P. *Nature* **286**, 414–418 (1980).
16. Farabaugh, P. J. & Fink, G. R. *Nature* **286**, 352–356 (1980).
17. Roeder, G. S. & Fink, G. R. *Cell* **21**, 239–249 (1980).
18. Roeder, G. S., Farabaugh, P. J., Chaleff, D. T. & Fink, G. R. *Science* **109**, 1375–1380 (1980).
19. Williamson, V. M., Young, E. T. & Ciriacy, M. *Cell* **23**, 605–614 (1981).
20. Williamson, V. M., Cox, D., Young, E. T., Russel, D. W. & Smith, M. *Molec. cell. Biol.* **3**, 20–31 (1983).
21. Errede, B. *Cell* **25**, 427–436 (1980).
22. Montgomery, D. L. *et al. J. biol. Chem.* **257**, 7756–7751 (1982).
23. Jauniaux, J.-C., Dubois, E., Vissers, S., Crabeel, M. & Wiame, J.-M. *EMBO J.* **1**, 1125–1131 (1982).
24. Scherer, S., Mann, C. & Davis, R. W. *Nature* **298**, 815–819 (1982).
25. Gafner, J., DeRobertis, E. & Philippsen, P. *EMBO J.* **2**, 583–591 (1983).
26. Goldberg, M. L. thesis, Stanford Univ. (1979).
27. Ikenaga, H. & Saigo, K. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4143–4147 (1982).
28. Snyder, M. P. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7430–7443 (1982).
29. Hayward, W. S., Neel, B. G. & Astrin, S. M. *Nature* **290**, 475–480 (1981).
30. Payne, G. S., Bishop, M. J. & Varmus, H. E. *Nature* **295**, 209–214 (1982).
31. Kuff, E. L. *et al. Nature* **302**, 547–548 (1983).
32. Schnieke, A., Harbers, K. & Jaenisch, R. *Nature* **304**, 315–320 (1983).
33. Messing, J. *3rd Cleveland Symp. Macromolecules: Recombinant DNA* (ed. Walton, A.) 143–153 (Elsevier, Amsterdam, 1981).
34. Smith, G. E. & Summers, M. D. *Analyt. Biochem.* **109**, 123–129 (1980).
35. Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).
36. Philippsen, P., Kramer, R. A. & Davis, R. W. *J. molec. Biol.* **123**, 371–386 (1978).
37. Sutcliffe, J. G. *Cold Spring Harb. Symp. quant. Biol.* **43**, 77–90 (1978).
38. Bolivar, F. *et al. Gene* **2**, 95–113 (1977).
39. Yang, R. C. A., Liz, J. & Wu, R. *Meth. Enzym.* **68**, 178–181 (1979).
40. Grunstein, M. & Hogness, D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961–3965 (1975).

Solution contact charging with respect to earthquake lights

LOCKNER *et al.*[1] have proposed a mechanism to explain the production of earthquake lights (EQLs) which strongly relies on the generation of electricity by the boiling of water in heated rocks in the shear zone. Using data from the original paper on saline charging[2] and spray electrification they explain the coronal origin of EQLs for magnitudes 6–7 but were unable to account for those of the Matsushiro swarm. More recent investigations of solution contact charging[3,4] have demonstrated that the charging mechanism is not as Blanchard originally speculated, that is, "mechanical pulverization of water" with charge values similar to those of spray electrification, but is a solid–solid contact mechanism. Whereas sprayed aqueous solution droplets carry charges of about 4×10^{-19} C, those from solution contact boiling are in the range 3×10^{-17} to 3×10^{-14} C (refs 4, 5). The charges of 10^{-7} C g^{-1} quoted from Blanchard related to saline solution undergoing transition boiling on a surface at approximately 350 °C. However, at temperatures cited by Lockner *et al.*, in excess of this, Leidenfrost boiling occurs to produce charges of 10^{-5} C g^{-1}. Although Leidenfrost boiling produces fewer droplets, each carries higher charge by factors of 3–5 above those on sprayed droplets.

I suggest that the higher values are more likely and may also account for the EQLs of the Matsushiro swarm, thereby bringing the latter within compass of the proposed mechanism. One further observation is that previous studies of electrification from boiling have been based on $NaCl_{aq}$. An extension of these investigations[6] has revealed that many aqueous solutions including samples of groundwaters will generate electricity by flash, transition and Leidenfrost boiling.

The more recent information would seem to strengthen the mechanism proposed for the production of EQLs though some modification is required in which spray electrification should be deleted where boiling is involved and the more suitable solution contact charging process substituted.

C. POUNDER

161 Cotmanhay Road,
Ilkeston,
Derbyshire, DE7 8QT, UK

1. Lockner, D. A., Johnston, M. J. S. & Byerlee, J. D. *Nature* **302**, 28–37 (1983).
2. Blanchard, D. C. *Nature* **201**, 1164–1166 (1964).
3. Pounder, C. *J. Electrostat.* **1**, 395–397 (1975).
4. Pounder, C. *J. Electrostat.* **9**, 159–175; 177–182 (1980).
5. Bright, A. W. *Proc. R. Instn Gt Br.* **53**, 267–269 (1981).
6. Pounder, C. *Proc. Electrostat. '83, Inst. P. Ser.* **66**, 221–224 (1983).

Carnivore social behaviour—does it need patches?

RECENTLY, Macdonald[1,2] presented an interesting model explaining the evolution of social behaviour in carnivores. Briefly, the model, called the resource dispersion hypothesis (RDH), is as follows. Important food for carnivores occurs in patches and food availability in these patches varies with, for example, weather and season. Frequently situations occur when only one of these patches yields the resources needed for the animals' maintenance. Such situations can occur during single nights or seasons. Hence, the carnivore has to include enough patches within its territory (see, for example, Fig. 1 of ref. 1) to reliably support its minimum needs in all such situations. Territory size and configuration is determined by "dispersion of transient patches of available prey (ephemeral from night to night, or shifting from one season to the next)"[1]. Group size, on the other hand, is positively correlated with the average patch quality (or quality of the least valuable patch, it is unclear which) (Fig. 1 of ref. 1).

Macdonald discusses predictions that are not necessarily borne out by the model, for example: "From the resource dispersion hypothesis one would not necessarily expect .... any relationship between group size and territory area, as the two are argued to be affected, largely independently, by the abundance and dispersion of available food respectively". The lack of such a correlation is then treated as a corroboration of the model[1]. However, as RDH cannot be falsified regardless of whether there is a correlation or not, this prediction is useless for testing the hypothesis. Here I will identify and evaluate one prediction that is an *invariable* consequence of RDH: when the temporal variation is such that access to a given patch is vital to the territory owners, then all group members are expected to exploit this patch simultaneously. If the group members *regularly* forage in different patches simultaneously then the basic assumption of ephemerally unique food patches falls and so does RDH. This point was stressed by Bradbury and Vehrencamp[3] when developing a model (very similar to RDH) to explain territory and group size in emballonurid bats. These authors[3] found that all members of a given group of bats foraged in a common patch at any time.

Does it happen regularly that group members of, for example, badgers or red foxes forage simultaneously in a common patch? Macdonald's suggestion[1] that it may be "on a bad night or a bad year" makes it difficult to evaluate this prediction. Individual badgers move and hunt solitarily inside a clan range[4] and individual foxes are in physical contact with one another for as little as 4 min each night[5]. Also, Macdonald[6] reported that dominant and subordinate red foxes in his study area outside Oxford generally foraged in different parts (and patches; Table 6 of ref. 6) of the group territory. Moreover, in the Revinge area in southern Sweden, breeding red foxes differed, in all seasons of the year, from their non-breeding group members in their use of hunting habitat[7]. Thus, on neither a nightly nor a seasonal basis do members of a given group seem to be restricted in their hunting to a given patch. It is not likely that a whole group should unite at a single food patch during a 'bad' year, unless one considers the entire territory as one patch, but then RDH is no longer valid. Macdonald[1] concludes that once group living in carnivores has evolved as an adaptation to resource dispersion then other benefits accruing from sociality *per se* (for example, cooperative hunting and defence of kills) further moulds the social organization in each population or species and that "the balance of these contemporary benefits is not necessarily the same as that which originally selected for group living". Unfortunately, this *ad hoc* argument makes every serious attempt to test RDH virtually impossible as each time data that would falsify the hypothesis are obtained, it could always be claimed that a particular population is no longer under the original selection pressure for sociality. Even so, this would not help either for the Oxford or for the Revinge red foxes, as both populations are believed to be at an early stage in the evolution of sociality[1,8].

Recently[8,9] I argued that permanently territorial altricial vertebrates that feed on an interannually fluctuating food resource, keep a constant territory size if the animals' lifespan is longer than the average time period of the food fluctuations. The animals' territory sizes will be so adjusted that they contain enough food for reproduction, or at least survival[10], during the bottle-neck years of food scarcity. The model predicts that as food abundance increases, the territory owners, for example the breeding pair, take advantage of this resource surplus by allowing their adult offspring to remain at home, thereby increasing the group size. When food abundance decreases the subordinate group members will be evicted from the territory by the breeding pair. Hence, in any given territory of, say, foxes or badgers, group size will be positively correlated with the annual food abundance whereas territory size will be constant from year to year. This model does not include any assumption about ephemeral prey patches. A simple field test to distinguish

0028-0836/84/040389—02$01.00
© 1984 Macmillan Journals Ltd

between this model and RDH would be to prevent (for example, by a fence) groups of badgers or foxes exploiting a given food patch within their territories. My model predicts that territory size will remain constant if the experiment is performed in territories having three or more group members whereas group size will decrease in proportion to the ratio P_p/P_t, where P_p = prey productivity of the enclosed patch, and P_t = prey productivity of the entire territory. It is only when this experiment is performed in territories occupied by a single pair that the territory owners will try to expand or abandon their territories. Macdonald's[1,2] model predicts that territory size will increase whereas group size may or may not remain constant (depending on the quality of the new food patch incorporated in the territory).

TORBJÖRN VON SCHANTZ
Department of Animal Ecology, Ecology Building, University of Lund, S-223 62 Lund, Sweden

1. Macdonald, D. W. *Nature* **301,** 379–384 (1983).
2. Maconald, D. W. in *Proc. Worldwide Furbearer Conf.* Vol. 2 (eds Chapman, J. & Pursely, D.) 918–949 (University of Maryland Press, 1981).
3. Bradbury, J. W. & Vehrencamp, S. L. *Behav Ecol. Sociobiol.* **1,** 337–381; 383–404 (1976).
4. Kruuk, H. *J. Zool.* **184,** 1–19 (1978).
5. Macdonald, D. W. *et al.* in *A Handbook on Biotelemetry and Radio Tracking* (eds Amlaner, C. J. & Macdonald, D. W.) 405–424 (Pergamon, Oxford, 1980).
6. Macdonald, D. W. *Z. Tierpsychol.* **52,** 171–200 (1980).
7. von Schantz, T. *Oikos* **37,** 63–68 (1981).
8. von Schantz, T. *Oikos* **42,** 48–58; 59–65 (1984).
9. von Schantz, T. thesis, Univ. Lund (1981).
10. Lindström, E. thesis, Univ. Stockholm (1982).

MACDONALD REPLIES—Although most easily visualized where only one patch is likely to be fruitful in each territory at a given time (refs 1 and 2; hence Fig. 1 in ref. 3), the resource dispersion hypothesis (RDH) does not require these conditions[4–8]. Rather, the hypothesis identifies conditions of resource dispersion where "the smallest home range with an economically defendable[9] configuration which will reliably support a pair of foxes (on a bad night or a bad year) may sometimes support additional foxes"[3].

In spatially patchy environments, convoluted territorial borders might encompass sufficient fragments of transient patches to yield food for only a pair of residents. RDH proposes that the defence of such ranges may not be economic[9] whereas a less convoluted configuration, perhaps including more resources, could be defendable. On this view, each territory may contain many patches, each being variously fruitful at any one time. Whether group members forage in a patch simultaneously, just as whether they travel and/or forage alone or together, will depend on the nature of the prey and on other selective pressures affecting their sociality[3].

I have suggested[8] that where prey were spatiotemporally uniform in their availability, there the smallest economically defendable range that could sustain a pair would not support additional adults (whether or not, and for how long, it would be advantageous to maintain territories larger than this minimum size would depend on such factors as the magnitude of pressure from intruders and the benefits of increasing group size). von Schantz's formulation concerns an intermediate case where, irrespective of its dispersion, prey is temporally variable. Again, the pattern of prey availability creates conditions where the minimum range which supports a pair (in a poor year) can sometimes support extra adults (in a rich year). The interannual mode[10,11] highlights the question of how social behaviour provides "a buffer to prevent spatial organization altering prematurely in response to ephemeral changes in food availability"[8]. von Schantz's[10] emphasis on the costs of territorial readjustment is compatible with my formulation.

Territory size and shape may sometimes be adapted to 'bottle-neck' periods in the abundance of available prey; it seems no less plausible (nor at variance with the data[4–8] to argue that they are sometimes further constrained by the dispersion of resources. von Schantz is mistaken in thinking that I treated independence of territory and group sizes as corroborating RDH; rather, I showed how this was accommodated by RDH. Experiments like that suggested are vital although the result would depend on which patch was fenced (due to interacting variation among dominance and patch richness and utilization[12]).

von Schantz's hypothesis requires group size to descend to two during the average breeding lifespan. Among species which form "spatial groups"[3], these conditions are probably not met in some carnivore populations[13], although they may be in others[6,10]. The spatiotemporal formulation of RDH is applicable to groups of stable or unstable membership whereas the purely temporal formulation may explain only those unstable groups whose membership periodically decreases to two. Some badger and fox groups reported in refs 4, 5, 8 and 13 have subsequently remained larger than 2 for over 10 yrs (H. Hofer, D. W. M., E. Neal and J. Phillipson, unpublished observations).

DAVID W. MACDONALD
Animal Behaviour Research Group, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

1. Bradbury, J. W. & Vehrencamp, S. L. *Behav Ecol. Sociobiol.* **1,** 383–404 (1976).
2. Clutton-Brock, T. H. *Nature* **250,** 539–542 (1974).
3. Macdonald, D. W. *Nature* **301,** 379–384 (1983).
4. Kruuk, H. *Behav Ecol. Sociobiol.* **4,** 75–89 (1978).
5. Kruuk, H. & Parish, T. J. *J. Zool.* **196,** 31–39 (1982).
6. Mills, M. G. L. *J. Zool.* **198,** 39–51 (1982).
7. Hersteinsson, P. & Macdonald, D. W. *Symp. zool. Soc. Lond.* **49,** 259–289 (1982).
8. Macdonald, D. W. in *Proc. Worldwide Furbearer Conf.* Vol. 2 (eds Chapman, J. & Pursely, D.) 918–949 (University of Maryland Press, 1981).
9. Brown, J. L. *Wilson Bull.* **70,** 160–169 (1964); *J. theor. Biol.* **95,** 793–810 (1982).
10. von Schantz, T. thesis, Univ. Lund (1981); *Oikos* **42,** 48–58; 59–65 (1984).
11. Lindstrom, E. in *The Red Fox* (ed. Zimen, E.) 177–184 (Junk, The Hague, 1980).
12. Macdonald, D. W. *Z. Tierpsychol.* **52,** 171–200 (1980).
13. Neal, E. *Badgers,* 321 (Blandford, Poole, Dorset, 1977).

111-day periodicity of X-ray transient A0535+26

PRIEDHORSKY AND TERRELL have recently argued about some special characteristics of X-ray transient A0535+26 (ref. 1) to justify their speculations: that (1) the optical counterpart HDE245770 of A0535+26 cannot be a BO Ve star; (2) it would be better if the terminal velocity of the stellar wind from HDE245770 were $<1{,}000$ km s^{-1}; (3) the B star must be rotating rapidly to lose matter from the equatorial region; (4) the companion HDE245770 must have a mass loss rate of $\sim 10^{-5}\ M_0\ \mathrm{yr}^{-1}$ to power the X-ray luminosity of A0535+26.

Furthermore, they point out that there is a discrepancy between evaluations of the mass loss rate from UV data and their own estimates (see point (4)).

These five points have been discussed elsewhere. Specifically: (1) HDE 245770, optical counterpart of A0535+26 (ref. 2), is an 0.9 IIIe star[3]; (2) the terminal velocity of the stellar wind in HDE245770 is ~ 630 km s^{-1} (ref. 4); (3) from the broadening of He(II) line (1,640 Å), the rotational velocity of HDE245770 is $v_e \sin i \cong 230$ km s^{-1} (ref. 5); (4) the mass loss rate of HDE245770, derived by IR data, is of the order of $10^{-6}\ M\ \mathrm{yr}^{-1}$ (ref. 6); (5) the discrepancy between evaluations of the mass loss rate derived from UV and IR data has been discussed in refs 5,7.

FRANCO GIOVANNELLI
Istituto di Astrofisica Spaziale, CNR, C.P. 67, I 00044 Frascati, Italy

1. Priedhorsky, W. C. & Terrell, J. *Nature* **303,** 681 (1983).
2. Bartolini, C., Guarnieri, A., Piccioni, A., Giangrande, A. & Giovannelli, F. *IAU Circ.* No. 3167 (1978).
3. Giangrande, A., Giovannelli, F., Bartolini, C., Guarnieri, A. & Piccioni, A. *Astr. Astrophys. Suppl.* **40,** 289 (1980).
4. Giovannelli, F. *et al. Space Sci. Rev.* **30,** 407 (1981).
5. Giovannelli, F. *et al. Proc. 3rd European IUE Conf.* ESA SP-176, 233 (1982).
6. Persi, P. *et al. Mon. Not. R. astr. Soc.* **187,** 293 (1979).
7. Snow, T. P. *Be Stars, Proc. IAU Symp.* No. 98, 509 (1982).

PRIEDHORSKY AND TERRELL REPLY—We thank Giovannelli for pointing out references relevant to our letter, some of which we may have overlooked even if space had permitted their inclusion. We had intended only to present new X-ray observations of A0535+26. It is satisfying that the IR studies cited by Giovannelli support our conclusion that the mean mass loss rate of HDE245770 to its companion A0535+26 is of the order $M \sim 10^{-5.5}$ $(v_{\mathrm{wind}}/1{,}000\ \mathrm{km\ s^{-1}})^4 \mathrm{M}_\odot\ \mathrm{yr}^{-1}$.

WILLIAM C. PRIEDHORSKY
JAMES TERRELL
Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA

0028-0836/84/040390—01$01.00

© 1984 Macmillan Journals Ltd

Consciousness and conscience

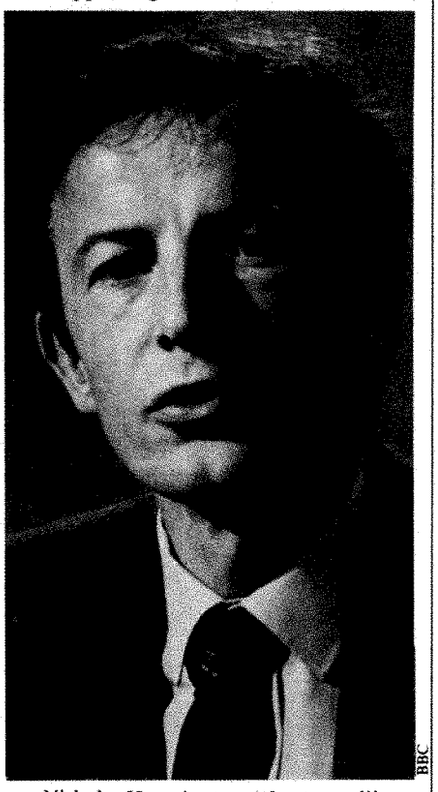
Stuart Sutherland

Consciousness Regained. By Nicholas Humphrey.
Oxford University Press: 1983. Pp.222. £12.95, $19.95.

MANY of the world's best minds have wrestled with the problem of consciousness; so for that matter have many of the worst. In *Consciousness Regained,* a collection of essays most of which have been published elsewhere, Dr Nicholas Humphrey joins the throng. He claims, unexceptionally, that since consciousness has presumably evolved, it must have a function, which is, he suggests, to enhance social interaction. He reaches this conclusion in two steps, the first of which concerns the intellect rather than consciousness.

According to Humphrey, the intellect in man and many other primates has been developed to a point far beyond that needed to deal with the material environment. He argues that chimpanzees and gorillas, who have invented a technology for subsisting, have less need for intelligence than do less technologically orientated primates. Merely by watching another member of its group, a chimpanzee can learn to poke a stick into a termites' nest and draw it out covered with a tasty feast. The individual animal does not have to use creative intelligence. The argument ignores the question of how the technique was invented in the first place: even if it was learned by trial and error, poking sticks into nests is a creative act and the chimpanzee has to make the connection between it and the resulting mouthful of termites. Moreover, although high intelligence might not be needed for day-to-day living, chimpanzees and gorillas must sometimes be subjected to novel and dangerous events which they can survive only by the use of intelligence. In the case of man, the argument is even weaker since his physical defences and resources are less and his survival — often under very adverse external conditions — depends entirely on his ability to manipulate the physical world by the use of intelligence. Although it is impossible to assess how far the intelligence of a given species evolved through pressure to cope with the physical world, there may be some truth in Humphrey's ingenious alternative — that in man and some other primates it evolved in order to help the individual deal with other members of the species.

There is, however, a big leap from this conclusion to the next step in his argument: he maintains that consciousness itself arose in order to help man (and possibly some other primates) understand the behaviour of others. Consciousness provides a model of the way in which people's own minds work — a model of their emotions, motives, thoughts and sensations: in so far as different minds are similar this model provides a basis for understanding others. There is, unfortunately, an obvious fallacy in the argument. The brain could represent the processes underlying motives, thinking and so on and could use this representation as a model for others' behaviour and the forces underlying it without the representation appearing in consciousness. Indeed,



Nicholas Humphrey — "fun to read".

Humphrey himself argues elsewhere that the brain contains a representation of the position of the parts of the body, but that this representation does not appear in consciousness. It is easy to invent functions that consciousness might subserve: what is difficult and has never yet been achieved is to show that they can be subserved only by consciousness.

There are three lesser problems with Humphrey's thesis. First, he does not sufficiently distinguish between consciousness and self-awareness. It is hard to believe that species other than primates lack consciousness, though they may lack self-awareness. Second, it is simply not true that in most of their dealings with others people use a conscious model either of their own behaviour or of that of others. People normally interact spontaneously and with little or no conscious thought. It is only when social interactions go wrong that they start to think consciously about them — often with unfortunate consequences. Third, if the reason why people are self-aware is to enable them to understand others, it is curious that self-knowledge should be so fallible. People's motives are often more transparent to others than to themselves.

For the rest, Humphrey is always stimulating and fun to read, though by its nature most of his book is rather bitty. He develops the far from novel idea that the reason why people engage in activities which seem to have no direct relevance to survival is that the activities provide novel experiences in terms of which people can better understand others. Some examples are play, initiation ceremonies, dreaming, keeping pets, watching dramatic performances and the manipulation of children by parents — particularly gratuitous cruelty. It is hard to accept that the function of such cruelty is to teach a child what it feels like to be unfairly treated, particularly as what evidence there is suggests that maltreated children tend to become poorly adjusted adults.

Another perennial problem that Humphrey tackles is that of the value of art. He argues that in classifying objects in the natural world, people look for sets whose members are similar in some respect to all the others, but differ in some way from the members of other sets. Much art (and, he might have added, almost all music) consists of variations on a theme and hence it teaches the viewer to spot similarities and differences and gives him practice in the task of classification. This is an ingenious account of the intellectual content of art, but leaves out its emotional force.

The book ends with a series of disconnected essays and book reviews, which are lively, but lightweight. Humphrey rightly and suavely demolishes the pretensions of such self-appointed gurus as Douglas Hofstadter and Gregory Bateson, and he provides a piece of impassioned rhetoric on nuclear war, in which he appeals to everyone to go out and stop it. He declaims "I have not heard of one good reason for not halting [nuclear armaments] tomorrow". Unfortunately he is so carried away by his own rhetoric that he omits to tell both the man in the street and the politicians exactly what it is they should do to prevent nuclear war.

Humphrey writes with elegance and force, and although he sometimes fails to follow his ideas through, they are always stimulating. Even the reader who disagrees with his arguments will derive pleasure from working out for himself why they are wrong. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

Tackling the supreme symmetry

M. J. Duff

Superspace or One Thousand and One Lessons in Supersymmetry.
By S.J. Gates Jr, M.T. Grisaru, M. Roček and W. Siegel.
Benjamin/Cummings: 1983. Pp.548. Hbk $48, £40.80; pbk $25, £21.25.

HAD Scheherazade tried to while away those Arabian nights with *One Thousand and One Lessons in Supersymmetry*, one has the feeling that long before the chapter on quantum superspace Schahriar would have cried "Hey, wait a minute! Where are the references?". The graduate student of theoretical physics or other inquisitive researcher, hoping to use this text as a guide to the literature on supersymmetry and supergravity, will be sadly disappointed. Not one reference to the original works is to be found in the book. And of the six references to conference proceedings, one is incorrect. (The title of the 1981 Trieste proceedings should be *Supergravity 81* and the editors were S. Ferrara and J. G. Taylor.) The authors, all of whom are well-known Sultans of Supersymmetry, apologize to their friends in the field and beg pardon for omitting the credits. While I suspect that no such pardon will be forthcoming, this is nevertheless a valuable addition to the introductory literature on the ideas of supersymmetry and superspace which will be essential reading for the devotee.

In the words of the Introduction, "Supersymmetry is the supreme symmetry: It unifies spacetime symmetries with internal symmetries, fermions with bosons, and (local supersymmetry) gravity with matter". About one-third of the current publications in theoretical elementary particle physics is devoted to supersymmetry or supergravity, and an increasing number of experimental papers deal with the search for the still unobserved (and perhaps unobservable) superparticles which the theory predicts. More and more PhD students are choosing this field for their research yet constantly one hears the complaint that there is no adequate starting point for the learner. *Superspace* will certainly be of help, but it is not for the casual browser or the dilettante.

Supersymmetry experts fall into two categories: those who treat the theory like any other quantum field theory, using separate "component" fields to describe both bosonic (integer spin) and fermionic (odd-half-integer spin) particles; and those who prefer the new-fangled approach in which bosons and fermions are combined into "superfields" which live in a strange world called "superspace". In superspace, the four spacetime dimensions are bosonic with coordinates obeying the usual multiplication $AB = +BA$ but extra fermionic dimensions are also introduced whose coordinates obey $AB = -BA$.

As their title suggests, the authors of *Superspace* are unashamedly in favour of these superfields and use the superspace formalism throughout. They have covered all aspects of superspace: superspace geometry, representations of supersymmetry, and global superfields, but their ultimate goal is the unification of gravity and matter via supergravity. At present supergravity is the best available candidate for the Unified Field Theory which will unite gravity with the other fundamental forces of nature. One of the problems in such a scheme is to avoid the ultraviolet divergences of quantum gravity. The authors present a convincing case that only with the techniques of super-Feynman diagrams (for which they themselves have been largely responsible) can we hope to answer this all-important question.

Superspace is an Aladdin's cave of highly-condensed information. But although it is written with an easy style and admirable clarity, it will require a keen mind and determination, rather than a simple "Open, Sesame", to extract the riches. □

Michael Duff is a Lecturer in Physics at Imperial College, University of London.

Jenner by himself

Donald R. Hopkins

Letters of Edward Jenner: And Other Documents Concerning the Early History of Vaccination.
Edited by Genevieve Miller.
Johns Hopkins University Press: 1983. Pp.145. $15, £12.75.

THE recent global triumph over smallpox has stimulated great interest in historical aspects of that disease and persons associated with it. So it is the right time finally to have published these letters of Edward Jenner from the Henry Barton Jacobs Collection at Johns Hopkins University. More than anyone else, it was Jenner who set the stage for the eventual eradication of smallpox with his epochal discovery of vaccination in 1796.

Because of Jenner's great historical importance and his broad interests, I have often wished I could hear him describe his discovery and the related events that followed, at first-hand, or at least in a taped interview. Fortunately, even though Jenner is of an earlier era and we have no taped interview, we do have many of his letters, some 600 of which are known to still exist. This book contains a good many (110) of them, most being published here for the first time.

As Genevieve Miller notes in her brief introductory essay, in these letters to 45 different correspondents we meet Jenner the naturalist, author, father, country gentleman, scientist, husband, horticulturalist, geologist and the medical practitioner. We also meet a man who, like all of us, was occasionally wildly mistaken. Most interestingly, however, since all but two of these letters are from the period after he discovered vaccination (when he was 47 years old and already had made several significant scientific discoveries



Cow pock and the fears of the public — Gillray's celebrated cartoon of 1802, lampooning the activities of the Anti-Vaccine Society.

which were overshadowed by his later work), we meet the humanist Father of Vaccination. From 1796, until his death in 1823, Jenner constantly and doggedly promoted his discovery in the face of persistent opposition.

Some of these encounters seem to me to have almost as much force as if they were face to face. We feel Jenner's exaltation, for example, when he learns of the successes of vaccination in Copenhagen and Ceylon, and of the spectacular expedition dispatched by the King of Spain under Francisco Xavier Balmis which brought vaccination to Spanish territories around the world:

> A few days since, I rec'd from Madrid a Document respecting Vaccination which fills me with more astonishment than anything that has yet reach'd me on that subject . . . . Would to Heaven the British Cabinet had shown the same Philanthropic Spirit as that of Spain! [p.31].

We also share his exasperation and frustration at the cost in lives and suffering as a result of the antivaccinists in his own country, while much of the rest of the world adopted his discovery with thankful alacrity:

> One would think the statement of Facts, as they now stand before the Public from every quarter of the Globe would blow away such stuff as these abominable people produce, like Chaff, but it is not so, or the Bills of Mortality would not exhibit weekly such horrid scenes of devastation from the Smallpox [p.70].

No biographer or historian could convey Jenner's feelings during this period of his life better than he does himself in these letters.

The format of the book is well chosen. The annotated letters are presented in chronological order, except for the six which are of unknown date. Miller introduces the correspondents, and other persons as they are first mentioned, with short biographical sketches. In helpful but unobtrusive footnotes, she also explains various phrases, objects, practices and so on which are unfamiliar to us. Easy access to the contents is assured by means of an index, and by the table of contents in which Miller lists the letters in order, with the name of the correspondent and the key subject(s) discussed in each.

Fifteen other documents which are included in the 20-page appendix, we are told, "constitute the remainder of the unpublished Jacobs material relating to Jenner or to contemporaries involved with vaccination" (p.xxvi).

My only real disappointment after reading the book is a direct result of its effectiveness: I came away yearning to see all of Jenner's letters compiled and published in a single work. Until then, however, this collection will do very well. □

Donald Hopkins is Assistant Director for International Health at the Centers for Disease Control, Atlanta, Georgia. He is author of Princes and Peasants: Smallpox in History *(University of Chicago Press, 1983).*

All of physics

R.H. Dalitz

McGraw-Hill Encyclopedia of Physics.
Editor-in-chief Sybil P. Parker.
McGraw-Hill: 1983.
Pp.1,337. $59.50, £43.75.

The McGraw-Hill Encyclopedia of Science and Technology is a well-known publication, unique in its coverage of all fields of science. It has been revised about every five years, the most recent edition being the fifth which was published in 1982 (for review see *Nature* **229**, 283; 1982). Its articles have developed over the years, so that they are now well pruned of excess material and unusually free from errors.

This *McGraw-Hill Encyclopedia of Physics* has been made up by selecting 760 articles dealing with aspects of physics and mathematics, taken from the 1982 edition of the larger work. About 40 per cent of these articles are quite short, occupying less than a column but with ample cross-reference to the longer pieces in which they find a natural place. About 12 per cent are more substantial, occupying more than 8 columns each. Some of these are much longer, for example *gas dynamics* (15 columns), *superconductivity* (17), *radioactivity* (35) and *ultrasonics* (15). To categorize the articles in this way is perhaps misleading, however, since some large topics have been broken up; thus for fluid flow we have *fluids* (4), *fluid dynamics* (1), *fluid flow* (9), *fluid flow principles* (2), *fluid mechanics* (3) and *fluid statics* (1), not to mention *hydrodynamics* (8), *turbulent flow* (5), *magnetohydrodynamics* (11) and *gas dynamics* (15). On the other hand, *particle accelerator*, amounting to 50 columns, is a single entry but has seven sections, each with a different author. Perhaps more of the shorter pieces could have been grouped together in this way, although this might have made them appear forbidding to inexperienced readers.

Many of the articles are excellent. Plasma physics and nuclear physics are particularly well-served, the accounts being quite up to date and clearly illustrated, but the areas of elementary particle physics and condensed matter are also very well covered, as are more traditional topics in articles such as *units of measurement, sound, molecular physics* and *gravitation*. The mathematical articles are compact but generally quite accessible. Their style varies considerably; for example *combinational theory, graph theory, statistics* and *trigonometry* are clear and readable whereas a few of them, while being excellent and authoritative, are rather technical for the average physicist, let alone a high-school student. All but the shortest pieces have an adequate bibliography, two to five sources being quoted, generally covering standard books on the subject as well as one or two recent review articles. The articles are well cross-referenced, often paragraph by paragraph, and at the end of the volume there is a detailed index.

The reader will generally start with the index, which will offer a possible article. In reading this, he will find cross-references to related articles. By proceeding systematically in this way, he will generally be led to what he needs. For example, I tried the term *eigenvalue*, for which there is no article. The index referred me to *quantum mechanics* and to *eigenfunction*, while the latter referred me to the former and to *non-relativistic quantum theory*, so I quickly reached my goal. However, it is possible for topics to slip through this overlapping net. For example, spontaneous decay of the proton, a central topic of current research, is not in the index. The article *proton* does not mention it. The index for proton lists *proton radioactivity* but this turns out to be nuclear decay by the spontaneous emission of a proton. After some looking around, I found the topic contained in the *baryon* article under the subheading *proton stability*, and in the *elementary particle* article under the subheading *grand unified theories*. Another example is cosmic radiation, although this subject may perhaps be considered more a part of geophysics or astrophysics. The index led me to *bremsstrahlung*, in which proton emission by cosmic ray particles is briefly mentioned. This article gives a cross-reference to *cosmic rays*, but there is no article of that title. The basic facts about cosmic rays really merit a place in this volume, since they have been historically of immense importance and still give the highest-energy nuclear collisions available for study.

It is natural to ask how this encyclopaedia compares with the *Encyclopedia of Physics* published by Addison-Wesley in 1981 (for review see *Nature* **290**, 657; 1981). The main difference is that the latter is really intended for other physicists, or at least for scientists in neighbouring fields, who seek enlightenment on a topic peripheral to their own area of expertise. It has fewer articles but they are longer, on average, and considerably more technical, with equations being used freely. However, it seems that many of the articles of the Addison-Wesley encyclopaedia were written not later than early 1977, so it is not up to date on topics in areas of rapid growth. The McGraw-Hill version would appear to be the most suitable available for the audience to which it is directed — high schools and colleges. But all concerned with mathematics, physics, chemistry or engineering will find much of interest to them in its pages, laid out in an attractive and easily digestible form. □

R.H. Dalitz is Royal Society Research Professor in Theoretical Physics at the University of Oxford.

Astrophysical fruit

Geoffrey Bath

The Left Hand of Creation: The Origin and Evolution of the Expanding Universe.
By John D. Barrow and Joseph Silk.
Basic Books/Heinemann: 1983. Pp. 256. $17.95, £9.95.

"THEORETICAL astrophysics is as exact a science as geometry", remarked the Oxford mathematician E. A. Milne in a paper in 1930. Milne's affirmation in the exactitude of astrophysics followed introductory remarks outlining the problem of radiative transfer through a spherical shell of gas. This introduction concluded with an outspoken defence of what Milne saw as one of the chief necessities for progress — namely, the need to simplify a complex problem into an analytically tractable set of conditions, in his case the simultaneous and self-consistent solution of radiative transfer through matter, including ionization and excitation. Milne confined his attention to "the simplest question that can be propounded". That is, the case of static grey material. Today those working in the field of radiative transfer are approaching the solution of non-LTE (local thermodynamic equilibrium), extended, relativistically expanding atmospheres. But no one working in the field would deny the truth of Milne's justification for a simpler approach in 1930. "Until we have fully and rigorously discussed the case of grey material we are unlikely to make useful progress with more complicated hypotheses."

It is salutary to reflect on the state of model atmosphere theory half-a-century ago in the context of developments within modern cosmology. In the past cosmology has all too frequently been dismissed as an insular, acroamatic subject; a science which, with only the one singular case open to investigation, was doomed to failure on the grounds of inadequate experimental verification. For after all, so the argument goes, how could Newton possibly have succeeded in his theory of gravitation with only the one instance of a single apple falling on his head as experimental proof? Amusing though the myth may be, it was the extensive studies of motion and dynamics for two thousand years, from Aristotle and Richard of Wallingford to Galileo and Newton himself, which permitted a precise theory of gravity to be formulated.

However to view cosmology as the study of one experiment of the Grand Designer is to mistake the vessel for the cargo it contains. Cosmology is not the science of one single aspect of the Universe. Rather it is all aspects, woven against the tapestry of time, space and the forces of nature. The attempt to understand the origin, development and ultimate fate of its constituents is no different in principle from the aim of chemistry to unravel the code of chemical bonding, or of molecular biology to tease out the molecular basis of life.

Barrow and Silk present this quest with wit and gusto. Confident and experienced guides, they conduct their readers around the great debates in contemporary cosmology. At issue are such questions as the origin of the lack of antimatter, the source of spatial fluctuations responsible for all present structure in the Universe, the quasi-supernatural, as they seem superficially, properties of the vacuum giving rise to the paradox of black hole evaporation, and the progressive decomposition of the cosmic soup as time is run backwards towards the Planck time when the Universe was 10^{-43} seconds old, complete symmetry held sway, and all forces were indistinguishable.

No new development in fundamental physics can take place today without its influence on the evolution of the Universe being tested. Increasingly cosmology, and the astronomical evidence at its core, plays a fruitful role as a guide to permissable physical theorizing, supplementing more orthodox laboratory experiment and verification. The resulting astrophysical theorems aim at an ultimate amalgamation of theory and observation which will account for the form and content of the Universe — a Universe seen as a necessary consequence of physical law, with only the most basic axioms as the starting point.

But do not turn to this book for proofs of those theorems. The excitement and challenge are captured, but the rigour is necessarily elsewhere. Just as for many young minds the bald statement of Pythagoras's theorem is the first step towards a clearer perception of the structure underlying the physical world, so this book is a primer to recent attempts at unification of the forces of nature, and the powerful patterns those attempts are creating in the science of astrophysics. □

Geoffrey Bath is a Research Fellow of Wolfson College, University of Oxford.

Fresh yeast

D.R. Harper

Yeasts: Characteristics and Identification.
By J.A. Barnett, R.W. Payne and D. Yarrow.
Cambridge University Press: 1983. Pp.811. £75, $150.

MANY of the outstanding publications dealing with the classification, nomenclature and identification of yeasts have, in the past, come from the Centraalbureau voor Schimmelcultures in Delft. Jacomina Lodder, of the Centraalbureau, edited what has for many years been considered as the definitive work on yeast taxonomy, *The Yeasts: A Taxonomic Study* (North-Holland, 1970), in which she provided the characteristics of 349 yeast species and described relevant laboratory methods. However, her scheme for identification placed emphasis on the ability of organisms to produce ascospores. This led, inevitably, to practical problems associated with demonstrating sporulation.

In England, J.A. Barnett and R.J. Pankhurst approached yeast identification in a different way, and in *A New Key to the Yeasts* (North-Holland, 1974) presented a computer-derived key which involved only physiological tests. *A New Key* was based on the information given in *The Yeasts* and was intended to be used in conjunction with it.

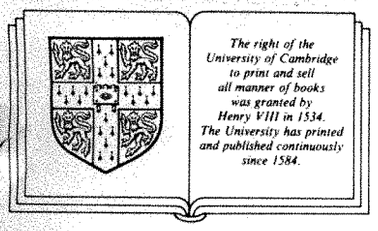
Five years later, *A Guide to Identifying and Classifying Yeasts* (Cambridge University Press, 1979) brought together as authors J.A. Barnett and R.W. Payne, with D. Yarrow from the Centraalbureau. Improved methods of computing were used to generate identification keys consisting of either physiological tests or both physiological and morphological observations. However, it was again necessary to consult Lodder's text for certain additional details.

The successor to *A New Key* and *A Guide* is *Yeasts: Characteristics and Identification*, an up-to-date book which does not rely on another work for any essential information. In it, Barnett, Payne and Yarrow discuss yeast classification and give commonly used procedures for yeast identification. In addition, they provide detailed accounts of 469 yeast species; responses to 83 tests and clear photomicrographs are also included.

The authors have revised the successful format of *A Guide* and incorporated it into this volume. They give 18 identification keys which deal with various groups of yeasts (for example, clinical isolates, yeasts associated with foods and beverages, and yeasts with particular physiological or morphological attributes). Lists of small numbers of tests for the identification of single species are provided. One chapter presents generic characteristics and another conveniently tabulates the characteristics of each species. The extra sections containing specific epithets and yeast names will undoubtedly prove invaluable given the confusion caused by nomenclatural changes in yeast taxonomy.

In producing this book, Barnett, Payne and Yarrow have met the daunting standards set by Lodder in 1970. *Yeasts: Characteristics and Identification* satisfies the requirements of being comprehensive and of containing easily accessible information. It will undoubtedly stand as *the* major text on yeast taxonomy for a considerable time to come. □

David Ross Harper is the microbiologist at the Metropolitan Police Forensic Science Laboratory, London.



CAMBRIDGE SCIENCE PUBLISHING

maintaining a great tradition

Reproduction in Mammals

Second Edition
Book 3: Hormonal Control of Reproduction
Edited by **C.R. AUSTIN** and **R.V. SHORT**

The latest volume in the thorough, updated revision of this classic work discusses the manifold ways in which hormones control the reproductive processes in both males and females.

0521256372 **Hard covers £22.50 net/ $39.95**
0521275946 **Paperback £8.95 net/ $16.95**

Animal Physiology

Adaptation and Environment
Third Edition
KNUT SCHMIDT-NIELSEN

The latest edition of this highly-praised, widely-used textbook has been completely revised and updated. In addition, new material on the brain and senses, and on nervous and endocrine functions is included.

0521259738 **£15.00 net/ $29.95**

Neural Origin of Rhythmic Movements

Edited by **A. ROBERTS** and **B.L. ROBERTS**

Thirty-seven contributors together provide a comprehensive and wide-ranging review of our basic knowledge of the way rhythmic movements originate within the nervous systems of both vertebrate and invertebrate animals.

Society for Experimental Biology Symposium 37

0521256348 **£37.50 net/ About $99.50**

The Physiology of Trematodes

Second Edition
J.D. SMYTH and D.W. HALTON

This detailed, revised account of our knowledge of one of the major groups of parasitic worms takes account of recent developments in our understanding of the ultrastructure, cytochemistry, biochemistry and immunology of the group.

0521222834 **Hard covers £30.00 net/ $59.50**
0521294347 **Paperback £11.95 net/ $23.95**

Progress in Anatomy, Volume 3

Edited by **V. NAVARATNAM** and **R.J. HARRISON**

This important review of recent research covers a variety of topics including primate evolution, quantification in microscopic studies, differentiation of the blastocyst, the intermediate lobe of the pituitary gland, primary sensory neurons mediating pain and prospects for regeneration in the mammalian central nervous system.

0521249538 **£37.50 net/ $74.50**

The Ecology of Phytoplankton

C.S. REYNOLDS

This comprehensive volume examines the life cycle of phytoplankton and considers the problems presented by a habitat without any solid surfaces: how can an organism stay buoyant in the water, gain sufficent nutrients and grow under difficult and diverse environmental conditions?

Cambridge Studies in Ecology

0521237823 **Hard covers £30.00 net/ About $49.50**
0521282225 **Paperback £12.50 net/ About $18.95**

Light and Photosynthesis in Aquatic Ecosystems

JOHN T. O. KIRK

In this unique general study, John Kirk examines in turn the physics of light transmission through water, the biochemistry and physiology of aquatic photosynthesis and the ecological relationships partly determined by the underwater light balance.

0521244501 **£37.50 net/ $74.50**

Eddington

The Most Distinguished Astrophysicist of His Time
S. CHANDRASEKHAR

A lucid non-technical account of the achievements of A.S. Eddington, for 31 years Plumian Professor of Astronomy at Cambridge during which time he made major contributions to astrophysics and general relativity.

0521257468 **£7.50 net/ $12.50**

Cecilia Payne-Gaposchkin

Her Autobiography and Other Writings
Edited by **KATHERINE HARAMUNDANIS**

The first woman professor and head of department at Harvard, Cecilia Payne-Gaposchkin can lay claim to be the greatest woman astronomer ever. This book traces her early life and academic career and is augmented by personal recollections of her daughter and historical appreciations from colleagues.

0521257522 **£19.50 net/ $34.50**

Ion-selective Electrodes

Second Edition
J. KORYTA and **K. STULIK**

This much-revised edition of a successful book presents a compact and unified treatment of the theory of ion-selective electrodes together with an updated account of their analytical, biomedical and physico-chemical applications.

0521238730 **£25.00 net/ $49.50**

Antarctic Earth Sciences

Proceedings of the Fourth International Symposium, Adelaide, 1982
Edited by **R. L. OLIVER**

Almost 100 scientists have contributed to this prestigious symposium which provides a comprehensive review of several seasons' research on all aspects of Antarctic earth sciences and Antarctic resources.

052125836 **£45.00 net/ $79.50**

Co-published with the Australian Academy of Science

Magnetic Oscillations in Metals

D. SHOENBERG

A systematic account of the nature of magnetic oscillation in metals, including descriptions of experimental techniques for the study of oscillations and the nature of their connection with the electronic structure of the metal concerned.

Cambridge Monographs on Physics

0521224802 **£55.00 net/ $97.50**

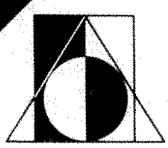
CAMBRIDGE UNIVERSITY PRESS

The Edinburgh Building, Shaftesbury Road, Cambridge CB2 2RU — 32 East 57th Street, New York, NY 10022

Circle No.03 on Reader Service Card.

Wiley announce tw

NEW



Diabetic Medicine



A Journal of the British Diabetic Association

EDITOR: Dr. John Ireland

Consultant Physician, Southern General Hospital, Glasgow G51 4TF, UK

EDITORIAL BOARD *will include*

K.G.M.M. ALBERTI	J.W. FARQUHAR	H. KEEN	R.B. TATTERSALL
D. ARMYTAGE	C.M. FLETCHER	JANET KINSON	J.D. WARD
A. BLOOM	C.N. HALES	P.H. SÖNKSEN	P.J. WATKINS

ASSOCIATE EDITORS

S.R. BLOOM	B.M. FRIER	R.J.JARRETT	W.M.G.TUNBRIDGE
J.L. DAY	E.A.M. GALE	SUE JUDD	S. WALFORD
C.J. FOX	D.R. HADDEN	J.I. MANN	P.H. WISE.

AIMS AND SCOPE

This journal is an information exchange on all aspects of diabetes mellitus and aims to interest everyone helping diabetic patients whether through fundamental research or better health care. The journal will provide a multi-disciplinary forum combining original articles, reviews, comment, news and corespondence by which it is hoped to increase knowledge about the aetiology and pathogenesis of diabetes and its complications and to promote new ideas about management and education.

EDITORIAL PHILOSOPHY

The aim of DIABETIC MEDICINE, as of the BDA itself, is to advance knowledge about diabetes and its management. DM should be of interest to those working in the centres of excellence, but the overwhelming need is to extend this interest to a wider audience and to generate a climate where diabetes will be identified as a subject deserving more health care resources.

PAPERS APPEARING IN EARLY ISSUES

REVIEWS

CONTROLLING THE ADOLESCENT DIABETIC, I.W. Farquhar, *Edinburgh*

NORMOGLYCAEMIC RE-ENTRY AND DIABETIC COMPLICATIONS, H. Keen, *London*

INDUCTION OF ALTERED IMMUNITY AND THE BETA CELL, G.F. Bottazzo, *London*

SYMPOSIUM

PUMPS IN PRACTICE: Practical Aspects of Continuous Subcutaneous Insulin Infusion, (CSII)

ROSTRUM

HARNESSING RESOURCES, P.R. Harsant, *Basingstoke*

CAREERS IN DIABETIC MEDICINE, J.K. Wales, *Leeds*

ORIGINALS

MICROALBUMINURIA PREDICTS MORTALITY IN NON-INSULIN DEPENDENT DIABETES, R.J. Jarrett et al, *London*

LEFT VENTRICULAR DYSFUNCTION IN YOUNG DIABETIC PATIENTS, R.J. Northcote et al, *Glasgow*

SUBSCRIPTION DETAILS

Bimonthly, starting April 1984 | Volume 1 (4 issues) | UK: £39.00 Elsewhere: US $69.00

For a detailed prospectus and/or specimen copy please write to the most convenient address below.

Journals Department,
John Wiley & Sons Ltd.
Baffins Lane · Chichester,
Sussex PO19 1UD · England



Subscription Department 'C',
John Wiley & Sons Inc.
605 Third Avenue · New York,
N.Y. 10158 · U.S.A.

Circle No.04 on Reader Service Card.

major medical journals

Now Published by Wiley

THE JOURNAL OF

Pathology

A Journal of the Pathological Society of Great Britain and Ireland

EDITOR-IN-CHIEF

Professor D.H. Wright, *Department of Pathology, University of Southampton, U.K*

EDITORS

Dr. A.G. MacIver, *Department of Pathology, University of Southampton, U.K.*
Professor F. Walker, *Department of Pathology, University of Leicester, U.K.*

EDITORIAL BOARD

P.P. ANTHONY, *UK*
C.L. BERRY, *UK*
J.W. BRADFIELD *UK*
T.J. CHAMBERS, *UK*
C.M. FENOGLIO, *USA*
C.H.W. HORNE, *UK*
P. ISAACSON, *UK*
J.V. JOHANNESSEN, *Norway*
E.L. JONES *UK*
J.W. KEELING, *UK*
I. LAUDER, *UK*
J.M. POLAK, *UK*
P.J. SCHEUER, *UK*
J.R. TIGHE, *UK*
P.G. TONER, *UK*
J.C.E. UNDERWOOD, *UK*
R.A. WALKER, *UK*
M.N.I. WALTERS, *Australia*
N. WOOLF, *UK*
N.A. WRIGHT, *UK*
A.H. WYLLIE, *UK*

AIMS AND SCOPE

THE JOURNAL OF PATHOLOGY is a journal of the Pathological Society of Great Britain and Ireland. From January 1984, it will incorporate the other affiliated journal, DIAGNOSTIC HISTOPATHOLOGY. The new group of Editors intend that the journal shall reflect the interests of the members of the Society. This will be no easy task since they cover the whole range of experimental and diagnostic histopathology.

High quality papers, written in English and dealing with descriptive histopathology will be welcome. Papers dealing with new techniques and those that elucidate the mechanisms underlying disease processes and histopathological appearance will be particularly encouraged.

Original articles in the field of experimental pathology, relevant to human disease, will be welcome. Review articles dealing with general and experimental pathology, as well as diagnostic histopathology, will be solicited. Unsolicited reviews will also be accepted if they are clearly written and topical. Editorials, a letter column and reviews of recently published books will form regular features of the Journal. Meetings abstracts of the Pathological Society of Great Britain and Ireland will be published as they become available and the annual C.L. Oakley Lecture will continue to be included.

FORTHCOMING ARTICLES

LIVER SINUSOIDAL CELLS by J.W.B. Bradfield

B_2 MICROGLOBULIN IN NON-MALIGNANT AND MALIGNANT HUMAN BREAST: A FEATURE OF DIFFERENTIATION by K. Sidky and R.A. Walker

THE EFFECT OF PROSTACYCLIN ON THE ADHESION OF LEUCOCYTES TO INJURED VASCULAR ENDOTHELIUM by G. Jones and J.V. Hurley

HISTOGENESIS OF GRANULAR CELL TUMOR - AN IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY by M. Miettinen, E. Lehtonen, H. Lehtola, P. Ekblom, V-P. Lehto and I. Virtanen

FIBRONECTIN IN DISEASE by A.J. D'Ardenne and J.O. McGee

SUBSCRIPTION DETAILS

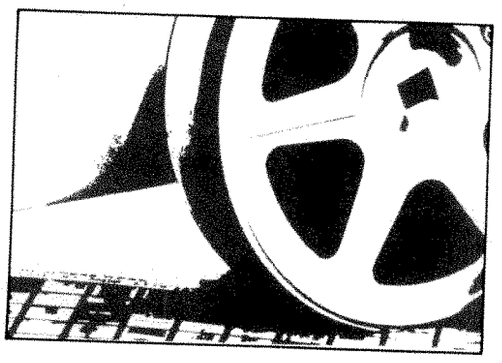
Volume 142-144 (1984) Monthly UK: £80.00 Elsewhere: US$149.00

For a detailed prospectus and/or specimen copy please write to the most convenient address below.

Journals Department,
John Wiley & Sons Ltd.
Baffins Lane · Chichester,
Sussex PO19 1UD · England

Subscription Department 'C',
John Wiley & Sons Inc.
605 Third Avenue · New York,
N.Y. 10158 · U.S.A.

nature
is available in Microform.



University Microfilms International

Please send additional information
for _____
Name _____ (name of publication)
Institution _____
Street _____
City _____
State _____ Zip _____

300 North Zeeb Road, Dept. P R., Ann Arbor, Mi. 48106

Circle No.66 on Reader Service Card.

Revised and updated edition

Photoelectron Spectroscopy

An introduction to ultraviolet photoelectron spectroscopy in the gas phase

J H D Eland, MA, DPhil,
Fellow of Worcester College, Oxford

When first published, *Photoelectron Spectroscopy* was praised by the *Journal of Electron Spectroscopy and Related Phenomena* as an "excellent treatise . . . a broad and lucid introduction suitable for chemists and physicists at advanced undergraduate and postgraduate level". It has now been carefully revised and updated for this new edition.

The purpose of the book remains unchanged; to provide the reader with a complete account of the subject at a practical level. The treatment is thorough but non-mathematical and the approach progressive, from a first familiarization with simple spectra through experimental methods and the photoionization process to the interpretation of ionization potentials and band shapes.

Hardcover 288 pages 215 × 138 mm
Illustrated 0 408 71057 8 £25.00 1983

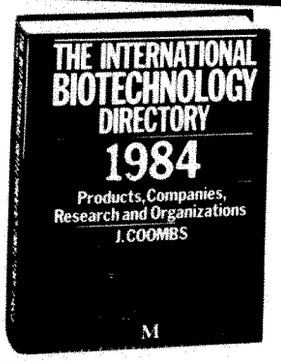
Available from your local bookseller or direct from the publishers.

Butterworths
Borough Green, Sevenoaks, Kent TN15 8PH

Circle No.12 on Reader Service Card

EXAMINE ON FREE APPROVAL WITHOUT COMMITMENT

TWO NEW reliable, up-to-date and complete biotechnology information resources.



Detailed information on every aspect of biotechnology worldwide

An indispensable guide to:
★ Products, services and research
★ Companies ★ Information sources.
★ Government and international agencies

THE INTERNATIONAL BIOTECHNOLOGY DIRECTORY 1984 —
- is thoroughly international
- features a unique BUYER'S GUIDE to products, research and services
- covers both the old and new biotechnologies

The Directory tells you:
★ Who your competition is, and what they are doing.
★ Who are potential customers and suppliers.
★ Where to buy equipment, chemicals and services.
★ Where culture collections are located and what cultures they hold.
★ Who imports/exports biotechnology products in over 20 countries.
★ Which government departments are associated with biotechnology.

All compiled by the eminent biotechnologist Dr. Jim Coombs and now available to you on **FREE APPROVAL.**

Macmillan Paperback 320pp **September 1983**
ISBN 0 333 36178 4 **£40.00/$80.00**

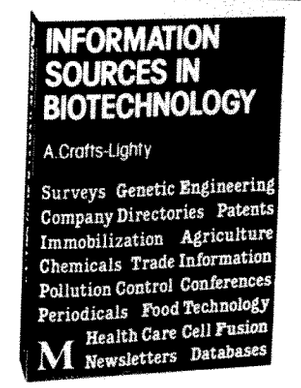
The ONLY guide to the selection of cost effective biotechnology information sources

★ covers both old and new technologies
★ covers technical and commercial literature
★ covers difficult and hard to trace sources
★ includes over 20 at-a-glance evaluation tables
★ provides an Index of publishers and their addresses

Dr. Crafts-Lighty, Head of Information and Library Services at Celltech Limited, systematically and critically reviews all the important information sources:
• books • conferences • trade periodicals • research periodicals • computer databases • patent • market surveys • directories • organisations • secondary sources

Macmillan Hardcover 400pp **November 1983**
ISBN 0 333 35140 1 **£50.00/$100.00**

Send no money now.
Examine on FREE APPROVAL
You can take these new books for 30 days examination without commitment. If you are not completely satisfied, just return the book(s) in good condition within 30 days and we will cancel your invoice.



Globe
Globe Book Services Limited,
Brunel Road, Houndmills,
Basingstoke, Hants RG21 2XS, England.
Telephone: Basingstoke (0256) 26222
A DIVISION OF MACMILLAN PUBLISHERS LTD

The Nature Press,
15 E 26 Street, New York, N.Y. 10010
Telephone: 212-532-4811

FREE APPROVAL ORDER FORM

To: Ian Jacobs, Globe Book Services Ltd., FREEPOST, Brunel Road, Houndmills, Basingstoke, Hants RG21 2BR, England.
The Nature Press, 15 E 26 Street, New York. N.Y. 10010. Credit card callers call toll — free 800-221-2123 (or in New York: 212-532-4811)

Please send me on 30 days FREE APPROVAL _____ copies of *The International Biotechnology Directory 1984* at £50.00/$100.00 each, and/or _____ copies of *Information Sources in Biotechnology* at £40.00/$80.00 each.

If I am not entirely satisfied, I may return any book in good condition within 30 days and you will cancel my invoice.

Postage and Packing
UK subscribers £2.00 (Overseas £3.00)
US Subscribers $1.50

Tick preferred method of payment
☐ Bill me. I need send no money now.
I enclose cheque for £/$ _____
Charge my credit card on publication £/$ _____
Visa Access/Mastercharge
Am. Express Diners
Card No. _____
Expiry Date _____
Signature _____
(Orders cannot be accepted without a signature)
Name _____ (PLEASE PRINT)
Address _____
_____
Postcode/Zip Code _____
Books will be delivered to you on publication.

UK Subscribers POST TODAY — NO STAMP NEEDED N2

Circle No.65 on Reader Service Card.

Psychology

BURROWS, G.D., NORMAN, T.R. and DAVIES, B. (eds). Antidepressants. Drugs in Psychiatry, Vol.1. Pp.350. ISBN 0-444-80474-9. (Elsevier Biomedical/North Holland: 1983.) $45, Dfl.130.

CLARK, J.H. A Map of Mental States. Pp.242. Pbk ISBN 0-7100-9235-0. (Routledge & Kegan Paul: 1983.) £6.95.

IVERSEN, L.L., IVERSEN, S.D. and SNYDER, S.H. (eds). Neuropeptides, Vol.16. Handbook of Psychopharmacology. Pp.577. ISBN 0-306-41048-6. (Plenum: 1983.) $65.

KILMANN, P.R. and MILLS, K.H. All About Sex Therapy. Pp.227. ISBN 0-306-41317-5. (Plenum: 1983.) $15.95.

OLLENDICK, T.H. and HERSEN, M. (eds). Handbook of Child Psychopathology. Pp.526. ISBN 0-306-40938-0. (Plenum: 1983.) $50.

REASON, J. and MYCIELSKA, K. Absent-Minded?: The Psychology of Mental Lapses and Everyday Errors. Pp.262. Hbk ISBN 0-13-001743-4; pbk ISBN 0-13-001735-3. (Prentice-Hall: 1982.) Hbk np; pbk £5.90.

RIEBER, R.W. (ed.). Dialogues on the Psychology of Language and Thought. Pp.166. ISBN 0-306-41185-7. (Plenum: 1983.) $19.50.

SMITH, T. The Homoeopathic Treatment of Emotional Illness: A Self-help Guide to Remedies Which Can Restore Calm and Happiness. Pp.208. Pbk ISBN 0-7225-0812-3. (Thorsons: 1983.) £5.95.

TALBOTT, J.A. and KAPLAN, S.R. (eds). Psychiatric Administration A Comprehensive Text for the Clinician-Executive. Pp.410. ISBN 0-8089-1529-0. (Grune & Stratton: 1983.) $38.50.

Biological Sciences

MURAMATSU, T. *et al.* (eds). Teratocarcinoma and Embryonic Cell Interactions. Pp.344. ISBN 4-7622-5294-8/0-12-511180-0. (Japan Scientific Societies Press/Academic: 1982.) $36.

NADELSON, C.C. and MARCOTTE, D.B. (eds). Treatment Interventions in Human Sexuality. Pp.488. ISBN 0-306-41082-6. (Plenum: 1983.) $42.50.

NOAKES, D.L.G. *et al.* (eds). Predators and Prey in Fishes. Proceedings of the 3rd Biennial Conference held May 1981, Illinois. Pp.230. ISBN 90-6193-922-8. (Dr W. Junk: 1983.) Dfl.150, $65.

NOGGLE, G.R. and FRITZ, G.J. Introductory Plant Physiology, 2nd Edn. Pp.627. ISBN 0-13-502096-4. (Prentice-Hall: 1983.) £27.85.

OPLATKA, A. and BALABAN, M. (eds). Biological Structures and Coupled Flows. Pp.519. ISBN 0-12-527520-4/0-86689-016-5. (Academic/Balaban ISS: 1983.) Np.

PARRISH, J.A. (ed.). Photoimmunology. Pp.304. ISBN 0-306-41280-2. (Plenum: 1983.) $39.50.

PAUL, W.E. *et al.* Annual Review of Immunology, Vol.1. Pp.666. ISBN 0-8243-3001-3. (Annual Reviews Inc: 1983.) $27 (US), $30 (elsewhere).

PAVONE-MACALUSO, M. and SMITH, P.H. (eds). Cancer of the Prostate and Kidney. NATO Advanced Science Institute Series, Vol.53. Pp.750. ISBN 0-306-41132-6. (Plenum: 1983.) $89.50.

PENTZ, L. The Biolab Book: Twenty-six Laboratory Exercises for Biology Students. Pp.127. Pbk ISBN 0-8018-2512-1. (Johns Hopkins University Press: 1983.) £8.50, $12.95.

PIANKA, E.R. Evolutionary Ecology, 3rd Edn. Pp.416. ISBN 0-06-045232-3. (Harper & Row: 1982.) £20.

Pocket Life Sciences Dictionary. Pp.270. Pbk ISBN 0-582-55656-2. (Longman: 1983.) 90p.

PRUDHOE, S. British Polyclad Turbellarians: Keys and Notes for the Identification of the Species. No.26. Pp.77. Hbk ISBN 0-521-25545-7; pbk ISBN 0-521-27076-6. (Cambridge University Press: 1982.) Hbk £12.50; pbk £5.95.

PUROHIT, S.S. (ed.). Aspects of Physiology and Biochemistry of Plant Hormones. Pp.345. No ISBN. (Kalyani, New Delhi: 1983.) Rs.35.

RAO, P.N. *et al.* (eds). Premature Chromosome Condensation: Application in Basic, Clinical, and Mutation Research. Pp.381. ISBN 0-12-580450-4. (Academic: 1982.) $45.

REA, A.M. Once a River: Bird Life and Habitat Changes on the Middle Gila. Pp.285. ISBN 0-8165-0799-6. (University of Arizona Press: 1983.) $24.50.

REBACH, S. and DUNHAM, D.W. (eds). Studies in Adaptation: The Behavior of Higher Crustacea. Pp.282. ISBN 0-471-89823-6. (Wiley: 1983.) £37.75, $56.50.

REDFERN, M. Insects and Thistles. Naturalists' Handbooks 4. Pp.64. Hbk ISBN 0-521-23358-5; pbk ISBN 0-521-29933-0. (Cambridge University Press: 1983.) Hbk £8; pbk £2.95.

REICHS, K.J. (ed.). Hominid Origins: Inquiries Past and Present. Pp.278. Hbk ISBN 0-8191-2864-3; pbk ISBN 0-8191-2865-1. (University Press of America: 1983.) Hbk $22.50; pbk $11.75.

RITCHIE, D.D. and CAROLA, R. Biology, 2nd Edn. Pp.644. ISBN 0-201-06356-5. (Addison-Wesley: 1983.) Np.

ROBBINS, C.T. Wildlife Feeding and Nutrition. Pp.343. ISBN 0-12-589380-9. (Academic: 1983.) $31.50.

ROBINSON, H. A Generic Review of the Tribe Liabeae (Asteraceae). Smithsonian Contribution to Botany No.54. Pp.69. No ISBN. (Smithsonian Institution Press: 1983.) Np.

ROGERS, A.W. Cells and Tissues: An Introduction to Histology and Cell Biology. Pp.256. Pbk ISBN 0-12-593120-4. (Academic: 1983.) £12.20 (UK only), $23.

RYLANDS, L. Animal Camouflage. Pp.24. Hbk ISBN 0-85122-388-5; pbk ISBN 0-85122-387-7. (Dinosaur Publications: 1983.) Hbk £2.75; pbk 95p.

SAKAI, H., MOHRI, H. and BORISY, G.G. (eds). Biological Functions of Microtubules and Related Structures. Pp.453. ISBN 0-12-615080-X. (Academic: 1982.) $32.

SCHIEMER, F. (ed.). Limnology of Parakrama Samudra — Sri Lanka: A Case Study of an Ancient Man-made Lake in the Tropics. Developments in Hydrobiology, Vol.12. Pp.238. ISBN 90-6193-763-9. (Dr W. Junk: 1983.) Dfl.150, $65.

SERRA, G.B. (ed.). The Ovary. Pp.446. ISBN 0-89004-248-9. (Raven: 1983.) $68.20.

SHEPPARD, C.R.C. A Natural History of the Coral Reef. Pp.152. ISBN 0-7137-1268-6. (Blandford Press: 1983.) £8.95, $16.95 (US), $21.95 (Canada).

SIGEL, H. (ed.). Metal Ions in Biological Systems, Vol.15: Zinc and Its Role in Biology and Nutrition. Pp.520. ISBN 0-8247-1750-3. (Dekker: 1983.) SwFr.198.

SIMS, R.W., PRICE, J.H. and WHALLEY, P.E.S. (eds). Evolution, Time and Space: The Emergence of the Biosphere. Pp.492. ISBN 0-12-6444550-8. (Academic: 1983.) £35, $57.75.

STEWARD, F.C. (ed.). Plant Physiology, Vol.7: Energy and Carbon Metabolism. Pp.553. ISBN 0-12-668607-6. (Academic: 1983.) $67.50.

STRAŠKRABA, M. and GNAUCK, A. Aquatische Ökosysteme: Modellierung und Simulation. Pp.279. No ISBN. (Gustav Fischer: 1983.) DDR 45 M.

SUN, G.Y. *et al.* (eds). Neural Membranes. Pp.608. ISBN 0-89603-052-0. (Humana: 1983.) $59.50 (US), $69.50 (elsewhere).

SVARE, B.B. (ed.). Hormones and Aggressive Behavior. Pp.595. ISBN 0-306-41055-9. (Plenum: 1983.) $59.50.

TORSSELL, K.B.G. Natural Product Chemistry: A Mechanistic and Biosynthetic Approach to Secondary Metabolism. Pp.401. Hbk ISBN 0-471-10378-0; pbk ISBN 0-471-10379-9. (Wiley: 1983.) Hbk £23, $44.50; pbk £9.50, $17.95.

VARI, R.P. Phylogenetic Relationships of the Families Curimatidae, Prochilodontidae, Anostomidae, and Chilodontidae (Pisces: Characiformes). Pp.60. No ISBN. (Smithsonian Institution Press: 1983.) Np.

VERNBERG, W.B. (ed.). Physiological Mechanisms of Marine Pollutant Toxicity. Pp.564. ISBN 0-12-718460-0. (Academic: 1982.) $45.

WALKER, R. The Molecular Biology of Enzyme Synthesis: Regulatory Mechanisms of Enzyme Adaptation. Pp.381. ISBN 0-471-06051-8. (Wiley: 1983.) £44.50, $66.45.

WEISS, L. (ed.). Histology: Cell and Tissue Biology, 5th Edn. Pp.1219. ISBN 0-333-35406-0. (Macmillan Press, London: 1983.) £30.

WILLMER, C.M. Stomata. Pp.166. Pbk ISBN 0-582-44632-5. (Longman: 1983.) £6.95.

YEO, P.F. and CORBET, S.A. Solitary Wasps. Naturalists' Handbook 3. Pp.64. Hbk ISBN 0-521-23387-9; pbk ISBN 0-521-29940-3. (Cambridge University Press: 1983.) Hbk £8; pbk £2.95.

History of Science

CASIMIR, H. Haphazard Reality: Half a Century of Science. Pp.356. US and Canada ISBN 0-06-015028-9; UK ISBN 0-06-337031-X. (Harper & Row: 1983.) £12.50.

SHARAF, M. Fury on Earth: A Biography of Wilhelm Reich. Pp.550. ISBN 233-97544-6. (Andre Deutsch: 1983.) £16.95.

Anthropology

BINFORD, L. R. In Pursuit of the Past: Decoding the Archaeological Record. Pp.256. ISBN 0-500-05042-2. (Thames & Hudson: 1983.) £12.50.

CHIPPINDALE, C. Stonehenge Complete. Pp.296. ISBN 0-500-05043-0. (Thames & Hudson: 1983.) £12.50.

DOUMAS, C.G. Thera: Pompeii of the Ancient Aegean. Excavations at Akrotiri 1967–79. Pp.168. ISBN 0-500-39016-9. (Thames & Hudson: 1983.) £16.

SALMON, M.H. Philosophy and Archaeology. Pp.203. ISBN 0-12-615650-6. (Academic: 1982.) $20.

STEPONAITIS, V.P. Ceramics, Chronology, and Community Patterns: An Archaeological Study at Moundville. Pp.375. ISBN 0-12-666280-0. (Academic: 1982.) $46.

Applied Biological Sciences

AGRIS, P.F. and KOPPER, R.A. (eds). The Modified Nucleosides of Transfer RNA, II: A Laboratory Manual of Genetic Analysis, Identification, and Sequence Determination including Cloverleaf Structures for 233 Sequences and the 1979-82 Bibliography. Pp.320. ISBN 0-8451-0225-7. (Alan R. Liss: 1983.) £29.

AHMAD, F. *et al.* (eds). From Gene to Protein: Translation into Biotechnology. Miami Winter Symposia, Vol.19. Pp.589. ISBN 0-12-045560-9. (Academic: 1982.) $45.

BELLMANN, K. (ed.). Molecular Genetic Information Systems: Modelling and Simulation. Pp.325. No ISBN. (Akademie-Verlag: 1983.) 60 M.

BONICA, J.J., LINDBLOM, U. and IGGO, A. (eds). Proceedings of the Third World Congress on Pain. Advances in Pain Research and Therapy, Vol.5. Pp.990. ISBN 0-89004-800-2. (Raven: 1982.) $109.12.

BOWERS, J.Z. and KING, E.E. (eds). Academic Medicine: Present and Future. Pp.241. Pbk ISBN 87470-037-X. (Rockefeller Archive Center: 1983.) Np.

BRADSHAW, R.A. *et al.* (eds)s. Proteins in Biology and Medicine, Vol.2. Pp.419. ISBN 0-12-124580-2. (Academic: 1983.) $31.50.

BUSVINE, J.R. Insects and Hygiene: The Biology and Control of Insect Pests of Medical and Domestic Importance, 3rd Edn. Pp.568. Hbk ISBN 0-412-15910-4; pbk ISBN 0-412-25360-7. (Chapman & Hall: 1983.) Np.

CHAZOV, E., SAKS, V. and RONA, G. (eds). Advances in Myocardiology, Vol.4. Pp.649. ISBN 0-306-40877-5. (Plenum: 1983.) $69.50.

CHEY, W.Y. (ed.). Functional Disorders of the Digestive Tract. Pp.366. ISBN 0-89004-859-2. (Raven: 1983.) $52.70.

CIBA FOUNDATION SYMPOSIUM 96. Fetal Antigens and Cancer. Pp.263. ISBN 0-272-79660-3. (Pitman: 1983.) £25, $35.

CIBA FOUNDATION SYMPOSIUM 97. Better Crops for Food. Pp.248. ISBN 0-272-79729-4. (Pitman: 1983.) £25, $35.

COATS, J.R. (ed.). Insecticide Mode of Action. Pp.470. ISBN 0-12-177120-2. (Academic: 1982.) $59.50.

COLLINS, C.H. Laboratory-Acquired Infections: History, Incidence, Causes and Prevention. Pp.277. ISBN 0-408-10650-6. (Butterworth: 1983.) £21.

DAMJANOV, I., KNOWLES, B.B. and SOLTER, D. (eds). The Human Teratomas: Experimental and Clinical Biology. Pp.416. ISBN 0-89603-040-7. (Humana: 1983.) $49.50 (US), $59.50 (elsewhere).

DEINHARDT, F. and DEINHARDT, J. (eds). Viral Hepatitis: Laboratory and Clinical Science. Liver: Normal Function and Disease Series, Vol.3. Pp.600. ISBN 0-82471801-1. (Dekker: 1983.) SwFr.238.

DETHLEFSEN, L.A. and DEWEY, W.C. (eds). Third International Symposium: Cancer Therapy by Hyperthermia, Drugs, and Radiation. National Cancer Institute Monograph 61. Pp.550. No ISBN. (US Department of Health and Human Services: 1983.) $21 (USA), $26.25 (elsewhere).

DRAPER, H.H. (ed.). Advances in Nutritional Research, Vol.5. Pp.257. ISBN 0-306-41095-8. (Plenum: 1983.) Np.

EISENSTEIN, T.K., ACTOR, P. and FRIEDMAN, H. (eds). Host Defenses to Intracellular Pathogens. Advances in Experimental Medicine and Biology, Vol.162. Pp.538. ISBN 0-306-41259-4. (Plenum: 1983.) Np.

FAHN, S., CAINE, D.B. and SHOULSON, I. (eds). Experimental Therapeutics of Movement Disorders. Advances in Neurology, Vol.37. Pp.339. ISBN 0-89004-695-6. (Raven: 1983). $52.08.

FALCONER, D.S. Problems on Quantitative Genetics. Pp.104. Pbk ISBN 0-582-44679-1. (Longman: 1983.) £3.95.

FRITZ, H. *et al.* (eds). Kinins-III, Part A and Part B. Advances in Experimental Medicine and Biology, Vol.156. Pp.1222. Set ISBN 0-306-41167-9. (Plenum: 1983.) Np.

GALANTER, M. (ed.). Recent Developments in Alcoholism, Vol.1. Pp.484. ISBN 0-306-41202-0. (Plenum: 1983.) $55.

GALLIN, J.I. and FAUCI, A.S. (eds). Chronic Granulomatous Disease. Advances in Host Defense Mechanisms, Vol.3. Pp.272. ISBN 0-89004-825-8. (Raven: 1983.) $55.80.

GEORGHIOU, G.G. and SAITO, T. (eds). Pest Resistance to Pesticides. Pp.809. ISBN 0-306-41246-2. (Plenum: 1983.) Np.

GORDON, I. Controlled Breeding in Farm Animals. Pp.436. Hbk ISBN 0-08-024410-6; pbk ISBN 0-08-024409-2. (Pergamon: 1983.) Hbk np; pbk £15, $30.

GRAHAM, P.H. and HARRIS, S.C. (eds). Biological Nitrogen Fixation Technology for Tropical Agriculture. Based on Papers presented March 1981, Columbia. Pp.726. ISBN 84-89206-22-8. (Centro Internacional de Agricultura Tropical: 1983.) $35.

HAYAISHI, O. and UEDA, K. (eds). ADP-Ribosylation Reactions: Biology and Medicine. Pp.698. ISBN 0-12-333660-0. (Academic: 1982.) $67.50.

HENDERSON, B.E. *et al.* (eds). Third Symposium on Epidemiology and Cancer Registries in the Pacific Basin. National Cancer Institute Monograph 62. Pp.216. No ISBN. (US Department of Health and Human Services: 1983.) $13 (USA), $16.25 (elsewhere).

HOLLAENDER, A., LASKIN, A.I. and ROGERS, P. (eds). Basic Biology of New Developments in Biotechnology. Basic Life Sciences, Vol.25. Pp.579. ISBN 0-306-41244-6. (Plenum: 1983.) Np.

International Nonproprietary Names (INN) for Pharmaceutical Substances, 1982: Cumulative List No.6. Pp.494. Pbk ISBN 92-4-056013-0. (WHO: 1982.) SwFr.55.

KERR, J.W. and GANDERTON, M.A. (eds). Proceedings of Invited Symposia. 11th International Congress of Allergology and Clinical Immunology held October 1982, London. Pp.560. Hbk ISBN 0-333-32550-8; pbk ISBN 0-333-32551-6. (Macmillan Press, London: 1983.) Hbk £60; pbk np.

LAWRENCE, C.W. (ed.). Induced Mutagenesis: Molecular Mechanisms and their Implications for Environmental Protection, Vol.23. Pp.433. ISBN 0-306-41163-6. (Plenum: 1983.) Np.

LENNERSTRAND, G., ZEE, D.S. and KELLER, E.L. (eds). Functional Basis of Ocular Motility Disorders. Proceedings of an International Symposium held August–September 1981, Stockholm. Pp.603. ISBN 0-08-029772-2. (Pergamon: 1982.) $100, £50.

LIEBERMAN, M. (ed.). Post-Harvest Physiology and Crop Preservation. NATO Advanced Study Series, Vol.46. Pp.572. ISBN 0-306-40984-4. (Plenum: 1983.) $67.50.

LURQUIN, P.F. and KLEINHOFS, A. (eds). Genetic Engineering in Eukaryotes. NATO Advanced Science Institute Series, Vol.61. Pp.282. ISBN 0-306-41275-6. (Plenum: 1983.) Np.

MATOREN, G.M. (ed.). Clinical Research Practices and Drug Regulatory Affairs, Vol.1. No.1. Pp.107. No ISBN. (Marcel Dekker: 1983.) Np.

McGRATH, J.J. and BARNES, C.D. (eds). Air Pollution — Physiological Effects. Research Topics in Physiology, Vol.5. Pp.353. ISBN 0-12-483880-4. (Academic: 1982.) $44.

METTLIN, C. and MURPHY, G.P. (eds). Progress in Cancer Control III: A Regional Approach. Progress in Clinical and Biological Research, Vol.121. Proceedings of the Annual Meeting held October 1982, Buffalo. Pp.342. ISBN 0-8451-0121-8. (Alan R. Liss: 1983.) £34.

MURRAY, F. (ed.). Fluoride Emission: Their Monitoring and Effects on Vegetation and Ecosystems. Pp.234. ISBN 0-12-511980-1. (Academic: 1982.) $29.50.

O'CONNOR, T.E. and RAUSCHER, F.J. Jr. (eds). Oncogenes and Retroviruses: Evaluation of Basic Findings and Clinical Potential. Proceedings of a Workshop held September 1982, Buffalo. Pp.304. ISBN 0-8451-0119-6. (Alan R. Liss: 1983.) £26.

OWENS, A.H. Jr., COFFEY, D.S. and BAYLIN, S.B. (eds). Tumor Cell Heterogeneity: Origins and Implications. Pp.520. ISBN 0-12-531520-1. (Academic: 1982.) $39.50.

PARISER, S.F., LEVINE, S.B. and GARDNER, M.L. (eds). Clinical Sexuality. Reproductive Medicine Series, Vol.3. Pp.224. ISBN 0-8247-1895-X. (Dekker: 1983.) SwFr.93.

PATEL, S.J. (ed.). Pharmaceuticals and Health in the Third World. Pp.328. Pbk ISBN 0-08-030210-6. (Pergamon: 1983.) £10.50, $21.

PEREZ, D.E. and ORDETX, G.S. Apicultura Tropical, 3rd Edn. Pp.486. Pbk ISBN 84-89400-17-2. (Editorial Technologica: 1983.) Np.

PIRCHNER, F. Population Genetics in Animal Breeding, 2nd Edn. Pp.414. ISBN 0-306-41201-2. (Plenum: 1983.) Np.

REID, E. and LEPPARD, J.P. (eds). Drug Metabolite Isolation and Determination. Pp.287. ISBN 0-306-41265-9. (Plenum: 1983.) Np.

RIPPERE, V. The Allergy Problem: Why People Suffer and What Should be Done. Pp.240. Hbk ISBN 0-7225-0867-0; pbk ISBN 0-7225-0796-8. (Thorsons: 1983.) Hbk £8.95; pbk £4.95.

RUSSEL, A.D. and QUESNEL, L.B. (eds). Antibiotics: Assessment of Antimicrobial Activity and Resistance. The Society for Applied Bacteriology Technical Series No. 18. Pp.386. ISBN 0-12-604480-5. (Academic: 1983.) £29, $48.

RYDER, R.D. Victims of Science: The Use of Animals in Research. Pp.180. Pbk ISBN 0-905225-06-6. (National Anti-Vivisection Society: 1983.) £3.75.

SALVATORE, M. and PORTA, E. (eds). Radioisotopes in Cardiology. Pp.329. ISBN 0-306-41267-5. (Plenum: 1983.) $45.

SARKAR, B. (ed.). Biological Aspects of Metals and Metal-Related Diseases. Pp.330. ISBN 0-89004-807-X. (Raven: 1983.) $64.48.

SCHATTENKIRCHNER, M. and MÜLLER, W. (eds). Modern Aspects of Gold Therapy. Rheumatology, Vol.8. Pp.232. ISBN 3-8055-3630-5. (Karger: 1983.) SwFr.130, DM 156, $78.

SCHMIDT, E.H.F. and HILDEBRANDT, A.G. (eds). Health Evaluation of Heavy Metals in Infant Formula and Junior Food. Pp.192. Pbk ISBN 3-540-11823-3/0-387-11823-3. (Springer-Verlag: 1983.) DM 38, $15.20.

SEGAWA, T., YAMAMURA, H.I. and KURIYAMA, K. (eds). Molecular Pharmacology of Neurotransmitter Receptors. Advances in Biochemical Psychopharmacology, Vol.36. Pp.320. ISBN 0-89004-736-7. (Raven: 1983.) $54.56.

SHANMUGARATNAM, K. *et al.* Cancer Incidence in Singapore 1968–77. IARC Scientific Publications No.47. Pp.174. Pbk ISBN 92-8-321147-2. (International Agency for Research on Cancer: 1983.) $15, SwFr.30.

SHULMAN, S. and DONDERO, F. (eds). Immunological Factors in Human Contraception. Pp.227. ISBN 88-7084-006-9. (Field Educational Italia: 1983.) L. 60.000.

SMITH, W.R. (ed.). Energy From Forest Biomass. Pp.279. ISBN 0-12-652780-6. (Academic: 1983.) $27.50.

TAAB, H.A. *et al.* Fortschrittsberichte Aus Naturwissenschaft und Medizin. Pp.352. Pbk ISBN 3-8047-0720-3. (Wissenschaftliche Verlagsgesellschaft mbH: 1983.) DM 32.

TURUSOV, V.S. (ed.). Pathology of Tumours in Laboratory Animals, Vol.3: Tumours of the Hamster. IARC Scientific Publications No.34. Pp.461. ISBN 92-832-1134-0. (International Agency for Research on Cancer: 1983.) $40, SwFr.80.

WATKIN, D.M. Handbook of Nutrition, Health, and Aging. Pp.326. ISBN 0-8155-0929-4. (Noyes: 1983.) $32.

WOOD-GUSH, D.G.M. Elements of Ethology: A Textbook for Agricultural and Veterinary Students. Pp.240. Hbk ISBN 0-412-23160-3; pbk ISBN 0-412-23170-0. (Chapman & Hall: 1983.) Hbk £15; pbk £6.95.

YOXEN, E. The Gene Business: Who Should Control Biotechnology? Pp.264. Pbk ISBN 0-330-28112-7. (Pan: 1983.) £3.95.

ZILLINSKY, F.J. Common Diseases of Small Grain Cereals: A Guide to Identification. Pp.141. No ISBN. (International Maize and Wheat Improvement Center Mexico: 1983.) $18.75.

General

BLAIR, I. Taming the Atom: Facing the Future with Nuclear Power. Pp.248. Hbk ISBN 0-85274-414-5; pbk ISBN 0-85274-483-8. (Adam Hilger: 1983.) Hbk £15; pbk £6.50.

BONNER, J.T. The Evolution of Culture in Animals. Pp.216. Pbk ISBN 0-691-02373-5. (Princeton University Press: 1983.) £8.60.

BOURNE, M.C. Food Texture and Viscosity: Concept and Measurement. Pp.325. ISBN 0-12-119060-9. (Academic: 1982.) $36.

BUCHANAN, A. Food Poverty and Power. Pp.123. Hbk ISBN 0-85124-351-7; pbk ISBN 0-85124-352-5. (Spokesman: 1982.) Hbk np; pbk £3.50.

CARBERRY, J.B. and ENGLANDE, A.J. Jr. (eds). Sludge Characteristics and Behavior. NATO Advanced Science Institute Series, Applied Sciences No. 66. Pp.450. ISBN 90-247-2831-2. (Martinus Nijhoff: 1983.) Dfl. 150, $65.50.

CENTRE NATIONAL D'ETUDES SPATIALES. Management of Large Space Projects. Pp.871. ISBN 2-85428-086-5. (Cepadues Editions: 1983.) FF 350.

DEBONS, A. and LARSON, A.G. (eds). Information Science in Action: System Design, Vols 1 and 2. NATO Advanced Science Institute Series: Applied Sciences, No. 59 and 60. Pp.916. Vol. 1 ISBN 90-247-2806-1; Vol. 2 ISBN 90-247-2807-X; Set ISBN 90-247-2808-8. (Martinus Nijhoff: 1983.) Dfl. 300, $130.

DEUDNEY, D. and FLAVIN, C. Renewable Energy: The Power to Choose. Pp.431. Pbk ISBN 0-393-01710-9. (W.W. Norton: 1983.) Np.

Down to Earth, Mud Architecture: An Old Idea, A New Future. Pp.192. Pbk ISBN 0-500-27277-8. (Thames & Hudson: 1983.) £6.95.

ELLIS, D.V. (ed.). Marine Tailings Disposal. Pp.368. ISBN 0-250-40614-4. (Ann Arbor: 1982.) £35.

ENGLAND, J.M. A Patron for Pure Science: The Natural Science Foundation's Formative Years, 1945–57. Pp.443. No ISBN. (Natural Science Foundation: 1982.) Np.

EVANS, G. The Yellow Rainmakers: Are Chemical Weapons Being Used in Southeast Asia? Pp.202. Hbk ISBN 0-86091-068-7; pbk ISBN 0-86091-770-3. (Verso: 1983.) Hbk np; pbk £3.95, $7.50.

EVENARI, M. *et al.* The Negev, 2nd Edn. Pp.437. ISBN 0-674-60672-8. (Harvard University Press: 1982.) £28.

FORD, K.W. (ed.). Nuclear Weapons and Nuclear War. Papers based on a Symposium held April 1982, Washington, D.C. Pp.35. No ISBN. (American Association of Physics Teachers: 1983.) Np.

GOETZMANN, W.H. and SLOAN, K. Looking Far North: The Harriman Expedition to Alaska 1899. Pp.244. Pbk ISBN 0-691-00591-5. (Princeton University Press: 1983.) £7.75.

GOWING, R. Roger Cotes — Natural Philosopher. Pp.210. ISBN 0-521-23741-6. (Cambridge University Press: 1983.) £22.50.

Second Generation
PATCH/WHOLE CELL CLAMP

LIST-MEDICAL
WEST-GERMANY

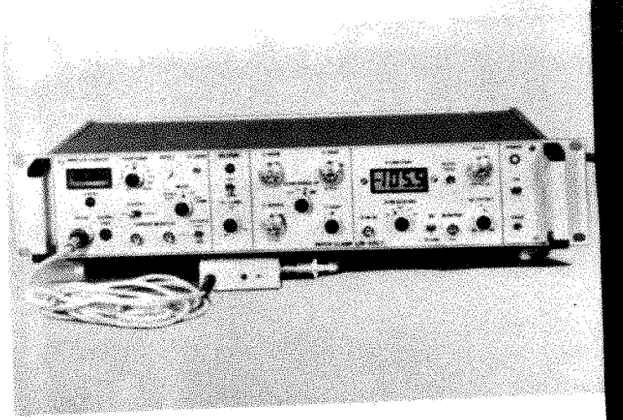
Unique new technology enables you to measure single channel and whole cell currents using one headstage–one experimental set-up lets you work with patches and cells up to 50 µm.

Instrument noise is so low that it eliminates the electronics as a source of experimental noise.

The EPC-7 is the second generation clamp design by Prof. E. Neher and Dr. F. J. Sigworth of the Max Planck Institute. It is state-of-the-art technology derived from years of pioneering experience with the practical application of patch clamps.

- Internal Resistance Switching 50 GΩ-500 MΩ
- 100 KHz Bandwidth
- Integrated Series-Resistance and Transient-Cancellation Function
- Noise is 0.05 pA RMS at 1 KHz
- Built-in Noise measuring meter
- ...And More

There is nothing else that even comes close ...



The new L/M - EPC - 7 System

List-electronic

Pfungstaedter Strasse 18 - 20
D-6100 Darmstadt/Eberstadt, West Germany
Phone (0 61 51) 5 60 00 + 5 60 60

Our representative in USA: MEDICAL SYSTEMS CORP.
239 Great Neck Road, Great Neck, N.Y. 11021

Our representative in Japan: SHOSHIN EM CORP.
23 Kitayashiki, Hashiramachi, Okazaki 444, Japan

Circle No.01 on Reader Service Card.

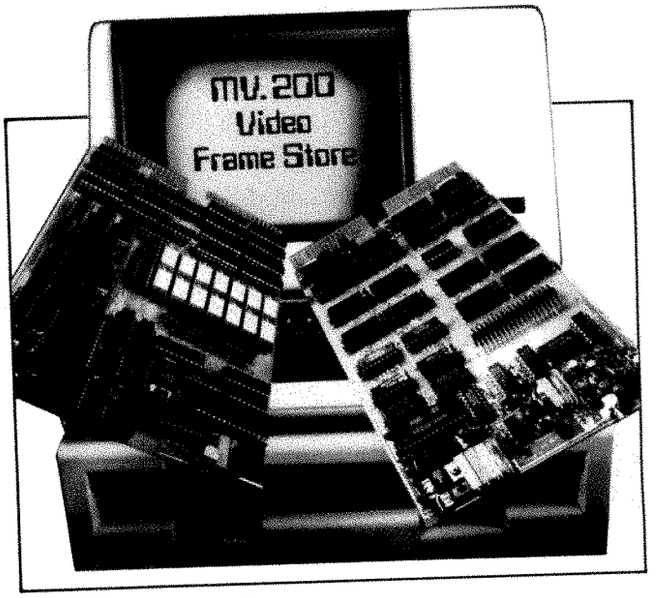
INDUSTRIAL IMAGE INSPECTION AT THE RIGHT PRICE

The MV200 system has been designed to provide a simple and inexpensive 'building block' concept for the electronic, electrical, robotic and mechanical industries; to enable a contactless measuring or data acquisition system to be assembled, using a C1700 solid-state camera or other TV camera as input.

☆ **Image enhancement** – for use in low light level applications, image recovery from noisy signals, X-ray processing and enhancement of X-ray or other film photographs.

☆ **Component, recognition menuing and inspection** – when used in conjunction with either solid-state or high stability Vidicon cameras, the systems can be used for component recognition, measurement, grading, counting, area measurement, orientation, positioning etc., and is a useful tool in quality assurance.

☆ **Designed and manufactured in the UK.** With the low cost of the MV200 system and wide range of standard software available, your very own custom-built system becomes economic and viable.



HAKUTO
HAKUTO INTERNATIONAL (U.K.) LIMITED Opto Electronics Division

HAKUTO INTERNATIONAL (UK) LTD.,
Hakuto House, 159a Chase Side, Enfield, Middlesex EN2 0PW.
Tel: 01-367 4633 Tlx: 299288

A member of SIRA

Circle No.02 on Reader Service Card.

They say all Image Analysers are equal, but MAGISCAN is more equal than others.



More applications...
More speed...
More power...
More reliability...
More answers

Magiscan gives you the answers because Magiscan sees, counts, measures and analyses – it interprets what it sees.

If you need to extract data from an image – Magiscan can do it.

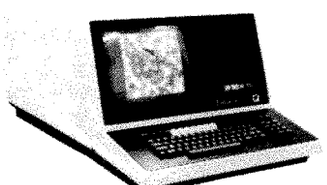
All over the world Magiscan is being used to automate time-consuming tasks such as counting asbestos fibres, analysing chromosomes, detecting cracks in vehicle manufacturing, research into timber cells or assessing paper quality – because Magiscan frees the operator to concentrate on the major decisions.

And you don't have to be a computer expert to use it. This compact image analysis system has the most sophisticated "menu" package available. Its clear prompt procedure guides you through each stage of the analysis – or you can leave it to run automatically.

Circle No.11 on Reader Service Card.

No other image analyser offers so much research capability in such a compact unit, so use the speed, power and flexibility of Magiscan – and give yourself more time.

MAGISCAN



Joyce-Loebl
A Vickers company

World leader in Image Analysis

Joyce-Loebl – Marquisway – Team Valley Gateshead – Tyne and Wear – NE11 0QW England.
Tel: 0632 822111 – Telex: 53257.

Joyce-Loebl GmbH – Emanuel-Leutze-Str. 1A – 4000 Düsseldorf 11 West Germany.
Tel: 0211/594091 – Telex: 8581998.

And our exclusive USA distributor **Nikon Inc.** – Instrument Division 623 Stewart Ave – Garden City New York 11530 – USA.
Tel: 516/222 0200 – Telex: 967806.

Image analysis

This week's feature focuses on recent developments in image analysis systems

● Spectragraphics has released a new type of computer graphics workstation: the system 1500 Multi-Station. The system 1500 provides a range of interactive graphics features as well as a raster graphics workstation. The system's controller supports from one to four workstations. When configured with a 'communications controller', the system 1500 can support up to 64 workstations. It is offered with a choice of 32-bit parallel interfaces for the IBM, Perkin-Elmer, and Digital Equipment host processors. The system 1500 Multi-Station is designed to accommodate graphics applications such as: computer aided design/computer aided manufacture (CAD/CAM), signal processing, seismic interpretation, command and control, simulation, mapping and molecular modelling. It is available in either monochrome or colour (16, 256, or 4096 simultaneous colours from palettes with up to 16 million colours). The workstation's cathode ray tube is a raster-scan 1024 × 1024 display. Objects can be rotated, scaled and translated. Multiple viewports can be defined and transformed images clipped to their boundaries at real-time rates. In addition, a range of input peripherals is available. This includes keyboards, joysticks, data tablets, digitizers, programmable function switches and programmable function dials.
Circle No. 100 on Reader Service Card.

● A series of image analysis systems is available from Artek. The system 810 is modular and is designed such that all the components are able to interface with peripherals and computers. The second system, the model 982, is Artek's basic image analysis building block. It is designed for use in particle sizing, producing histograms, edge or density counting, area measurement and microscopic applications. Also available is the digitized video system with the 940 memory and image editor. This consists of the video from the model 982 which is digitized into a 512 × 512 × 1 format. The computer functions it can perform include selecting and rejecting objects, filling holes and drawing a measuring frame to match a sample.
Circle No. 101 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● Two new 6-bit and 8-bit video frame memories have been manufactured by Hamamatsu and are available in the UK through Hakuto International. The units, designated the models C1900 and C1900-01, will digitize and store analog video signals from a television camera and will provide video outputs of the stored image in real time. The C1900 has a 6-bit memory (64 levels) for storing the intensity level of each picture element. It is intended for use with general television cameras as well as Hamamatsu's own range, the C1000 series. The C1900-01 has an 8-bit memory (256 levels) and its own synchronous generator. The camera head of the C1000 or C1145 series non-interlaced television cameras with 512 scanning lines can be connected to the C1900-01 directly, thus eliminating the need for a camera control unit. Image preprocessing functions such as integration, subtraction and image enhancement are not available. These processes may be programmed and commanded by a separate computer.
Circle No. 102 on Reader Service Card.

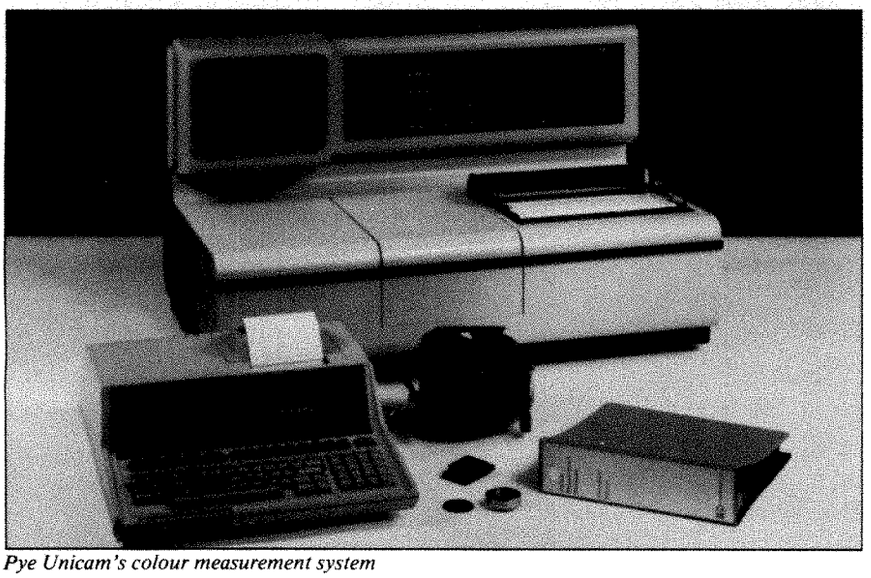
● Two image analysis systems are available from Artek that can be used in conjunction with the light microscope. The Artek 880 and 980 image analysers allow a choice of scanning field shape, size and position. The systems can analyse three operations — numerical count, object area and aperture area — and these can be reselected using a mode selector. Both the 880 and 980 have bottom light and darkfield illumination capacities. The bottom light can be used when light is able to penetrate the objects being analysed and the darkfield can be used when the contrast between the object and the background is poor. In addition, the 880 has a top light.
Circle No. 103 on Reader Service Card.

● The Photonic microscope system from Hamamatsu allows organisms such as motile cells to be viewed with an optical microscope, something that had hitherto been difficult. The system can be used with optical microscopes with differential interface contrast facilities. A television camera is fitted to a conventional optical microscope equipped for differential interference contrast (DIC) and controlled by an image processor incorporating video frame memories. A range of image processing functions can be performed including background subtraction, integration, contrast and edge enhancements.
Circle No. 104 on Reader Service Card.

● The Zeiss Videoplan 2 is an interactive image analysis system which can perform quantitative analysis of geometric characteristics and data evaluation in the life and material sciences. The system evaluates photographic materials, including X rays, and is equally effective in analysing direct images from video images or light and electron microscopes. Videoplan 2 consists of a digitizer tablet, a microcomputer, a video monitor and a portable keyboard. Dual, double-sided, high-density disk drives and a 64-K user memory plus a 16-K video memory are included. As a stand-alone system, Videoplan 2 can be programmed for turn-key use or it can function as a data generator that interfaces with other computers. The updated Videoplan 2 software includes an array of data acquisition programs, statistics, stereology, data editing and graphic routines for distribution and correlation analyses. Specialized programs are available as options. The computer uses a CP/M operating system and can be programmed in several languages.
Circle No. 105 on Reader Service Card.



The Videoplan 2 interactive image analysis system from Zeiss



Pye Unicam's colour measurement system

● A new colour measurement system is available from Pye Unicam. It is based on the PU 8800 spectrophotometer which uses a software package for the HP 85 computer. The PU 8800 range of instruments incorporates a video display, a built-in recorder and printer options and a choice of side or end-window photomultipliers. The colour measurement software is configured for operation with an HP 85 and coupled through the PU 8800's bi-directional RS 232C interface. Available on tape and disk, the software provides measurement of colour or colour difference in up to three modes — diffuse or total reflectance and transmission. Illuminants A, C, D_{65} and D_{75} are offered, all weighted at 2° and 10° observer angles. Reflectance data on up to 99 standards or samples can be stored on file for immediate recall and the system allows editing of stored black and white calibration values if required. The results can be produced in both tabular and graphical form, along with a listing of the system and calculation conditions, operator identity and date. The instrument can store up to ten user programs.
Circle No. 106 on Reader Service Card.

● Micromeritics has produced an interface for linking a computer to its SediGraph system. The interface, called SediComp, is designed to allow any SediGraph 5000ET to be adapted to Apple II + and Apple IIe microcomputers. SediComp expands the SediGraph data output capability so that it can provide tabular and graphic data in printed or video display format. Programs and data are stored on standard 5¼-inch floppy disks. The SediGraph automates the classical sedimentation technique. The unit can analyse particle sizes in the range from 0.1 μm to 100 μm in a single analysis. The SediComp package includes Apple personal computer boards, master program disks, connecting cables and a user manual.
Circle No. 107 on Reader Service Card.

● Zenith Data Systems has launched two monochrome video monitors known as the ZVM-122 and ZVM-123 green phosphor display, depending upon visual preference. The new monitors are compatible with most business microcomputers and video games, such as the Apple II and III, the IBM personal computer, the Commodore 64 and Vic 20, Texas Instruments' 99/4 and the Atari 800 and 1200, as well as Zenith's own Z100 desktop computer. Designed with a bandwidth of 15 MHz that gives the ability to display more than 800 lines horizontally, the ZVM-122/123 monitors have a rise time of 30 ns. The instrument's 12-inch diagonal screen offers a display with up to 25 lines containing 80 characters each. Each monitor is also equipped with a 40 to 80 character switch. This enables the character size to be increased to accommodate a video format when the computer has a 40-column display.
Circle No. 108 on Reader Service Card.

● The Hamamatsu C1901 and C1901-01 are 16-bit video frame memories with real-time image preprocessing functions. The C1901 is intended for use with raster-scan television cameras, including Hamamatsu's C1000 series and the C1145 range of non-interlaced TV cameras with 512 scanning lines. Since the C1901 contains its own synchronous generator, it is directly compatible with the camera head of the C1000 or C1145. A camera control unit is not required. In contrast, the C1901-01 is designed for use with Hamamatsu's C1181-01 random access camera (image dissector camera) operating in the photon counting mode. It can also be modified for slow scan inputs for the scanning electron microscope. Both models have real-time image preprocessing functions such as integration subtraction, shading correction and image enhancement controlled by using keyboard strokes. In addition, programmable red, green and blue (RGB) outputs are provided.
Circle No. 109 on Reader Service Card.

● Micro Consultants has introduced IDAS, a new generation of digital fluorography equipment. IDAS is a digital fluorography system that interfaces directly to any modern X-ray equipment. The fluoroscopic television output is connected to IDAS via a single coaxial cable. The system is mobile, being trolley mounted. It uses the graphics technology of Micro Consultants' sister company Quantel, and can be expanded with modular software packages. IDAS digital image storage capability can be expanded by interfacing to Centinel, MicroConsultants' medical central image library system. Centinel provides a digital centralized storage and filing system for both pictures and patient records. Images can be stored on Winchester disks and on removable cartridge disks. It can support a number of remote image terminals which have fast access to 500,000 (base system) digitally-stored images.
Circle No. 110 on Reader Service Card.



IDAS from Micro Consultants

● Dapple System's Imageplus + is a software-based image analysis system that can be used in conjunction with a standard Apple IIe computer with 64 K of RAM. The system is boosted with hardware and software options that provide a 256 K memory so that the computer operates at over three times the speed of a standard Apple IIe! This increased capability enables all 64 grey levels to be acquired and stored simultaneously. The system first acquires the image through a video camera, then provides measurements such as area, length, breadth, perimeter and orientation of each feature. Facilities are provided for both manual and automatic modification of the raw image data. Imageplus + can also provide a range of statistical and graphical operations. These include correlation between variables, means, variances and multiple regression. Imageplus + is available in Europe from Miller Systems.
Circle No. 111 on Reader Service Card.



NEW EDITION!

How Britain runs its science...

This highly successful wallchart has now been revised and updated to take account of changes in structure and personnel since the previous edition was published in 1981. It includes the ministerial and other changes which followed the British General Election on 9 June, 1983.

The guide provides a comprehensive survey of the administration of British science, showing the interlocking relationship between science, engineering and medical establishments. It gives a full account of the funding of science in Britain, with details of money spent by government, research councils, industry and universities.

Designed as a fold-out sheet, the 75 × 95cm chart can be carried easily to meetings or hung as a wall poster for permanent reference. The new edition is available at £3.50 or US$7.95 from Nature's offices in Basingstoke or New York.

In the UK and elsewhere: Nature, Brunel Road, Houndmills, Basingstoke, Hants, RG21 2XS, England.

In the US and Canada: Nature, Suite 1503, 15 East 26th Street, New York, NY 10010, USA.

Cheques should be made payable to Nature.

Please send me

____ Chart(s) of **'How Britain Runs its Science'**

I enclose a cheque for _____

Name _____

Address _____

Please allow 6 to 8 weeks for delivery.

nature

Circle No.95 on Reader Service Card.

LONDON OFFICE Keith Walesby 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024)
AMERICAN OFFICES NEW YORK Miss Gene Fein 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900
SAN FRANCISCO JJHS (415) 392 6794 ●**PASADENA** JJHS (213) 796 9200
CHICAGO Didier & Broderick (312) 498 4520 ● **BOSTON** CEL Assoc (617) 848 9306
CANADIAN OFFICE TORONTO Peter Drake 17 Pine Crescent, Toronto, Ontario M4E 1L1 (416) 690 2423

RATES UK — Rest of world — £11.00 per centimetre. Minimum 3 cm. £2 is charged for the re-direction of replies to advertisements with a box number.

CONDITIONS. The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.

Scientist-Biotechnology

The Unilever Research Laboratory at Vlaardingen is one of the Concern's three large laboratories. Its 1000 employees are engaged in research into food and detergent products and process development.

They carry out industrial research on a multidisciplinary basis. Close contacts are maintained with operating Companies, sister laboratories abroad and Universities.

The job

Our involvement in biotechnology is growing fast. Therefore we would like to contact candidates for a post as

Biotechnologist

in the Process Engineering research group.

Within this group, the section engaged in bioprocessing consists of a staff of 25, including some experienced biotechnologists.

We expect the successful candidate to:

- be knowledgeable in the areas of fermentation and downstream processing, with a good track record as demonstrated by publications, etc.
- be able to identify new opportunities in these areas
- have at least five to ten years industrial research experience
- have managerial experience
- have excellent skills in communication, in order to stimulate further growth of biotechnology within Unilever

If you can meet these requirements please contact Ir R. Keuning, a Director of the Unilever Research Laboratory, who will be able to give further information. Telephone no. 3110 353000 (extension no. 2015).
Written applications in English should be addressed to one of the addresses below:

Citizens of EEC-countries:
Unilever Research Laboratory, Personnel & Organization Department.
Attn. Mrs M. Veenendaal, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands.

US residents:
Lever Research Inc., Attn. Ms Bette Droge, Placement Manager, 45 River Road, Edgewater N.J. 07020, USA.

Unilever.

(W687)A

UMM AL — QURA UNIVERSITY
FACULTY OF APPLIED SCIENCES & ENGINEERING
DEPARTMENT OF MEDICAL SCIENCES

Assistant or Associate Professor in:-

1. **Haematology**
2. **Physiology**
3. **Medical Parisitology**
4. **Pathology**

Qualification: PhD. (Preferably Med-Quat) M.D.

Applications are invited for the above positions tenable immediately for 4 years with the possibility of renewal. Salary depends on qualification and experience.

Applicants are expected to teach and train undergraduate students as well as initiate independent research and participate in working research groups.

Please send CV, and arrange for 2 letters from 2 referees to be sent to:

Dr A. Sultan (Head of Department)
UMM AL-QURA UNIVERSITY
Faculty of Applied Sciences and Engineering,
Department of Medical Sciences,
PO Box 3711, Makkah — Saudi Arabia.

(W693)A

DEPARTMENT HEAD

DEPARTMENT OF BIOLOGICAL SCIENCES DREXEL UNIVERSITY

Applications and nominations are invited for the position of Head of the Department of Biological Sciences beginning September 1, 1984. Drexel University, which emphasizes programs in science and technology and has been a leader in cooperative education for more than sixty years, has approximately 12,000 students in seven colleges. It is situated in the center of Philadelphia not far from a variety of medical schools and other universities. The Biological Sciences Department, one of four departments offering BS, MS, and PhD degrees in the College of Science, is to be expanded considerably in the direction of molecular biology, biochemistry, cell biology, immunology, and other aspects of modern biology. The new Department Head will have the responsibility for recruiting a senior faculty member to fill an endowed Chair and to recruit a number of other persons to fill tenure track positions within the next few years.

The successful candidate is expected to (a) have an earned doctorate in the biological sciences or in a related field; (b) be qualified for tenure as a full professor; (c) have had significant scientific accomplishments; (d) be actively engaged in funded research; (e) have knowledge of funding sources generally in the biological sciences; (f) be experienced in directing graduate research in a PhD granting department; and (g) show evidence of leadership and of a commitment to strong graduate and undergraduate program in modern biological science.

Applications and correspondence should be addressed to Dr. W. William R. Nes, Chairperson, Department Head Search Committee, Department of Biological Sciences, Drexel University, Philadelphia, Pennsylvania, 19104. Applicants should include the names and addresses of three to five persons from whom they will request letters of recommendation. Drexel University is an Affirmative Aciton/Equal Opportunity Employer.

(NW243)A

Leukaemia Research Fund Centre at the Institute of Cancer Research

(Director: Dr. M. F. Greaves)

A Leukaemia Research Fund Centre is being established at the Chester Beatty Laboratory of the Institute of Cancer Research in the Fulham Road, London. The Centre will carry out research into the Cellular and Molecular Biology of Human Leukaemia. The following technical and post-doctoral posts will be available from 1st April, 1984.

1. Research Assistant

at the technician or research officer level to help organise molecular biology laboratory and actively participate in its research programme. Experience in recombinant DNA techniques essential. **Ref. 301/B/43.**

2. Research Assistant

at the technician or research officer level to be responsible for monoclonal antibody production. Experience in hybridoma techniques essential. **Ref. 301/B/44.**

3. Research Assistant

at the technician or research officer level to operate Fluorescence Activated Cell Sorter and participate in research programme on haemopoietic progenitor cells. **Ref. 301/B/45.**

Candidates for the above posts should have a degree, HNC or equivalent qualification in appropriate subject. Salary depending on qualifications and experience will be in the range of £5,491 — £7,098 p.a. plus London Allowance of £997 p.a.

4. Research Fellowship

A three year post-doctoral research fellowship is available for studies on the biochemistry of membrane associated proteins of normal and malignant haemopoietic cells. Experience in immunochemistry and membrane protein analysis will be required. Salary in range £7,630 — £8,975 p.a. plus London Allowance of £1,186 p.a. **Ref. 300/G/39.**

Applications for any of the above posts should be sent with a full CV in duplicate to **the Personnel Officer, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU,** quoting the appropriate reference number.

(1078)A

Faculty Positions in Media Technology

The Massachusetts Institute of Technology has created a new interdisciplinary laboratory to address the invention and creative use of new media. As part of this Media Laboratory's teaching responsibilities, six faculty positions are open in the Department of Architecture. Candidates qualified for any one or more of these openings are invited to submit resumes and any information by February 15, 1984:
Professor Nicholas Negroponte
Director, Media Laboratory
Room NE43-205
Massachusetts Institute of Technology
545 Technology Square
Cambridge, Massachusetts 02139

The positions are in the discipline areas of:

1. film and video engineering
2. computer/graphics
3. personal computation
4. photo-electronics
5. epistemology and learning
6. acoustics and audio engineering

Duties include 50% research and 50% teaching. In each case teaching will include three commitments:

1. an introductory subject in the field
2. a graduate seminar in the faculty person's particular field
3. thesis supervision

Faculty are expected to build research programs from the following existing platforms of sponsored research: human interaction with computers, broadcast technology, learning, signal processing, computer music, electro-optics, graphics and image making.

Applicants must possess a PhD or equivalent, and must have demonstrated ability and interest in research. Teaching experience at the University level is desirable. The positions respectively require professional experience in:

1. television engineering, digital video, telecommunication
2. computer animation, image processing, artificial intelligence
3. human/machine interface, experimental psychology
4. electronic imaging, display technology, 3-D
5. developmental psychology, social science, computation
6. digital audio, real-time signal processing, spatial sound, speech processing, performance technology

Each position can be at either the Assistant or Associate Professor level; salary competitive and to be negotiated. The opening is specifically for the academic year starting March 15, 1984. An earlier starting date is possible.

MIT IS AN EQUAL OPPORTUNITY/ AFFIRMATIVE ACTION EMPLOYER.

(NW240)A

DIRECTOR BIOTECHNOLOGY CENTER

The Ohio State University

The Ohio State University invites applications and nominations for the position of Director of the University's interdisciplinary Biotechnology Center. The candidate should have an earned doctorate and an outstanding record of achievement in an area of Biotechnology such as with microorganisms (including viruses), plants, animals, enzymes, nucleic acid and protein biochemistry, or biochemical engineering, as evidenced by publications, receipt of research grants and activities in national and international scientific organizations. The candidate should also have a demonstrated ability and willingness to interact with other scientists in a University setting.

The Director will provide leadership for and will coordinate the development of interdisciplinary programs in Biotechnology, conduct an active research program in a particular area of interest, and will draw on resources of existing programs in the Colleges of Agriculture, Biological Sciences, Mathematical and Physical Sciences (Chemistry) Engineering, Medicine, Veterinary Medicine and Pharmacy. The Director will have resources to appoint several additional faculty members for the Center beyond those appointments in Biotechnology being made by individual departments.

The Director will provide active leadership in promotion, direction and support of research and instruction activities of the Center, enhance academic excellence, maintain a high level of morale among its faculty, and encourage a spirit of learning among its students.

The Director is responsible for the development and advancement of the research, instruction and service activities of the Biotechnology program, for administering, where appropriate in coordination with other Directors or Deans, the personnel affairs, staffing patterns, budgetary projections and allocations of the Center. The Director reports to the Provost who is the chief academic officer of the University.

The position is available July 1, 1984, or at an earlier date. Salary is highly competitive and commensurate with background and experience.

Applications and nominations should be addressed to Dr. Patrick R. Dugan, Chairman, Search Committee for a Director of Biotechnology Center, The Ohio State University, 203 Bricker Hall, 190 North Oval Mall, Columbus, Ohio 43210. Deadline for applications and nominations is March 1, 1984.

The Ohio State University is an Affirmative Action/Equal Employment Opportunity Employer. (NW233)A

Am Institut für Med. Mikrobiologie der Johannes Gutenberg-Universität (Fachbereich 04 Medizin) ist eine

C3 Professur (Beamtenstelle auf Lebenszeit) für das Fachgebiet

MOLEKULARGENETIK

erstmals zu besetzen. Bewerber (Mediziner oder Naturwissenschaftler) sollen auf dem Gebiet der Prokaryonten und/oder Eukaryonten international ausgewiesen sein. Darüberhinaus wird Bereitschaft zur Kooperation mit den Forschungsgruppen des Institutes auf den Gebieten "Immunologie" und "Mikrobielle Pathogenitätsfaktoren" vorausgesetzt. Laborarraum von 250 qm und apparative sowie personelle Grundausstattung sind vorhanden.

Bewerbungen mit den üblichen Unterlagen sind bis vier Wochen nach Erscheinen dieser Anzeige zu richten an den Dekan des Fachbereichs Medizin der Johannes Gutenberg-Universität Mainz, Am Pulverturm 13, 6500 Mainz, Tel: 06131/17-3180. (W682)A

des Chercheurs qui trouvent !

Ils existent, nous travaillons avec eux...
Si la découverte de MEDICAMENTS vous tente,
rejoignez notre Centre de Recherche situé à Paris.

Nous créons encore des départements en

pharmacologie

Nous recherchons leurs

Directeurs

L'un sera spécialisé en Rhumatologie, il aura des connaissances approfondies • en immunologie, • sur les phénomènes biochimiques associés à l'inflammation, • en analgésie.

L'autre sera spécialisé en Neuro-Psychopharmacologie privilégiant l'abord biochimique.

Pour un département existant, nous recrutons un

Cadre de recherches

en Pneumologie (niveau 3e cycle en Sciences ou équivalent).

Il aura des connaissances en physiologie ou pharmacologie respiratoire, circulation pulmonaire, exploration fonctionnelle et pathologie expérimentale.

Dernière précision, notre Entreprise est française, du secteur privé. Elle s'enorgueillit d'être active au niveau mondial puisqu'elle exporte la moitié de ses découvertes.

Merci d'adresser lettre manuscrite rédigée en français, C.V. + photo, sous réf. 5500 à Média-System, 104 rue Réaumur 75002 Paris qui transmettra.

(W699)A

UNIVERSITY OF BERGEN, NORWAY

UNIVERSITY LECTURERSHIP IN SEDIMENTOLOGY

A position as *university lecturer* is vacant at the Geological Institute (Dept. A), University of Bergen, Norway. Applicants must have an MSc, PhD or equivalent qualifications and competence in sedimentology (preferably clastic sedimentology). Experience in sedimentary mineralogy and geochemistry will be advantageous.

The vacant position is in association with the research group in sedimentology/petroleum geology which is composed, in addition to the advertised position, of a scientific staff of 5. The successful candidate must be able to take part in teaching at both undergraduate and postgraduate levels according to the requirements of the institute and follow the regulations which apply for the position. Applications from women are encouraged. Further particulars about the lectureship may be obtained from the Secretary of the Faculty of Sciences, PO Box 25, 5014 Bergen Universitet, Norway. The salary will be in the range NKr. 123.496 — NKr. 162.917.

Applications, with a complete curriculum vitae, copies of degrees and testimonials together with one set of scientific publications and a list of these in triplicate should be sent to the University of Bergen, Personnel Department, PO Box 25, 5014 Bergen — Universitet, Norway, by 20th March 1984.

(W677)A

HERIOT-WATT UNIVERSITY
DEPARTMENT OF PHYSICS

RESEARCH ASSOCIATES — SEMICONDUCTOR OPTICAL BISTABILITY

The bistability research team, under the direction of Professor S D Smith FRS, presently numbers fifteen scientists. Research objectives include studies of the nonlinearities that are the physical origin of semiconductor optical bistability, assessment of new materials for bistability in the infrared, near-infrared and visible spectral regions, investigations of bistable characteristics, and the development of novel devices. Achievement of the optically bistable switch has caused speculation of device applications ranging from fibre communication link repeater elements to optical parallel processing and optical signal computing. The group has a number of collaborative projects, both with Industry (through the Joint Optoelectronic Research Scheme) and with Universities throughout Europe (through the European Joint Optical Bistability Project).

Applications are invited from candidates with PhD experience in semiconductors/optical properties, optoelectronic devices, laser physics, or theory of optical properties of semiconductors.

Salaries will be in the range £7,190 to £11,615, according to age and experience.

Informal enquiries may be made to Dr B. S. Wherrett, Department of Physics — Tel: 031-449 5111 ext 2223.

Application forms are available from the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX (please quote ref. no. 3/84).

(1043)A

Research Scientist- Animal Physiology

Harefield, Middx. c.£14,000

Glaxo Animal Health Limited, part of the well known Glaxo Group, is a young, highly autonomous and rapidly expanding company with UK market leadership in ethical veterinary products, and worldwide responsibility for the Group's animal health interests.

An opportunity now exists for a highly capable research scientist to join the company's physiology team within a department which is responsible for researching and developing innovative products designed to increase animal production.

Candidates should have gained a PhD in genuinely innovative research — preferably in the field of biochemistry, cell biology, metabolic science or nutritional physiology. Particular areas of interest include intermediary metabolism, muscle nutrition and development, digestion and absorption, fat or muscle cell culture, in vitro tissue culture, tissue residues studies, and protein and fat metabolism.

In addition to an original, fresh approach to the study of metabolism and growth, candidates should possess the ability to operate effectively with the minimum of supervision in a committed, small-team environment, together with communication/presentation skills of the highest order.

A starting salary of around the level indicated is envisaged, though this need not prove a limiting factor for high-calibre candidates. Generous benefits include guaranteed bonus, site allowance, free pension scheme, and, if appropriate, assistance with relocation; excellent sports and social facilities are also available.

For an application form, please contact Mrs Moira Model, Company Personnel Officer, Glaxo Animal Health Limited, Breakspear Road South, Harefield, Uxbridge, Middlesex UB9 6LS. Telephone: Ruislip (089 56) 30266.

Glaxo Animal Health Limited (1050)A

National Hellenic Research Foundation

Two research assistants, PhD, Molecular Biology, to take part in programs on Structure and function of nuclear RNP/carcinogens and hormonally regulated genes.

Send full curriculum to: Mrs. Th. Stefanou, Institute of Biological Research, National Hellenic Research Foundation, Vassileos Constantinou 48, Athens, Greece. (W680)A

MEMORIAL UNIVERSITY OF NEWFOUNDLAND
Sir Wilfred Grenfell College
The Department of Physics invites applications for a tenure-track position at the rank of

ASSISTANT OR ASSOCIATE PROFESSOR

at the Sir Wilfred Grenfell College campus of the University, located in Corner Brook, Newfoundland, subject to final budgetary approval. The College offers a full range of courses at the first and second-year levels. The primary responsibilities of the position will be instruction in the first and second-year Physics courses. The campus accommodates about 700 students in a residential setting and is located in a community of about 30,000 population.

Applications, including curriculum vitae and the names of three referees, should be sent to: Dr. D. H. Rendell, Head, Department of Physics, Memorial University of Newfoundland, St. John's, Nfld. A1B 3X7. (NW239)A

SOUTH MANCHESTER HEALTH AUTHORITY
CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE
PATERSON LABORATORIES

A POST-DOCTORAL RESEARCH POSITION

is available in the Department of Experimental Haematology to work with Dr T M Dexter on a project funded by the Cancer Research Campaign. The aim is to characterise the mode of action of various regulatory molecules and cell matrix components in haemopoietic cell development. Preference will be given to candidates who have experience in one or more of the following fields: monoclonal antibodies/receptor analysis, biochemical studies in hormone messenger systems using intact cells or isolated membrane.

Starting salary in the range £7,190 — £10,710.

Applications including full curriculum vitae and names and addresses of two referees should be made to Dr T M Dexter, Department of Experimental Haematology, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Wilmslow Road, Withington, Manchester M20 9BX. (1086)A

THE UNIVERSITY OF MANCHESTER
DEPARTMENTS OF CHILD HEALTH AND SURGERY

RESEARCH ASSISTANT

Re-advertisement

Applications are invited for a 3 y post to investigate the role naturally-occurring polypept growth factors in lung maturati This study will involve both rac immunoassay and chemical as techniques and the ability to w with laboratory animals is essent The work will divided between Medical School and Profes Boyd's Department of Child He at St. Mary's Hospital. There is opportunity for a suitable applic to register for a higher degree.

Applicants should have a g degree, ideally in Biochemist Pharmacology/Physiology and h an enthusiasm for research. In salary will be £6,310 pa on Research and Analogous Staff grade 1B.

Applications, including 2 copie the curriculum vitae and the name 2 referees should be sent to Dr Keys, Department of Surge Medical School, The Univers Manchester M13 9PT by Febru 10th, 1984. (1077)

THE UNIVERSITY OF NEW ENGLAND

DEPARTMENT OF ANIMAL SCIENCE

LECTURESHIP IN GENETICS

pplications are invited for tenured ctureship in Genetics. Duties will lude undergraduate teaching in netics and animal breeding, pervision of honours and post-duate students and research.

The applicant should have a broad owledge of genetics, with part-lar expertise in quantitative netics and its applications in mal breeding, some preference l be given to applicants with earch experience in theoretical antitative genetics. Applicants uld have a Science, Agriculture or terinary degree and appropriate st-graduate qualifications. The partment is well-equipped for etic research and is responsible all genetics teaching in the versity.

Appointment to commence as n as possible.

Further information may be tained from the Head of partment, Professor J S F Barker, writing or telephone (067) 73 2225. ary: A$23,394 — A$30,734 per um. Closing Date: 5th March, 4. Position No: 405.

The appointment will be to the manent staff but the University erves the right to make the ointment probationary where it siders this appropriate.

Other conditions include super-nuation, assistance with travel and oval expenses and with buying or ding a house in Armidale.

Applications, including the names addresses of three referees and ting the position number should be t to the Staff Officer, University New Englnd, Armidale, NSW 1, prior to the closing date. plicants should forward a copy of advertisement to their referees ask them to send their reports to the Staff Officer, Mr B G ner, marking their envelopes ivate and Confidential" and ting the position number, before above closing date so as to edite the appointment process.

Armidale, NSW 2351 Australia. (W681)A

THE UNIVERSITY OF SYDNEY

POSTDOCTORAL FELLOW PLANT BREEDING INSTITUTE CASTLE HILL

pointee to investigate the genetic is of resistance to stem rust in icale and to carry out routine ing of breeding materials for stralian triticale breeding groups. pointment for a maximum of 3 rs. Enquiries to Dr R A McIntosh) 634 2552. Salary: $A20,164 — 22,513.

Applications to Registrar, Univer-y of Sydney, NSW 2006, Australia 20 February 1984.

Equal employment opportunity is iversity policy. (W695)A

ROYAL POSTGRADUATE MEDICAL SCHOOL

(University of London)

PROSTAGLANDIN AND LEUKOTRIENE RESEARCH

Applications are invited for the position of Post-doctoral Research Fellow (Senior Research Officer) in the Department of Clinical Pharmacology. They successful candidate will work on an MRC funded project into the role of eicosanoids in pulmonary anaphylaxis. The research entails isolated tissue experiments (animal and human) and will involve collaboration with both clinical and scientific members of the Department. Excellent analytical facilities for prostaglandins and leukotrienes are available.

Salary £8,376 to £8,816 on Range 1A (inclusive of London Allowance).

Application forms and further particulars can be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London, W12 0HS, quoting reference number 20/216.

Closing date: 16 February 1984. (1081)A

UNIVERSITY OF CHICAGO

ASSISTANT OR ASSOCIATE PROFESSOR IN THE RESEARCH ASSOCIATE TRACK

to assume major responsibility in a cellular electrophysiology laboratory studying arrhythmogenesis and the actions of antiarrhythmic drugs. Experience in voltage clamping, cable analysis, ion sensitive microelectrodes, and computer programming preferable. Excellent scientific environment.

Renewable term appointment with full fringe benefits. Immediate availability.

Please send curriculum vitae, representative reprints, and names of three references to: Morton F Arnsdorf, MD, Chief, Section of Cardiology, University of Chicago Hospitals, Box 423, 950 E 59th Street, Chicago, Ill 60637. (NW245)A

ST THOMAS'S HOSPITAL MEDICAL SCHOOL

(United Medical & Dental Schools)

POSTDOCTORAL POSITION IN HUMAN NEUROPHYSIOLOGY

Applications are invited for a post-doctoral position at the Sherrington School of Physiology to study the organization of central synaptic input to motoneurones in man. The position is supported by the MRC and will be available from January 1984. Knowledge of spike train analysis would be an advantage but is not essential.

For more details please contact Dr John Stephens, Sherrington School of Physiology, St Thomas's Hospital Medical School, London SE1 7EH. (1002)A

Director

MRC Biostatistics Unit

The Medical Research Council invites applications for the post of full-time Director of the Biostatistics Unit to succeed Dr I Sutherland.

The person appointed will have an established reputation as a scientific investigator in biomedical statistics, be able to develop the Unit's research programme, and have the ability to lead, manage and co-ordinate the activities of a research team. It is not essential that he or she possess a medical qualification.

The Unit is concerned with the development and advancement of statistical methodology, both fundamental and as applied to medical problems, and with research into the epidemiology of disease. An important function at present is the provision of statistical advice to Council Units, Council Committees, and other scientific workers. The precise scope and emphasis of the Unit's future programme will be a matter for discussion between the successful candidate and the Council, but it is expected that the programme will include fundamental studies, both purely theoretical and problem-related. A candidate who wished the Unit to specialise in a particular line of biomedical research in which statistical methods play a prominent part should put their proposals forward.

The Unit is at present accommodated in the MRC Centre at the University Medical School, Cambridge; however, new accommodation may need to be sought on the appointment of a new Director. The present staff complement is 15, including 5 scientific staff.

An appointment to the Council's staff will be in accordance with the Council's terms and conditions of service, and with superannuation under the MRC Pension Scheme. The salary will be within the Council's Special Appointments Grade, which is equivalent to the Universities' professorial range. If required, assistance may be given with house sale/purchase expenses. The present Director is not due to retire until September 1986, but it is hoped that his successor will be able to join the Unit as Director-designate before that date.

Applications in the form of a short statement (not exceeding 1,000 words) outlining in general terms the scientific programme which the applicant would propose for the Unit, together with a *curriculum vitae*, list of publications, and the names of three referees, should be submitted not later than 16 March 1984 to the Secretary of the Council at the address below.

Additional information, including details of the current programme of the Unit, may be obtained from Mr K Hammond, MRC Headquarters Office, 20 Park Crescent, London W1N 4AL; telephone 01-636 5422 ext. 336.

MRC Medical Research Council

(1052)A

BIOTECHNOLOGISTS!

Are you reading Bio/Technology, the International Journal for Industrial Biology?

For a free sample copy of this new monthly Journal from the Publishers of *Nature*, please write or phone:

Mirta Soto
Bio/Technology
15 East 26th Street
New York 10010
USA
Tel: (212) 689 5900

Felicity Parker
Bio/Technology
Houndmills Industrial Estate
Brunel Road, Basingstoke
Hampshire, UK
Tel.: (0256) 29242

AGRICULTURAL BIOTECHNOLOGY

Shell Development Company is seeking scientists with significant records of achievement who are interested in applying their skills to agronomic research.

Positions are available for outstanding Ph.D.-level scientists with training in one of the following:

- Agricultural Microbiology
- Plant Molecular Biology
- Plant/Invertebrate Pathology
- Plant Cell and Tissue Culture

Shell Development Company at Modesto, California offers an outstanding research environment, a reasonable cost of living, and attractive salaries and benefits. Modesto is close to the San Francisco Bay Area and the Sierra Nevada Mountains. Shell's current staff includes a strong team of innovative scientists with a record of technical accomplishment and commercial success in agrochemicals research.

Applicants should be U. S. Citizens or have permanent resident visa. Interested applicants should send a résumé, in confidence, to: Manager, Employee Relations, Shell Development Company, Biological Sciences Research Center, P.O. Box 4248, Modesto, CA 95352.

Shell Development Company

An Equal Opportunity Employer M/F

(NW247)A

Analytical Chemist
Saudi Arabia
c. £18,250 p.a. tax-free

IAL have been providing high technological expertise to countries throughout the world for over 35 years. We are presently engaged in an environmental protection programme in Saudi Arabia and can offer a new opportunity to an experienced Analytical Chemist.

Based in Jeddah, you will be visiting locations all over the country to monitor the operations of fixed air quality stations. From mobile laboratories, equipped to the highest standards, you will be collecting and analysing field samples for standard air and water parameters. You will also be transferring your expertise to Saudi staff, giving support and assistance to on-the-job training programmes.

You should have a degree in Chemistry, ideally with experience in Analytical Chemistry and some basic knowledge of Biology. You should also have at least five years experience in either a modern analytical laboratory or in the laboratory services of a statutory body dealing with the monitoring of air or water quality.

As well as an attractive tax-free salary, paid in Saudi Riyals (at the currrent exchange rate of SR5.0=£1), you will enjoy excellent benefits such as rent free accommodation, life insurance and generous leave entirlement.

Please apply to the Senior Recruitment Officer, IAL, Aeradio House, Hayes Road, Southall, Middx. UB2 5NJ. Tel: 01-574 5134. Please quote Ref. S006.

IAL

THE HIGH TECHNOLOGY TASK FORCE

(W698)A

COMMUNICATIONS SYSTEMS
COMPUTER SYSTEMS AND SERVICES
MEDICAL SERVICES
AVIATION SYSTEMS AND SERVICES-WORLDWIDE

THE UNIVERSITY OF ADELAIDE

invites applications from both m and women for the following position

LECTURER IN GEOLOG (A1694) (TENURABLE)

in the areas of igneous petrology c metamorphic petrology. Applica should be engaged actively in f and laboratory studies in one of th areas. An interest in isotopic meth would be an advantage. T successful candidate will teach several levels in the undergradu programme and will be required supervise honours and postgradu students.

The position is available from August 1984.

Detailed information on resea and teaching facilities in Department of Geology and Mine ogy (which will be amalgamated v the Department of Econo Geology to form a single Departm of Geology in 1985) and in University's Electron Optical Ce may be obtrained from the Chair of the Department of Geology Mineralogy, Dr J B Jones.

It is University policy to encou women to apply for consideration appointment to, in particul tenurable academic positions.

Holders of full-time tenured tenurable academic appointme have the opportunity to take le without pay on a half-time basis f specific period of up to ten ye where this is necessary for the car children.

Further information about general conditions of all appo ments may be obtained from Senior Assistant Regist (Personnel) of the University.

Salary per annum: $A23,394 — $A30,734.

Applications, in duplicate, quo reference number and giving personal particulars (includ citizenship), details of acad qualifications and names addresses of three referees, sh reach the Senior Assistant Regis (Personnel) of the University Adelaide, GPO Box 498, Adela South Australia 5001 (Tel UNIVAD AA89141), not later t 20 February 1984.

The University reserves the r not to make an appointment o appoint by invitation.

(W697)

Please mention

nature

when replying to these advertisements

HERIOT-WATT UNIVERSITY

PARTMENT OF BREWING AND BIOLOGICAL SCIENCES

CTURESHIP IN MARINE BIOLOGY

tions are invited for the st which is available from 1st ember 1984. Applicants should ess an Honours Degree and have ied out postgraduate research in siology, preferably on the siology of marine animals.

he initial salary will be within the ge £7,190 — £9,425 on the urer salary scale.

rther particulars and applica- forms may be obtained from the f Officer, Heriot-Watt Univer- Chambers Street, Edinburgh 1HX, with whom applications ld be lodged not later than 29 ruary 1984, quoting ref /84. (1075)A

THE UNIVERSITY OF BRITISH COLUMBIA

RESEARCH ASSOCIATE

areer opportunity is available in field of regulation of phospha- lcholine biosynthesis. The essful applicant must have a , at least two years of post- oral work and experience in the fication of membrane-bound mes. Salary will be in the range 21,000 — $25,000, plus fringe fits. Deadline for application is ch 1, 1984.

bmit Curriculum Vitae, list of ications and the names of 3 rees to: Dr Dennis Vance, artment of Biochemistry, versity of British Columbia, couver, BC V6T 1W5 Canada. In rdance with Canadian immigra- requirements, priority will be n to Canadian citizens and anent residents of Canada. (NW234)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF GEOLOGY

Applications are invited for a

LECTURESHIP IN GEOPHYSICS

able from 1 September 1984. licants with research interests in area of geophysics will be sidered but the successful lidate will be required to devise teach a geophysics syllabus ropriate to the Hons. Geology ee course. He/she must also be ared to contribute to other ses at the first year level and to st with undergraduate field es.

lary is within the range £7,190 to 125 per annum.

urther details and application ns may be obained from the Staff ointments Officer, University of tingham, University Park, tingham NG7 2RD. Ref No 914. sing date for applications: 14 ruary 1984. (1065)A

QUEEN MARY COLLEGE

CHEMISTRY DEPARTMENT

HIGH FIELD (400 MHz) MULTINUCLEAR FT-NMR SERVICE

Technician Grade 5 required to assist in the operation of this University of London NMR Service (Bruker WH-400 Spectrometer). Experience in the operation of FT-NMR spectrometers preferred, but sound general experience with computer controlled instrumentation would be acceptable. Salary £7,529 to £8,582 per annum, inclusive of London Weighting. Flexi-time working required. 5 day, 37 hour week.

Written applications together with the names of 2 referees to be sent to Miss Garner, Personnel Assistant, Queen Mary College, University of London, Mile End Road, London E1 4NS, stating age, qualifications and previous experience. Closing date 10th February 1984. (1089)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF GEOLOGY

Applications are invited for a

LECTURESHIP IN SEDIMENTOLOGY

tenable from 1 September 1984. In addition to teaching sedimentology at all levels the successful candidate will also be required to contribute to other courses at the first year level and to assist with undergraduate field classes.

Salary is within the range £7,190 to £14,125 per annum.

Further details and application forms may be obained from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref No 913. Closing date for applications: 14 February 1984. (1064)A

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF PHYSICS

RESEARCH ASSOCIATE IN LASER RESEARCH

Applications are invited for two posts to work on gas lasers and new laser techniques. This project is part of a larger research programme into lasers and their applications. Applications are invited from candidates with PhD or MSc or with a first degree and post-degree experience.

Experience in fundamental or applied laser research would be an advantage. The posts are tenable immediately for a period of two years in the first instance. Salary range pa: £7,190 — £11,615 (Superannuation). More senior applicants may be considered for the post of Research Fellow (salary scale £10,710 — £14,125 pa).

Applications by letter, with curriculum vitae and the names of two referees should be sent to Dr T A King, Physics Department, The University, Manchester M13 9PL as soon as possible but not later than February 17th, 1984. (1066)A

NATIONAL UNIVERSITY OF SINGAPORE

DEPARTMENT OF BOTANY

Applications are invited for appointment to a

LECTURESHIP

in the Department of Botany

Candidates should have a PhD degree in plant and/or microbial genetics with good background or working experience in plant molecular biology, gene transfer and other aspects of plant biotechnology.

Preference will be given to candidates with both teaching and research experience in these fields.

Annual emoluments range from S$28,340 to S$58,750, the initial amount depending on the candidate's qualifications and experience.

Leave and medical benefits are provided. Under the University's Academic Staff Provident Fund Scheme, the staff member and the University are each required to contribute at the present rate of 23% of his salary, the staff member's contribution being subject to a maximum of S$920 a month. The sum standing to the staff member's credit in the Fund may be withdrawn when the staff member leaves Singapore/Malaysia permanently.

Other benefits include: a settling-in allowance of S$1,000 or S$2,000, subsidized housing at rentals ranging from S$100 to S$216 p.m., education allowance in respect of children's education subject to a maximum of S$12,000 p.a., passage assistance and baggage allowance for the transportation of personal effects to Singapore. Staff members may undertake consultation work, subject to the approval of the University, and retain fees up to the equivalent of 60% of annual gross salary in any one year. (STG£1 = S$3.00 approximately).

Application forms and further information on terms of appointment may be obtained from:

The Director
Personnel Department
National University of Singapore
Kent Ridge
Singapore 0511

NUS Overseas Office
5 Chesham Street
London SW1
United Kingdom
Tel: (01) 235 4562

(W688)A

ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

DEPARTMENT OF CLINICAL PHARMACOLOGY

PROTEIN CHEMIST CHARACTERIZATION OF HUMAN CYTOCHROMES P-450 SENIOR RESEARCH OFFICER

A postdoctoral person with experience of protein chemistry is required to join a multi-disciplinary team involved in the purification and characterization of human hepatic cytochromes P-450. The project will involve structural analysis of proteins by biochemical and immunological techniques. The post is supported for a period of 5 years with a salary in the range of £7,190 to £11,615 plus £1,186 London Allowance a year.

Further details of the research programme may be obtained from Professor D S Davies or Dr A R Boobis (Tel: 01-743 2030 ext 454).

Application forms and further particulars may be obtained from the Personnel Office, Royal Postgraduate Medical School, Ducane Road, London W12 0HS, quoting reference number 20/104.

Closing date: 16 February 1984. (1082)A

UNIVERSITY OF LONDON

British Postgraduate Medical Federation

THE CARDIOTHORACIC INSTITUTE LECTURER (NON-CLINICAL) IN ALLERGY AND CLINICAL IMMUNOLOGY

Applications are invited for a Scientific Assistant (Lecturer grade) under a Medical Research Council programme grant entitled "Allergy and the Eosinophil". Applicants should normally be under the age of 32 and have a PhD (or equivalent) in pharmacology, biochemistry, immunology or cell biology. The post is tenable for up to five years. Applications from scientists in industry, on secondment, are also invited. It is hoped that this post will be taken up as soon after 1st May as possible. Salary will be on the non-clinical lecturer scale currently £7,190 + £1,186 London Allowance, rising to £14,125 + £1,186.

Applications in duplicate in the form of a curriculum vitae giving full details of experience, and the names and addresses of two referees, should be sent to the Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP, from whom further details are available. The closing date is 1st March, 1984. (ACM2). (1070)A

UNIVERSITY OF PAPUA NEW GUINEA — PORT MORESBY

PROFESSOR OF HUMAN BIOLOGY

(P821001/84) (Department of Human Biology)
(Re-advertisement)

Applications are invited for the above post.

The Department of Human Biology comprises the pre-clinical disciplines of anatomy, physiology, biochemistry and pharmacology, and is responsible for the teaching of both undergraduate and postgraduate medical and dental students. The Department aims to present the basic medical sciences in an integrated fashion, with emphasis on clinical relevance. Clinical contact is introduced from the outset of the undergraduate course. An active programme of population-based and laboratory research is underway in the Department.

The successful applicant will have a distinguished academic record with one of the pre-clinical sciences as their main field of interest, but applications from clinicians with an especial interest in pre-clinical medical education will be particularly welcomed. A medical qualification would be a distinct advantage, although a person who is not medically qualified may apply. Teaching and/or research experience in a developing country would also be an advantage.

The appointee will provide academic leadership, participate in teaching in the basic medical sciences, and stimulate research. He will also promote the development of Papua New Guinean national academic staff. Opportunities will exist for maintaining links with clinical medicine by suitably qualified practitioners, and this would be welcomed in the interest of maintaining close correlation between the pre-clinical teaching programme and clinical practice. The Professor will not necessarily have any administrative responsibilities, although he can be elected to positions carrying such responsibilities.

Salary: K22,820 per annum, plus gratuity.

OTHER CONDITIONS: The successful applicant will be normally offered a contract for a three year appointment ending at the end of a semester. The gratuity entitlement is based on 24% of salary earned and is payable in instalments or a lump sum and is taxed at a flat rate of 2%. In addition to the salaries quoted above, the main benefits include: support for approved research; rent-free accommodation; appointment and repatriation airfares for appointee and dependants; financial assistance towards the cost of transporting personal effects to and from PNG: 6 weeks annual recreation leave with home airfares available after each 18 months of continous service; generous education subsidies for children attending schools in PNG or overseas; a salary continuation scheme to cover extended illness or disability. Applicants who wish to arrange secondment from their home institutions will be welcomed. The terms and conditions are currently under review.

Applications will be treated as strictly confidential and should include a full curriculum vitae, a recent small photograph and the names and addresses of three referees. In order to expedite the appointment procedure, applicants are advised to ask their referees to send confidential reports directly to the University without waiting to be contacted.

Applications quoting position number, should be forwarded to the Assistant Secretary (Staffing), University of Papua New Guinea, PO Box 320, UNIVERSITY, Papua New Guinea, to reach him no later than *31 March 1984*.

Candidates in the UK should also send a copy of their application to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W692)A

ZOOLOGICAL INSTITUTE
UNIVERSITY OF BASEL
SWITZERLAND

TWO ASSISTANTS IN EVOLUTIONARY ECOLOGY

required, starting 1 April 1984, or as soon as possible thereafter. Qualifications: doctorate, 2-3 years of postdoctoral experience, ability to teach in German. One position will be in ethology and sociobiology, the other in some other area — e.g., life-history evolution, sex-allocation theory, community ecology. Main duties: undergraduate course in area of expertise, participation in field course, development of independent research programme. Period of employment: 3-5 years. Applications with CV, publicationslist and names of referees to: Erziehungsdepartement, Personalabeteilung I, Münsterplatz 2, CH-4051 Basel, Switzerland. (W683)A

UNIVERSITY
OF CAMBRIDGE
CAVENDISH LABORATORY

SENIOR ASSISTANT IN RESEARCH

(Electron Microscopy)

The Laboratory expects shortly to be able to fill the above University post for duties involving the supervision of new applications of analytical electron microscopy as well as the design and commissioning of improved equipment. Excellent research facilities and technical support are available. The appointment is for a five year period but is renewable and carries a stipend in the range £8,530 to £10,710 pa.

Potential applicants should write to Dr A Howie, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE from whom further information can be obtained. (1092)A

HOUGHTON POULTRY RESEARCH STATION

GRANT-AIDED BY THE AGRICULTURAL & FOOD RESEARCH COUNCIL

IMMUNOLOGIST

The Institute is seeking an Immunologist in the Microbiology Department to provide immunological input to a multi-disciplinary project on the use of the techniques of genetic manipulation to produce novel vaccines for poultry. Specific duties will be to examine the local and systemic immune response of the chicken to viral and other antigens presented to mucosal surfaces but the appointee will also be expected to develop an interest in other immunological problems that are being investigated on the Station.

Applicants should have a first or upper second class honours degree in a biological subject and preferably a PhD, and will have had several years' post-graduate experience in immunology. At least 2 years relevant post graduate research experience is required for HSO grading; 4 years for SSO.

The appointment will be made in the Higher Scientific Officer or Senior Scientific Officer grade, according to experience. Salaries HSO: £7,149 x 6 to £9,561; SSO: £8,970 x 7 to £11,476.

For further details and application form please contact the Secretary, John Andrews, at Houghton Poultry Research Station, Houghton, Huntingdon, Cambs PE17 2DA. Tel St Ives (0480) 64101. (1088)A

THE UNIVERSITY OF MUNICH

POSTDOCTORAL POSITION IN NEUROBIOLOGY

Applications are invited for a postdoctoral scientist to work on the localization of transmitters in the extrapyramidal system using mainly immunocytochemical techniques. Experience in EM and/or behavioural methods would be an advantage.

Position funded by the German Research Association (BAT II a, appr. DM 60 000 p.a. before tax) is available immediately for 1 year with possible extension for further two years.

Send complete curriculum vitae and 3 letters of reference to: PD Dr C. Nitsch, Anatomische Anstalt, Pettenkoferstr. 11, D-8000 München 2, West-Germany. (W636)A

FRESHWATER BIOLOGICAL ASSOCIAT

VACANCY FOR PHYSIC LIMNOLOGIST AT WINDERMERE

Applications are invited fo year post in physical limn work on exchanges of ene matter across water-atmosphere water-sediment interfaces transport of dissolved and partic matter in lake ecosystems. Pote candidates should possess at le good honours degree in ph and/or mathematics and sh ideally have some experienc environmental data collection processing.

Salary and conditions of se are analogous to those of Scie Officer (£5,682 — £7,765) or Hi Scientific Officer (£7,149 — £9 in the Civil Service, gross depending on qualifications experience.

Full details and application f from the Secretary, Freshwater logical Association, The F House, Ambleside, Cumbria L 0LP (tel: 09662 2468). Closing 24th February 1984. (1084

CHIEF, NATIONAL GEODETIC SURVEY DIVISION

The National Oceanic Atmospheric Administra (NOAA) announces Senior Exec Service Vacancy, for Chief, Nat Geodetic Survey Division, Nat Ocean Service, Locatio Rockville, Maryland. Duties in providing scientific and tech management of a large and graphically dispersed scie organization dedicated development, improvement maintenance of the Nati Geodetic Reference System.

The pay rate for this SES pos will be commensurate with ground and experience (actua rate to be determined).

Apply to NOAA/NOS, Executive Boulevard, Rock Maryland 20852, Attn: PER1 Standard Form 171's mus received by 2/3/84 to be consid Equal Opportunity Employer. (NW246

UNIVERSITY OF CALIFORNIA
Berkeley

POSTDOCTORAL RESEARCH

Applications are invited from persons having a PhD in Biochemistry or related fields for postdoctoral positions in the laboratories of Drs Bruce Ames, Lester Packer and Martyn Smith. These positions are sponsored by the National Foundation for C Research. Research will be dir towards understanding the ro oxygen radicals in cancer and a Preference will be given to can with expertise in HPLC.

Please send curriculum vitae M T Smith, BEHS, School of P Health, University of Califc Berkeley, CA 94720. Telephone 642-8770. The Universit California is an Affirmative A Equal Opportunity Employer. (NW225

UNIVERSITY OF NATAL
DEPARTMENT OF BIOCHEMISTRY
PIETERMARITZBURG
SOUTH AFRICA

ications are invited from ly qualified persons regard- s of sex, religion, race, colour national origin, for appointment to the post of

PROFESSOR

e Department of Biochemistry. Department is responsible for teaching of Biochemistry to lents in both the Faculty of nce and Agriculture. The post become vacant on 1st January 5.

salary will be in the range ,882 — R33,885. This includes a pensionable allowance.

commencing salary notch will lependent on the qualifications or experience of the success- pplicant. In addition, an annual ice bonus of 93% of one nth's salary is payable, subject reasury regulations.

lication forms, further particu- of the post and information on sion, medical aid, group rance, staff bursary, housing and subsidy schemes, long e conditions, and travelling nses on first appointment are inable from Mr G J Henning, th African Universities Office, chester House, 278 High born, London WC1V 7HE, with m applications, on the cribed form must be lodged not than 9th March, 1984 quoting ence PMB 6/84.

(W684)A

THE UNIVERSITY OF ALBERTA
BONE STOMORPHOMETRIST SSISTANT/ASSOCIATE PROFESSOR
($29,720 — $41,820; $37,420 — $53,658)

lications are invited for a ion in a Bone Research Group as e Histomorphometrist. The ssful candidate must have MD, , and/or PhD degree, and post- oral research experience in the of quantitative bone histo- hometry.

e University of Alberta is an l opportunity employer, but in rdance with Canadian igration requirements this rtisement is directed to lished investigators who are idian citizens or permanent ents. This position will be ed in the long term through an nal agency, but the incumbent enjoy all the privileges of the sity academic rank at which pointment is made.

didates should forward their culum vitae, an outline of their rch interests, and names of three nces to: Dr T R Overton, rtment of Applied Sciences in cine, 10-102 Clinical Sciences, ersity of Alberta, Edmonton, rta, T6G 2G3, before 31 March

(NW242)A

MEDICAL RESEARCH COUNCIL LABORATORIES
The Gambia, West Africa

Research Assistant in Clinical Immunology

Applications are invited for a post of research officer in clinical immunology at the Medical Research Council Laboratories. Candidates will be directly involved in a research project run by the Director and in charge of a small clinical laboratory using radioimmunoassay and ELISA techniques. The successful candidate must have a relevant degree or the AIMLS or equivalent qualification followed by at least 2 years non-routine laboratory work and previous experience in clinical immunology.

MRC
Medical Research Council

An initial contract of 2 years will be offered. Salary will be determined by qualifications and experience.

Further details and applications forms may be obtained from Miss K. Douglas. The closing date for applications is 14 February 1984. (W686)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
POSTDOCTORAL RESEARCH FELLOW
(Immunology Department)

Required for 2 year CRC funded project to work on the in vitro immune response of lymphocytes from patients with Chronic Lymphocytic Leukaemia. Applicants should have a background in Immunology or Biochemistry with tissue culture experience. Initial salary up to £7,630 pa plus £1,186 pa London allowance.

Further details and application form from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace SW17 0RE. Closing date 22nd February. (1083)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF MEDICINE
MEDICAL LABORATORY SCIENTIFIC OFFICER

to assist in a Medical Research Council supported project to investigate the metabolism of vitamin D in health and in clinical disorders of calcium homeostasis. The work will include isolation of the metabolites by high pressure liquid chromatography and their measurement by radioimmunoassay.

Applicants should have a good Honours Degree in Biological Sciences. The appointment is for three years commencing early 1984. Salary is in the range of £7,140 — £7,434 (including London Allowance), according to age and experience.

Applications (including telephone numbers) with details of two referees to: professor J L H O'Riordan, Department of Medicine, The Middlesex Hospital, Mortimer Street, London W1N 8AA.

(1059)A

GRADUATE Research Assistant funded by the Cancer Research Campaign required to work on the pharmacokinetics of cancer chemotherapy in man in the joint departments of Clinical Pharmacology and Medical Oncology with particular reference to analytical and electrochemical methods. The post is available immediately, for two years, with the possibility of a third year. Salary scale: £6,310 — £7,190 plus £1,186 per annum London Allowance, and superannuation. Apply in writing to the Deputy Secretary, United Medical and Dental Schools, Guy's Hospital Medical School, London Bridge, London SE1 9RT, quoting Ref. MO1. (1062)A

POST-DOCTORAL POSITIONS

Two post-doctoral positions are currently available in the area of cardiovascular research.

One position is available for an applicant with a background in pharmacology and smooth muscle research and will involve the functional characterization of alpha adrenoceptors in vascular smooth muscle.

The second position is for someone with a neuroscience/anatomy background and an interest in pursuing projects related to a number of morphological aspects concerning cardiovascular control mechanisms (brainstem and spinal cord structure and function, peripheral autonomic innervation, role of substance P in cardiovascular formation, and structure of the heart and blood vessels).

The annual salary, depending on experience, will commence at $18,000, and partial re-location expenses are available.

Send curriculum vitae, statement of research interests, and the names of three academic references to: Dr C R Triggle, Associate Dean of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3V6.

(NW232)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following position:

FACULTY OF SCIENCE
DEPARTMENT OF PHYSICS
RESEARCH FELLOW

The appointee will be required to participate in and supervise the research with free-piston shock tunnel and shock tube facilities. The research interests include: high enthalpy hypersonic flows, non-equilibrium processes in these flows, shock-propogation, and problems in fundamental atomic physics and astrophysics related to shock heated gases.

The appointee should have a PhD and experience in a field relevant to the above research.

During 1984 the appointee will be required to participate in the teaching within the Department and in fields related to the above research.

The appointment is to 31 December 1986.

Closing Date: 28 February 1984.

Conditions of appointment: Salary will be in accordance with qualifications and experience within the range: Research Fellow $A23,394 — $A30,734. Current exchange rate is $A1 = UK62p.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia, or to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before submitting applications.

(W696)A

Medical Research Council Laboratories
The Gambia

Immunologist

Applications are invited from post-doctoral immunologists for a post at the MRC Laboratories, Fajara, The Gambia. The successful candidate for this post will join a group studying the immune response to malaria in man and he or she will be encouraged to study some of the soluble factors produced by human mononuclear and adherent cells which control the growth of malaria parasites *in vitro*. Previous experience in biochemistry and immunochemistry is, therefore, required.

Virologist

Applications are invited for a post of virologist at the MRC Laboratories, Fajara, The Gambia. The successful applicant for this post will join a team embarking on a new study of acute respiratory infections in Gambian children and he or she will be responsible for viral isolations and identification. Previous experience of respiratory virus isolation is required.

An initial contract of 2 years will be offered. Salary will be determined by qualifications and experience.

MRC
Medical Research Council

Applications should be submitted to Miss M. Grenham from whom further details may be obtained. Medical Research Council, 20 Park Crescent, London W1N 4AL. The closing date for applications is 29th February, 1984.

(W694)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PHYSIOLOGY

LECTURER

Applications are invited fo above post available fro September 1984.

Previous experience of experimental techniques of micropuncture or renal micr fusion in vitro would be an adva both in relation to research an teaching duties of the post.

Salary on the scale for Lect (£7,190 — £14,125) according t qualifications and experience.

Informal enquiries may be ma Professor B R Jewell (tel 431751 ext 7267).

Application forms and fu particulars may be obtained fro Registrar, The University, Leed 9JT quoting reference no 104/ Closing date for applica 20 February 1984. (109

BIRKBECK COLLEGE
(UNIVERSITY OF LONDON)
SERC DARESBURY LABORATORY

JOINT APPOINTMENT OF POSTDOCTORAL RESEARCH FELLOW IN PROTEIN CRYSTALLOGRAPHY

Applications are invited from protein crystallographers who have recently completed their PhD's for a five-year joint appointment. The successful applicant will be responsible for developing the use of synchrotron X-radiation for the study of protein crystals with especial emphasis on high resolution data collection and solution of the phase problem using anomalous dispersion techniques, with particular regard to projects developed at Birkbeck College by the Molecular Biology Laboratory of the Department of Crystallography. The successful applicant will need to be prepared to spend his/her time equally between Birkbeck where he/she will be responsible for preparation of crystals and development of the analyses, and Daresbury where X-ray data will be collected and experimental techniques developed. When at Daresbury, the successful applicant will be a member of the group responsible for providing general support to protein crystallographers using the synchrotron radiation source.

Initial salary, according to age and experience, will be between £8,376 pa and £9,716 pa including London Weighting.

Applications including CV and names of two referees should be sent to the Assistant Secretary (Personnel), Birkbeck College, Malet Street, London, WC1E 7HX, England. (1080)A

UNIVERSITY OF WARWICK

POSTDOCTORAL FELLOWSHIP

Applications are invited for a two year postdoctoral fellowship to study the synthesis and metal ion coordination chemistry of new polyazamacrocyclic ligands. Salary on the Research Range IA scale: £7,190 — £11,615 pa.

For further details contact Dr P Moore, Department of Chemistry and Molecular Sciences, University of Warwick, Coventry CV4 7AL. (1051)E

UNIVERSITY OF ABERDEEN
DEPARTMENT OF BIO-MEDICAL PHYSICS AND BIO-ENGINEERING

LECTURESHIP IN MODERN ELECTRONICS

Internationally recognised Department seeks Lecturer with interests in modern electronic techniques and their applications to teach and practice electronic design, particularly digital, and to join in the activities of a well equipped electronics workshop designing, fabricating and servicing modern electronics in bio-medical research and routine hospital work.

Opportunities for research. Additional experience in eg vacuum, high frequency, microwave and high voltage technology helpful but not essential.

Salary on Lecturer scale (£7,190 — £14,125 per annum) with appropriate placing.

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 Copies) should be lodged by 17 February 1984. (1054)A

POST DOCTORAL RESEARCH ASSOCIATE

to conduct experiments in which neurotransmitters and enzymes are measured in experimental animals or human volunteers after being subjected to a variety of nutritional conditions including trace element deficiencies. Requires PhD in Biochemistry with experience in enzymology, neurochemistry and HPLC. Prefer one-two years post doctoral experience. Anticipated beginning date for appointment is May 1, 1984. Salary $15,0 $17,000 plus benefits, one appointment with possibilit renewal.

Send CV, names of references, and relevent publica to: Ms Alice Hoffert, H Nutrition Research Center, PO 7166, University Station, Forks, ND 58202.

An Equal Opportunity/A ative Action Employer. (NW23

UNIVERSITY OF BATH
SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH OFFICER

required to prepare monoclonal antibodies to identify lipases of yeast and bacterial origin in foods. Applicants for this project, funded by MAFF, should be postdoctoral or have equivalent experience and have a background in biochemistry or microbiology with practical experience of monoclonal antibody production. The appointment will be for one year in the first instance with the possibility or renewal for a further year.

Salary up to £8,530 pa according to qualifications and experience.

Application forms and further particulars from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting ref. No. 84/16. Closing date: 22.2.84. (1095)A

UNIVERSITY OF CINCINNATI MEDIC CENTER

PHYSIOLOGIST — ENDOCRINOLOGIS

To work on structure-fu relationships in gastrointestin neuronal peptides using sy partial sequences and analogu measuring pancreatic and secretion, in collaboration research team which in synthetic chemists and bioch The work will be also suppor immunometric analyses. position is for three years beg as soon as possible after A 1984.

Applicants should s curriculum vitae and nam referees to Dr R F Murphy, D ment of Surgery, Univers Cincinnati Medical Cente Bethesda Avenue, Cincinnati 45267. (NW2

THE UNIVERSITY OF TASMANIA

HAIR OF BIOCHEMISTRY

plications are invited for appoint- it to the Chair of Biochemistry, ch became vacant on the irement of Professor E S dsworth.

pplications are invited from didates with an established itation in research in any field of hemistry and experience in hing and administration.

he present professorial salary is 8,997 pa. In addition, a ically qualified appointee will be led to receive an appropriate ing.

ndidates who wish to apply ld in the first instance write to Registrar, University of nania, Box 252C, GPO, Hobart, nania, Australia 7001, or to the etary-General, association of mmonwealth Universities ts), 36 Gordon Square, London H 0PF, to obtain a detailed ement of the conditions of intment and application forms. lications close on 30 March 1984. (W691)A

C CLINICAL RESEARCH CENTRE
Northwick Park Hospital)
Watford Road, Harrow,
Middx HA1 3UJ

DIVISION OF IMMUNOCHEMICAL GENETICS

lications are invited for a 3 year doctoral appointment in the m of immunochemical genetics tudies on the genes coding for syltransferase enzymes involved e biosynthesis of carbohydrate ens on cell surfaces and in ted glyco-proteins. This is an sion of the areas of research at nt under investigation in the ion and it is essential that the cant has previous experience in a e of recombinant DNA niques and in RNA and DNA encing. Opportunities are able for close interaction with cular geneticists working in divisions in the clinical research e.

lary scales are closely related to for university non-clinical emic staff and will depend on age experience. In addition to the salary there will be a London ance of £1,186 a year.

didates who wish to apply d send a curriculum vitae and names of two referees to Dr fred M Watkins, Division of unochemical Genetics, MRC al Research Centre, Watford , Harrow, Middlesex HA1 3UJ n three weeks of the appearance he advertisement. Reference /4053. (1074)A

Marketing Opportunities in Animal Health

Continued expansion of our animal health business has led to the creation of three challenging new positions for which we now seek applications.

Product Executives

Two people required to assist the Marketing Manager in extending our share in the market for a wide range of veterinary pharmaceuticals and biologicals. Candidates must show the ability to provide a comprehensive marketing resource and to draw up and implement effective marketing plans.

Product Development Executive

To assist the Product Development Manager in preparing data and dossiers for new products for the animal health market. Some years of marketing experience in industry with a qualification in marketing or a relevant university degree are a pre-requisite for all positions.

The company is a multi-national and a leader in this field offering an attractive salary, contributory pension scheme with free life assurance, free membership of P.P.P. and a generous allowance for anyone who has to relocate. The location is Milton Keynes where housing is readily available for purchase or rent.

For an application form write or telephone A. Forrest, Personnel Manager, Hoechst UK Limited, Walton Manor, Walton, Milton Keynes, MK7 7AJ. Telephone 0908 665050.

Hoechst

(1094)A

INSTITUTE OF CHILD HEALTH
University of London

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a post-doctoral research fellow to join a small, multi-disciplinary highly motivated, group working on early post-natal structural development of the pulmonary vasculature with reference to changes observed during the development of pulmonary vascular disease in children with congenital heart disease. Practical experience in immunohistochemical and immunofluorescent techniques is essential. The position is available for two years. Initial salary £8,376 per annum (inclusive of London Weighting) in accordance with University Scale 1A for Academic Related Research Staff.

Applicants should send curriculum vitae and the names of two referees to Dr Sheila G Haworth, Department of Paediatric Cardiology, The Hospital for Sick Children, Great Ormond Street, London WC1. (1067)A

ST MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Norfolk Place,
London W2 1PG
DEPARTMENT OF CLINICAL PHARMACOLOGY

POST-DOCTORAL RESEARCH FELLOW (NON-CLINICAL) IN CARDIOVASCULAR PHARMACOLOGY

The appointment is for one year in the first instance, with a possible extension to a maximum of three years.

The research programme involves the investigation of drug actions on isolated human blood vessels. Applicants with previous experience in adrenergic and/or cardiovascular pharmacology will be preferred.

Salary range: £6,310 — £8,530 + £1,186 London Allowance.

Applications and names of two referees should be sent to the Assistant Secretary (Personnel) at above address no later than 3 February 1984. (998)A

UNIVERSITY OF EDINBURGH
DEPARTMENT OF CLINICAL SURGERY
Applications are invited for the post of
DATA MANAGER/ COORDINATOR

for a research project within the University Department of Clinical Surgery. Applicants need not be medically qualified, but should have a scientific training and knowledge of, or experience in, the medical field.

Salary will be at an appropriate point on the scale £7,190 — £10,250. A full-time appointment would be preferred, but part-time would be considered. The appointment will be for one year in the first instance.

Further particulars are available from Professor A P M Forrest, University Department of Clinical Surgery, The Royal Infirmary, Edinburgh EH3 9YW, with whom applications, (2 copies) including curriculum vitae and the names of two referees, should be lodged not later than 17th February, 1984.

Please quote reference no 7042. (1063)A

STATE OF KUWAIT
UNIVERSITY OF KUWAIT
FACULTY OF MEDICINE — HEALTH SCIENCE CENTRE

Advertisement

CHIEF TECHNICIAN IN THE DEPARTMENT OF PHARMACOLOGY

Applications are invited for the post of Chief Technician in the Department of Pharmacology. Candidates should have experience in modern analytical techniques, especially Gas-Chromatography and Mass-Spectrometry and hold the F.I.M.L.S., or equivalent qualification, with fifteen years' experience including training.

Salary will be in the range KD 450-512 per month, (KD 1 = £1.8, US$ 3.5, approx.). There is no income tax in Kuwait and currency is transferable without restriction. Free, furnished, air-conditioned accommodation is provided and electricity and water supplied free of charge. Sixty days paid annual leave for each completed year of employment, and annual economy class return air tickets to the country of citizenship or permanent residence are provided for the appointee, spouse and three dependent children. Free medical treatment is available under the State Health Service.

Applications should be submitted to: The Dean, Faculty of Medicine, University of Kuwait Health Science Centre, PO Box 24923 Safat, Kuwait, with detailed curriculum vitae in duplicate, recent passport photograph, and the names of three referees, to arrive no later than 15th February 1984.
(W689)A

THE UNIVERSITY OF MANITOBA
FACULTY OF MEDICINE
has positions available as

ASSISTANT PROFESSOR

These positions are available in response to the development of a multi-disciplinary program in the area of recombinant DNA technology as applied to neurosciences or endocrinology. The successful applicant(s) will be expected to develop an independent research program utilizing recombinant DNA techniques.

Send curriculum vitae including a brief outline of future research interest to Dr. Henry G. Friesen, Professor and Head, Department of Physiology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba R3E 0W3 Canada. (NW225)A

UNIVERSITY OF OXFORD

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT — 1A
(Ref: B/R/97)

Required to work with Dr. E. A. Newsholme on the metabolic basis for changes in insulin sensitivity in muscle and other tissues. This is a 3 year appointment supported by a grant from the MRC. Experience in metabolic studies advantageous.

Salary on the scale £7,630 p.a. — £8,530 p.a.

Applications, with names and addresses of two referees to: Dr. E. A. Newsholme, Department of Biochemistry, South Parks Road, Oxford OX1 3QU. (1085)A

KING'S COLLEGE HOSPITAL DENTAL SCHOOL
Denmark Hill, London SE5

Department of Oral Pathology
HISTOPATHOLOGY

A Vacancy has arisen for a Medical Laboratory Scientific Officer to participate in routine histopathology.

Good facilities exist for becoming acquainted with a wide range of modern morphological techniques including histochemistry and electron microscopy. Salary on scale £5,491 — £7,741 + £1,042 London Weighting.

Further details from Mrs M Ayling, tel: 01-274 6222 ext 2861.

Closing date 20th February 1984.
(1057)A

ASSISTANTSHIPS

UNIVERSITY OF OXFORD — DEPARTMENT OF THEORETICAL PHYSICS

POSTDOCTORAL RESEARCH ASSISTANTSHIP IN THEORETICAL ASTROPHYSICS

An SERC-funded postdoctoral position in theoretical astrophysics is available in the Department of Theoretical Physics from 1 October 1984. This temporary appointment will be for a period of not more than three years. Candidates who are interested in working on the internal dynamics of galaxies, or on the formation and evolution of galaxies, including their interaction with their environments, should send a curriculum vitae, a list of publications and a synopsis of any unpublished work to Dr. J. J. Binney, Department of Theoretical Physics, 1 Keble Road, Oxford OX1 3NP, by 29 February 1984, and arrange for two letters of recommendation to reach him by that date. (1073)P

GRANTS and SCHOLARSHIPS

UNIVERSITIES FEDERATION FOR ANIMAL WELFARE
UFAW VACATION SCHOLARSHIPS FOR 1984

Applications are invited from undergraduates in agricultural, biological, medical and veterinary sciences for financial support to undertake supervised research projects lasting from 4 to 6 weeks, exceptionally 8 weeks, during UK academic vacations. Heads of department, lecturers etc who believe they can find suitable candidates for particular projects may also submit applications.

Projects should have relevance to improving the welfare of farm, laboratory, companion or wild animals and be agreed by the proposed supervisor at the University or College etc where the work will be carried out. Closing date 30 March 1984.

Awards will be £38 per week subsistence allowance and £12 per week departmental expenses.

Application form from: UFAW(VS1), 8 Hamilton Close, South Mimms, Potters Bar, Herts. EN6 3QD. (1071)H

INSTITUTE OF CHILD HEALTH
University of London
ELECTRONMICROSCOPY RESEARCH ASSISTANT

Applications are invited from graduates with a good honours degree in biological sciences for a research post in electronmicroscopy in the Department of Paediatric Cardiology. The position is for one year in the first instance. Postgraduate experience in electronmicroscopy, both practical and theoretical is essential. Initial salary will be according to age and experience within University Scale 1B for Academic Related Research Staff £7,496 — £8,376 per annum (inclusive of London Weighting).

Applicants should send curriculum vitae and the names of two referees to Dr Sheila G Haworth, Department of Paediatric Cardiology, The Hospital for Sick Children, Great Ormond Street, London, WC1. (1068)A

FELLOWSHIPS

UNIVERSITY OF BIRMINGHAM AND SERC DARESBURY LABORATORY
CENTRE FOR MATERIAL SCIENCE AND DEPARTMENT OF PHYSICS
TWO POST-DOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for two p available from April 1 1984 o 3-year SERC supported Rese Programme: "Electronic Struc and Properties of Rare Earth Me and Compounds".

The project will involve collat ation between the Centre Materials Science and the Dep ment of Physics, University Birmingham and SERC Daresb Laboratory.

One post will require an exp mentalist. The successful appli will be a member of staff of Centre for Materials Science and be required to conduct angle-reso photoemission measurements in Centre and at the Daresb Laboratory.

The second post is for theore work. It will be concerned with b structure calculations of the earth metals and their applica both for the interpretation of photoemission data and other tronic properties, in cooperation the band structure group at Daresbury Laboratory. The suc ful applicant will be a member o Physics Department of Birmingh but will be expected to sp approximately equal amounts of in the Department and at Daresb

Applicants should have a PhD be about to submit a thesis) in Phy or an allied subject. Relev experience with photoelect spectroscopy, band the techniques or rare earths, etc w be an advantage.

Salary on the Research Fellow scale £7,190 — £11,615 plus su annuation.

For further particulars ph 021-472 1301 ext 2559, quo reference X1.

No formal application form. T copies of application, including Curriculum Vitae and naming t referees to Assistant Regist (Science and Engineering), PO 363, Birmingham B15 2TT by February 1984. (1061

Continued on pages 13, 14

SEMINARS and SYMPOSIA

St George's Hospital Medical School Cell Surface Research Fund

The second annual Symposium will be held on Friday 13 April 1984 at St George's HMS, London SW17 on

THE ELECTRICAL PROPERTIES OF THE CELL SURFACE AND NEUROLOGICAL DISEASE

Chairmen: Lord Adrian FRS (Cambridge) & J. Newsom-Davis (Royal Free HMS, London)

Speakers: S. Bevan (UC, London), J. O. Dolly (Imp. Coll, London), A. de Hemptinne (Ghent), D. A. Heyden FRS (Cambridge), J. S. Kelly (St George's HMS, London), R. McBurney (Newcastle), C. A. Pasternak (St George's HMS, London) and T. A. Sears (Inst. Neurol., London)

Intending participants are invited to present posters at the meeting: brief abstracts should be sent before 1 March to Mrs V. Marvell, Department of Biochemistry, St George's HMS, London SW17 0RE; Tel: 01-672 1255, ext 4733, from whom further information can be obtained.

The Symposium forms part of the UNESCO Cooperative Research Programme in Biomaterials and Biotechnology in the European and North American Region.

The Symposium will be preceded by a Cell Surface Research Fund — UNESCO advanced training course on

MODERN METHODS FOR MEASURING MEMBRANE AND SURFACE POTENTIALS

from Monday 9 April — Thursday 12 April 1984 at St George's HMS

Instructors: C. L. Bashford, T. B. Bolton, J. M. Graham & J. S. Kelly

Course fee, inclusive of full board and lodging from Sunday 8 April — Friday 13 April (incl. attendance at the Symposium), £250

Financial assistance towards travel and other costs for applicants from outside UK is available.

Application forms and other information before 1 March from Dr C. L. Bashford, Department of Biochemistry, St George's HMS, London SW17 0RE, Tel: 01-672 1255 ext 5012

(1079)M

THE JOHNS HOPKINS SCHOOL OF HYGIENE SYMPOSIUM

Johns Hopkins Center for natives to animal testing is soring its 3rd annual symposium progress report from the center ternative methods in toxicology. Symposium will be held in more on October 23-24, 1984. encourage the submission of ers presentations. To be placed ne mailing list or for instruction posters please contact Ms ara J Williams, Secretary to the tor, The Center for Alternatives Animal Testing, The Johns kins School of Hygiene & Public th, 615 North Wolfe Street, more, Maryland 21205.

(NW235)M

POINTMENTS WANTED

rtise your qualities and qualifi- ns through the most influential ce weekly in the world at a al reduced cost of *25p a word*. onal Box Numbers £1). rtisements must be pre-paid and to: Nature Classified (Appts 4 Little Essex Street, London R3LF. (890)B

TDOCTORAL Scientist ish) age 34 seeks post in Drug ery (Oncology/Antiviral/Im- ology fields). Box 1058 c/o re, 4 Little Essex Street, London R3LF. (1058)B

FRENCH SCIENTIFIC
is looking for a situation in US, MD, Epidemiologist, Statistician, Data Analysist,
Please answer to Dr J F Phillippon 15, Route du Velodrome, Nouméa, New Calédonia, South Pacific.

(W690)B

FELLOWSHIPS *continued*

ESF PROGRAMME OF RESEARCH FELLOWSHIPS IN TOXICOLOGY (PGT)

Short-term (up to 6 months) and Long-term (6 to 12 months) Research Fellowships (deadline: 30 June 1984) and Short-term (up to 1 month) Visiting Fellowships (no deadline) are offered to scientists working in European laboratories to go to laboratories in other European countries. No condition of age, level or field of training, provided the project contributes to furthering research in TOXICOLOGY in Europe.

Information and application forms can be obtained from: Dr Bernard Munsch, European Science Foundation, 1 quai Lezay-Marnesia, F-67000 STRASBOURG, France; tel. (88) 35.30.63; telex 890440.

(W701)E

Friedrich Miescher-Institut, Basel, Switzerland
POSTDOCTORAL FELLOWSHIP IN MOLECULAR NEUROBIOLOGY

Applications are invited for an internally funded fellowship, of three years maximum duration, to work on the expression of neuron-specific proteins in developing brain using recombinant DNA techniques. A good training and demonstrated ability in molecular biology are required. The Institute provides outstanding facilities in a purely research environment. **Inquiries should be addressed to Dr. A. Matus, Friedrich Miescher-Institut, PO Box 2543, CH-4002, Basel, Switzerland.** (W700)E

E M B O

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1984 AWARDS

Next deadline: February 16, 1984

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is February 16, 1984. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on April 27, 1984.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany. (W531)E

EMBL

European Molecular Biology Laboratory

PREDOCTORAL FELLOWSHIPS

The European Molecular Biology Laboratory is to award a limited number (6-8) of predoctoral fellowships for graduates wishing to study for a PhD at EMBL Heidelberg or its outstations at DESY, Hamburg or ILL, Grenoble. The areas of research in Heidelberg include membrane biology, cellular differentiation, analysis of the structure of membrane proteins and protein nucleic acid complexes and the structure and regulation of genes in procaryotes and eucaryotes. At the EMBL outstations the research centres on the elucidation of biological structures using either synchrotron radiation (Hamburg) or neutron beams (Grenoble).

To be eligible, candidates must hold, or anticipate receiving in 1984, a first or upper second class honours degree, a masters degree or a diplom in biological sciences, physical sciences or mathematics. Preference will be given to candidates from the member states of EMBL and EMBC namely: Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom.

Short listed candidates will be asked to visit EMBL (travel expenses reimbursed) for an interview. Those awarded predoctoral fellowships will be expected to begin working at EMBL in September 1984. The normal duration of the fellowships will be 3 years subject to annual review.

The closing date for completed applications is February 1st 1984. Successful candidates will be notified by the end of April 1984.

Application forms and a catalogue giving further information about the research at EMBL and the groups participating in the Laboratory's predoctoral programme is available on request from Dr J. Tooze, Predoctoral Programme, EMBL, Postfach 10.2209, 6900 Heidelberg, F.R. Germany. (W511)E

DEPARTMENT OF EDUCATION, DUBLIN

POST-DOCTORAL RESEARCH FELLOWSHIPS 1984/85

Applications are invited for research fellowships in science (excludin medical and social science), engineering and architecture tenable in an one of the following institutions in Ireland:-

University College, Dublin.
University College, Cork.
University College, Galway.
Trinity College, Dublin.
St. Patrick's College, Maynooth.
The National Institute for Higher Education, Dublin.
The National Institute for Higher Education, Limerick.
An Foras Talúntais
(The Agricultural Institute), Dublin 4.
The Institute for Industrial Research and Standards, Dublin 9.
An Foras Forbartha, Dublin 4.
The Colleges of Technology, Kevin St. and Bolton St., Dublin.

Fellowships will be tenable for a period of two years with the possibilit of an extension for a third year.

Applicants should hold the degree of PhD or have equivalent researc experience. Applications will be considered from engineers, architect or technologists with suitable industrial experience. Candidates mus be under the age of 30 years and preferably under the age of 28 years or 31 March, 1984.

Appointments will be effective from a date not earlier than 1st October 1984. The initial salary, which will depend upon qualifications an experience, will normally be within the range IR£6,961 to IR£7,876 pe annum, but awards of up to IR£9,097 may be made in the case o engineers, architects and technologists whose industrial experience o other special circumstances warrant it.

Full particulars of the scheme including application forms ma be obtained from the Secretary, Department of Education (Headquarters Section 3), Marlboro' Street, Dublin 1, to whom applications for fellowships should be forwarded. The latest date for the receipt of applications is 31st March, 1984. (1091)E

LSB LEAKEY FOUNDATION RESEARCH FELLOWSHIP FIELD STUDIES OF THE GREAT APES

The Fellowship will be awarded to an individual to facilitate the establishment of a long-term research project on a wild population of great apes. Preference will be given to studies with significance for understanding human origins and to those which explore the causes of variation in ape behaviour, particularly in previously unstudied habitats. The Fellowship will begin on October 1, 1984 and the award, worth up to $20,000, is for field expenses only.

Applicants should submit a curriculum vitae and one page statement of research goals by April 1, 1984 to the L S B Leakey Foundation, Foundation Center 1-7, Pasadena, CA 91125. Eligible candidates will be asked to provide further details by July 1. (NW244)E

INTERNATIONAL UNION OF BIOCHEMISTRY FELLOWSHIPS

for attendance at the 13th International Congress of Biochemistry

Amsterdam, The Netherlands, 25-30 August 1985

IUB and the Organizing Committee of the 13th IUB Congress will together make available awards to assist younger biochemists who wish to attend the Congress. Primary consideration will be given to residents of countries where the practice of the science of biochemistry is in the early stages of development.

The Fellowships will support part of the cost of travel (normally up to a maximum of half the listed economy air fare or 70% of the Apex fare, whichever is lower) and subsistence during the Congress. The Organizing Committee of the Congress will waive the registration fee for Fellowship holders.

Applications should include the following information:

1. Name and date of birth; 2. Citizenship, residence; 3. Place of work, including full postal address; 4. Nature of work; 5. List of publications; 6. Do you intend to present a contribution at the 13th Congress? If so, indicate its tentative title; 7. Support available or expected to be available to the applicant; 8. Names of three referees, one of whom should, if possible, not live in the applicant's country of residence.;

This notice should be shown to the referees, and applicants must ask them to write to Dr. R. L. Hill, at the address below, to reach him by 15 August 1984.

Applications must be made in triplicate and as early as possible, arriving no later than 15 August 1984, to Dr. R. L. Hill, Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710, USA.

It is hoped to make known the decisions of the Selection Committee by 1 December 1984. (NW137)E

UNIVERSITY OF ABERDEEN

DEPARTMENT OF MICROBIOLOGY

TWO POSTDOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for two postdoctoral Research Fellowships to join the group led by Professor W A Hamilton working on the role of sulphate reducing bacteria.

One post (ref Mic 1) financed by the SERC Marine Technology Directorate will be concerned with a study of corrosion processes associated with steel structures in the offshore oil industry, and will attempt to relate microbiological activities of biofilms with the electrical polarisation characterisation of naturally fouled steel surfaces.

A second post (ref Mic 2) is financed by BP Petroleum Development and is designed to strengthen basic continuous culture studies on the enzymology and physiology of growth of sulphate reducing bacteria on hydrogen.

Appointments will be from 1 March 1984 and will be for a minimum of one year at this stage.

Salary within range £7,190 to £8,530 per annum, with appropriate placing, within IA Scale for Research and Analogous Staff.

Further particulars and application forms from The Secretary. The University, Aberdeen, with whom applications (2 copies), specifying references no(s), should be lodged by 10 February 1984. (1019)E

QUEEN MARY COLLEG

University of London

APPLIED MATHEMATICS DEPARTMENT

Theoretical Astronomy Un

Applications are invited for 1 or 2 year

RESEARCH FELLOWSHI

(with possibility of extension fo year). The core staff of the comprises 6 permanent membe the Academic Staff (Prof. Roxbu Drs MacCallum, Papaloiz Rowan-Robinson, Schwartz, Williams) while 4 other s members are engaged in rel research activities.

A Royal Society Research Fel several postdoctoral Assista visitors and research students are involved in the Unit, where rese interests span a wide range inclu solar and stellar physics, solar sy dynamics, interplanetary pla physics, interstellar mat extragalactic astrophysics cosmology, relativity gravitation. The Unit is also invo in the analysis of IRAS data.

Initial salaries in range £8,3 £10,161 pa (1 post may be at a senior level).

Applications, enclosin curriculum vitae, list of publicat description of recent and pla work and names of 2 referees, sh reach the College Secretary, Queen Mary College, Mile Road, London E1 4NS, by February. (1069

IIVERSITY OF READING

:PARTMENT OF PHYSIOLOGY & BIOCHEMISTRY

TWO RESEARCH FELLOWSHIPS

lications are invited for two post-toral University Research wships. The appointments will n the areas of (i) Molecular gy/Genetics: candidates should a background in molecular or atic cell genetics and should be rested in applying these niques to fundamental problems nimal growth and development mportance in agriculture. R2A).

leuropeptides: candidates should a background in the identifi-n and quantification of neuro-e peptides and an interest in ying these techniques to under-ding their role in neuro-crinology and neurophysiology. R3A).

ie Fellows will be expected to lop an independent research ramme within the structure of ting research groups. Both ointments will be for a fixed term hree years to start as soon as ible. Starting salary up to £9,425 SS superannuation.

pply, quoting appropriate Ref for application form to onnel Office, University of ding, Whiteknights, Reading 2AH. Further details can be ined in this way or by contacting R R Dils (telephone 0734 875123 600). (1055)E

IIVERSITY OF WARWICK POSTDOCTORAL FELLOWSHIP

lications are invited for a two postdoctoral fellowship to study nthesis and metal ion coordina-chemistry of new polyazamacro-c ligands. Salary on the Research ge IA scale: £7,190 — £11,615 pa.

or further details contact Dr P re, Department of Chemistry Molecular Sciences, University arwick, Coventry CV4 7AL. (1051)E

Atlas Research Fellowship

Pembroke College, Oxford University

The Rutherford Appleton Laboratory of the Science and Engineering Research Council in conjunction with Pembroke College, invite applications for a joint research fellowship. The aim of the Atlas Fellowship is to encourage studies, particularly those involving computers, in an area of research supported by the Rutherford Appleton Laboratory.

Topics include the following: study of condensed matter by scattering of neutron beams; some aspect of information technology including software engineering, IKBS, use of electron beam lithography in the fabrication of semi-conductor devices, image processing, computer aided design including electrical circuit design; finite element calculations; electro-magnetic systems; theoretical studies of the atmsophere and climate; remote sounding from space; image processing related to space astrophysics data reduction.

The successful candidate will be expected to undertake research work and have ideas on the programme he/she wishes to follow. Fellows have the rights and privileges of research fellows of Pembroke College, including the possibility of some teaching, and will be expected to spend the greater part of the time at Rutherford Appleton Laboratory.

There will be access to exceptionally powerful computer facilities, and our involvement with numerous university research groups gives an opportunity for contact with others engaged on a wide range of exciting research programmes.

The fellowship will be tenable for a period of three years and may be extended up to a period not exceeding five years. The stipend will depend upon the age and experience of the candidates selected, within the broad range £9,500 to £14,800.

For details of research fellows' rights at Pembroke College and application forms, write to Recruitment Office Personnel Group, Rutherford Appleton Laboratory, Chilton, Didcot, Oxon OX11 0QX, or telephone Abingdon 0235 445435 quoting reference No: VN 200.

The closing date for applications is: 17th February 1984 (1053)E

serc Rutherford Appleton Laboratory

UNIVERSITY OF READING

DEPARTMENT OF BOTANY

POSTDOCTORAL RESEARCH FELLOWSHIP

(Plant Cell Biology)

Applications are invited for a University postdoctoral Research Fellowship to investigate either gene expression during gametogenesis or molecular interactions involved in plant self-incompatibility mechanisms. Experience in cell biology is essential, and some familiarity with immunosystems would be advantageous. This fixed term, three year Fellowship will commence as soon as possible. Starting salary in range £7,190 — £9,425 pa plus USS.

Apply, quoting Ref R4A, for application form to Personnel Office, University of Reading, Whiteknights, Reading RG6 2AH. (1056)E

CALIFORNIA INSTITUTE OF TECHNOLOGY

POSTDOCTORAL FELLOWSHIP AT THE PALOMAR OBSERVATORY

A postdoctoral fellowship is available in the Astronomy Department, California Institute of Technology. The successful candidate will be expected to devote 50 percent of their time to work on the new Polomar Sky Survey which is to commence in early 1985. Duties would include: helping in establishing procedures for the survey; making observations for the Sky Survey for a few nights each month; quality control of the exposed plates. The Fellow would be expected to spend the other 50 percent of his or her time on science associated with the Survey. Applicants should have a good knowledge of astronomical photography and be familiar with some field of astronomy appropriate to the Survey.

The initial appointment is for 1 year, renewable for a further 2 years and starting October 1, 1984. The starting salary is $22,000. Applications should be sent by March 15, 1984 to: W. L. W. Sargent, Department of Astronomy 105-24, California Institute of Technology, Pasadena, CA 91125. Applications should include a curriculum vitae, bibliography of publications and preprints of manuscripts not yet published. Candidates should arrange for three letters of recommendation to be sent directly to Caltech.

Applicants will be considered without regard to race, religion, sex, national or ethnic origin, or non-disqualifying handicaps, in accordance with existing laws and regulations. Caltech is an Equal Opportunity Employer. (NW238)E

MAX-PLANCK-INSTITUT FUER ZUECHTUNGSFORSCHUNG COLOGNE

POST-DOCTORAL FELLOWSHIP IN MOLECULAR GENETICS

A post-doctoral molecular geneticist, preferably with experience in studying at gram-negative bacteria, is required to investigate the molecular basis of competition amongst rhizobium leguminosarium stains.

Position is available for a fixed term at two years beginning April, 1984 and carries a salary related to that at German Government employes.

Applications including a curriculum vitae, a full statement of research interests and experience, and the names of two references should be sent without delay to Dr. W. J. Broughton, Max-Planck-institut Fuer Zuechtungsforschung, D-5000 Koeln 30, FRG. (W685)E

CONFERENCES and COURSES

CHELSEA COLLEGE
(University of London)

MSc DEGREE IN BIOCHEMISTRY, IMMUNOLOGY, PHARMACOLOGY, BIOPHYSICS AND BIOENGINEERING

The following courses, leading to MSc by examination, will commence in October 1984.

MSc GENERAL BIOCHEMISTRY
2 academic years part-time (2pm-9pm, 1 day/week) or 1 year full-time. Candidates should be honours (first or second class) graduates in Chemistry, Pharmacy or a Biological Science, or hold a degree in Medicine or Veterinary Medicine.

MSc IMMUNOLOGY
2 academic years part-time (10am-5pm 1 day/week). Candidates should be honours graduates in a Biological Science, or hold a degree in Medicine or Veterinary Medicine.

MSc PHARMACOLOGY
1 academic year full-time. Candidates should be honours (first or second class) graduates in Science, Pharmacy or Medicine.
This course has been accepted by the SRC for the tenure of Advanced Course Studentships.

MSc BIOPHYSICS AND BIOENGINEERING
2 academic years full-time or 3 years part-time. Candidates should be honours graduates in Science, Engineering or Medicine.
This course has been accepted by the MRC for the tenure of Advanced Course Studentships.

For further information and application forms, write to the Secretary of the relevant department, Chelsea College, Manresa Road, London SW36LX. (1076)C

CHARLES COULSON SUMMER SCHOOL IN THEORETICAL CHEMISTRY

will be held at the Unitersity of Oxford from 9 — 19th September 1984.
The lecturers will be Dr. P. W. Atkins, Dr. D. L. Cooper, Professor N. H. March (Oxford) and Dr. N. C. Handy (Cambridge).
Further details and application forms can be obtained from Dr. P. J. Grout, Theoretical Chemistry Department, 1 South Parks Road, Oxford OX1 3TG.
Closing date for applications 1st June 1984. (1072)C

JAPANESE BIOTECHNOLOGY STUDY TOUR

An in-depth series of site visits, conferences and seminars with some of Japan's leading industrial scientists and executives in biotechnology.

This exclusive study tour is designed to give western scientists and executives an opportunity to become acquainted with the Japanese marketing and research capacity. The mission will be led by Christopher G. Edwards, Editor in Chief of BIO/TECHNOLOGY, and organized by the Technology Transfer Institute. Visits will be conducted throughout Japan from March 31–April 15.

For further information, contact: Technology Transfer Institute—New York, One Penn Plaza, Suite 1411, 250 W. 34th St., New York, N.Y. 10119. 212-947-2648 telex 420057TTIUI. Attention: Mr. Hashizume.

(NW174)C

STUDENTSHIPS

SOUTH MANCHESTER HEALTH AUTHORITY

CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE

STUDENTSHIPS FOR TRAINING IN RESEARCH

Five Cancer Research Campaign financed studentships will be awarded in these multidisciplinary laboratories for full time studies leading to higher degrees in research fields relevant to cancer. Successful applicants will work in one of the following divisions; biophysical chemistry, radiobiology, experimental chemotherapy, carcinogenesis, cell biology, experiment haematology, cell and tissue kinetics, ultrastructure, immunology and molecular biology.

Awards will be tenable for three years from 1 October 1984, with a tax-free grant of £2,880. Applications are invited from candidates who have or expect to obtain a first or upper second class honours degree.

Applications, which should include a detailed curriculum vitae and the names of three academic referees, should be sent to the Director, The Paterson Laboratories, Christie Hospital and Holt Radium Institue, Wilmslow Road, Withington, Manchester M20 9BX (Tel. 061-445 8123 ext 400) as soon as possible and in any case not later than 1 March 1984. (1087)F

UNIVERSITY OF LEICES

DEPARTMENT OF PHYSIC

Applications are invited fo

RESEARCH STUDENTSH

tenable from October 1984 leading to the degree of PhD i following areas.

CONDENSED MATTER PHYSICS
IONOSPHERIC PHYSICS
NUCLEAR ELECTRONIC
QUANTUM MOLECULAI PHYSICS
RADIATION PHYSICS
THEORETICAL PHYSICS
X-RAY ASTRONOMY

The studentships provide opportunity to join an ac department engaged in researc topics of fundamental technological interest. They suitable for graduates with a honours degree (I or IIi) in Phy Chemistry, Mathematics Engineering.

Applications, which sh include the names of two refe should be sent to Dr C. Nc Department of Physics, Universi Leicester, Leicester LE1 7RH. (1060

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMIST

SERC STUDENTSHIP I MICROBIOLOGICAL BIOCHEMISTRY

Applications are invited f graduates with a first or upper sec class honours degree for an av immediately available and ten for three years. The succes applicant will be involved in a s of the regulation of extracel toxin formation in Staphyloc aureus.

Applications giving detail academic record and the name two referees should be sent to I Coleman, Department of Bioc mistry, Nottingham Unive Medical School, Queen's Me Centre, Nottingham NG7 2UH. (1090

POSITIONS WANTE

PhD organic chemistry, MSc or technology, four years postdoc experience in organic, pep enzyme syntheses; solid backgr in modern synthetic and analy methods; several publications. S permanent position, prefer organic synthesis. Will relo Reply to PO Box 12842, Lexing Ky 40583-2842. (NW237

MISCELLANEOUS

SPONSORS to match small fu for private laboratory, hu cerebral electrophysiology; I EPogram, TRFC (Başar, 198 Hz in Alzheimer's; basic rese Southeast USA. 912-452 2537. (NW23

Typesetting: J.W. Arrowsmith Ltd, Bristol and Outline Graphics Ltd. London EC4Y 0HP. Printing: Kingsdale Press Ltd, Reading, Berks, in association with Godfrey Lang Ltd. London WC1R 4E and The William Byrd Press Inc., Richmond, Virginia, USA Published by MACMILLAN JOURNALS LTD, 4 Little Essex Street, London WC2R 3LF. — 26 January 1984.



nature is keeping ahead. Are you being left behind?

research results, the very latest international science s, key papers by the world's leading scientists and d of publication ensure *Nature* remains one of oremost weekly scientific journals in the world.

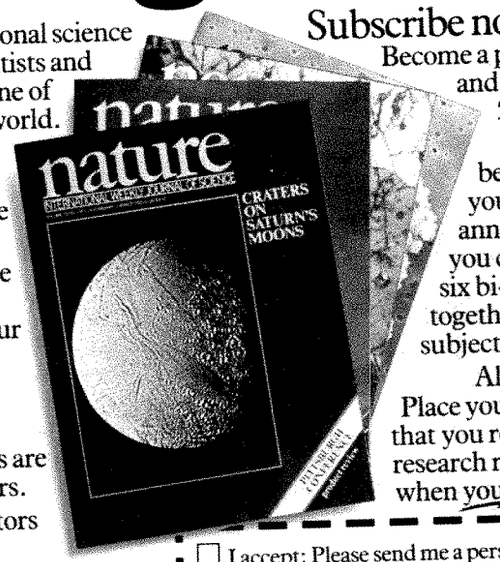
But if you're tied in to a circulation list ork, all this vital weekly information will oubtedly reach you late, often incomplete sometimes not at all.

With your own personal copy, you solve roblem. Every week you'll have all the research results and science news in your is within days of publication:

- **s** reports from throughout the world of ce and technology.
- **ews and Views** section, where new ideas are sed and discussed by leading researchers.
- **les and Letters** in which key investigators ose their work for the first time.
- **Reviews** – Eminent figures offer expert judgments wide variety of recent publications.
- **ial Issues** providing an in-depth treatment of topics portance.
- **luct Reviews** – Detailed descriptions of laboratory oment newly available.
- **sified Advertisements** for a wide variety of positions er the world.

You will no longer be dependent upon other people down the 'list' copy, and because you will natically receive every issue, you won't risk missing le copy through absence from work. Moreover, you all this key information for permanent reference.

rough your door every week



Subscribe now – at Half Price

Become a personal subscriber now and you will receive a discount of 50% off the full rate.

All 51 weekly issues will be delivered to the address of your choice. You can start your annual subscription with any issue you choose. And you will receive six bi-monthly author indexes, together with the annual index of subject and author.

All this for just 94p a week.

Place your order now and make certain that you receive the scientific news and research results crucial to your work when you want them.

☐ I accept: Please send me a personal subscription to *Nature* for one year (51 issues) at the special half-price rate of £49.00.

☐ I enclose my personal cheque for £49.00 made payable to *Nature*. (Note: Institutional cheques cannot be accepted at this special rate).

☐ Please charge to my credit card account.
Access/American Express/Visa/Diners Club.

Account No._____

Card Expiry Date_____

Signature_____

Name_____

Address_____

_____

_____

_____

Date_____ 7B18

Please return this form to:
Circulation Manager
Nature
Brunel Road, Houndmills
Basingstoke
Hants RG21 2XS

This offer applies to UK and North America only. Please allow approximately 4 weeks for delivery of the first issue in your subscription year.

nature

Circle No.90 on Reader Service Card.

Don't waste your time with inadequate alkaline phosphatases

order Phosphatase, alkaline

special quality for molecular biology, 1000 U, Cat. No. 713023

from Boehringer Mannheim

advantages:

★ **easy to use straight from the vial**
★ **efficient removal of 5'-phosphates**
★ **guaranteed free of exonucleases**
★ **guaranteed free of endonucleases**
★ **guaranteed free of nicking activities**
★ **guaranteed free of RNases**
★ **precise termination of activity**

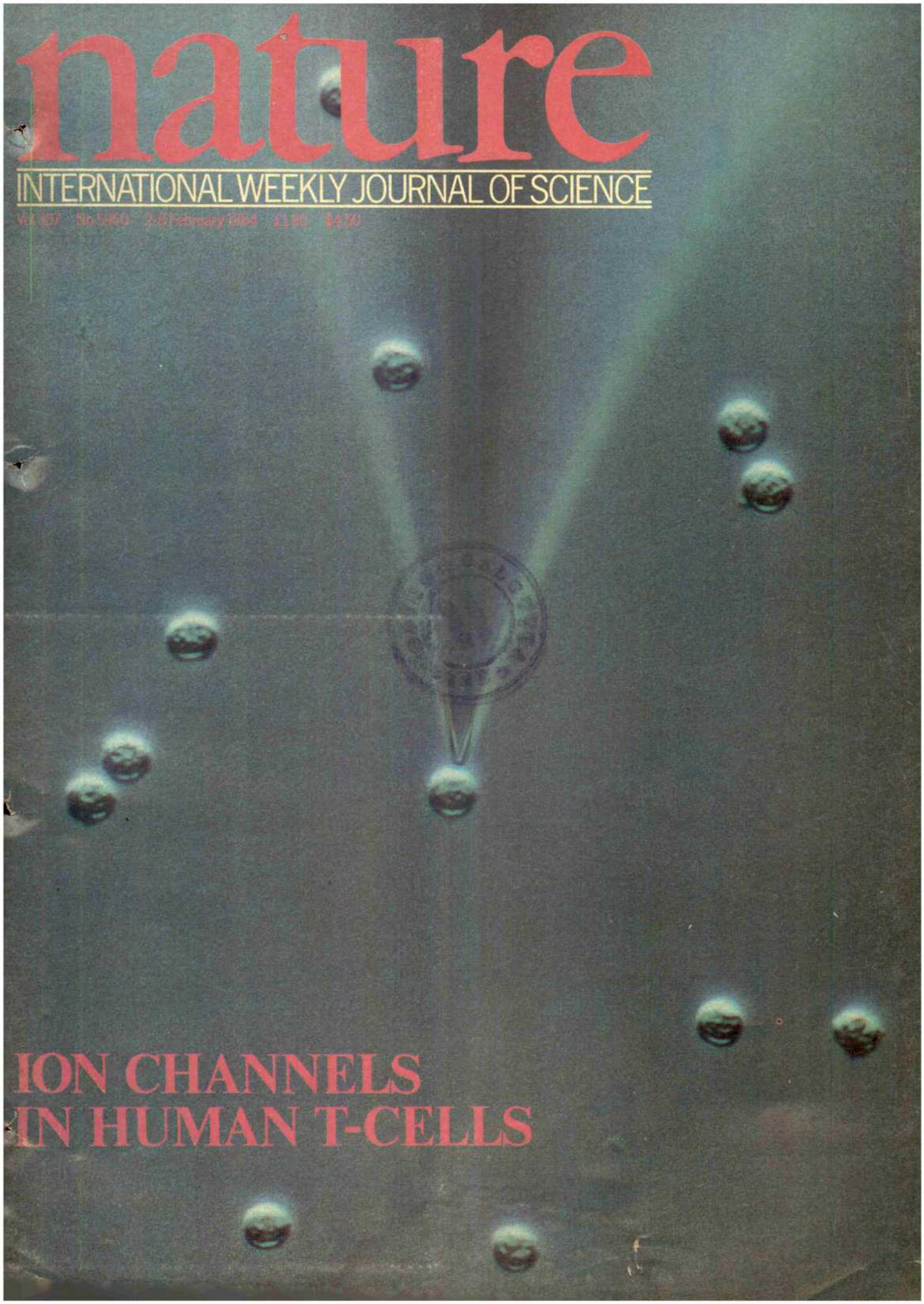
Please contact your local representative or

mannheim boehringer

Boehringer Mannheim GmbH
Biochemica
P.O. Box 310120
D-6800 Mannheim
W. Germany

Circle No.09 on Reader Service Card.

nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Vol 307 No 5950 2-8 February 1984 £1.90 $4.50



ION CHANNELS IN HUMAN T-CELLS

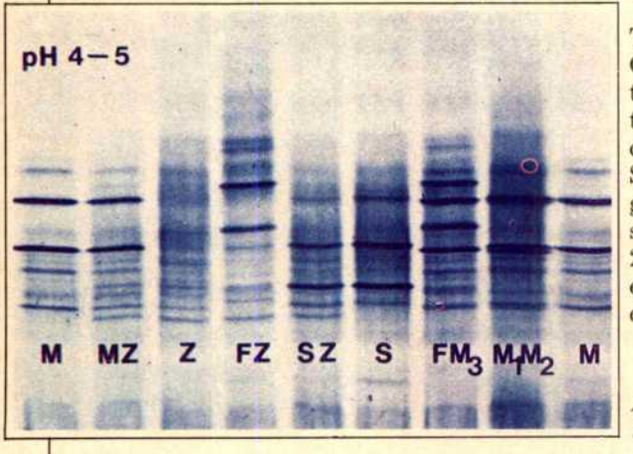
Only with the new LKB Horizontal

Unequalled flexibility i

READY-TO-USE GELS FOR MAXIMUM CONVENIENCE

All the owner of the LKB Horizontal Electrophoresis System needs to cover a wide range of electrofocusing applications is a supply of the popular and successful LKB Ampholine PAGplates. These allow the researcher to avoid the time and trouble involved in casting gels in the laboratory, while guaranteeing both high resolution and excellent reproducibility. As many as 96 samples can be separated simultaneously on a single PAGplate, enabling users with high workloads to spend their time screening samples instead of casting gels.

Eight phenotypes of α_1-antitrypsin are clearly identified using one of the convenient LKB Ampholine PAGplates ▼



ULTRATHIN GELS FOR MAXIMUM ECONOMY

Anticipating the need for less expensive electrofocusing gels, LKB developed the new UltroMould: a simple device designed to cast bubble-free gels as thin as 0.1mm in less than 30 seconds. Based on a patented sliding plate method, UltroMould is easy to use, producing perfectly accurate and uniform gels and ensuring a high standard of reproducibility. Adding UltroMould to the LKB Horizontal Electrophoresis System means that significantly less gel solution and smaller sample volumes are required, resulting in far more economical electrofocusing.

LKB UltroMould casts bubble-free ultrathin gels in only 30 seconds, making gel casting quicker, easier and more certain ▶



THICK GELS FOR MAXIMUM CAPACITY

Original electrofocusing techniques used thicker gels, and even today you may still wish to work this way in order to achieve higher capacity. The LKB Horizontal Electrofocusing System lets you easily cast and run Ampholine gels up to 2 mm thick, allowing much greater sample volumes to be analyzed successfully. The 2 mm thick gels are also ideal if you are new to electrofocusing and are following a procedure described in the literature.

After the thick gel has been cast, the sample is introduced with the help of the sample application foil ▶



This is one in a series of advertisements describing the unrivalled versatility of the LKB Horizontal Electrophoresis System. For further information about analytical electrofocusing, and the many other applications of the system, send today for our brochure.

THE LKB SYSTEM

Only LKB can offer the research scientist a complete, integrated system in which each component works with and complements all the others. Based on the new and improved Multiphor II, the system also includes a 5KV power supply, thermostatic circulator, laser densitometer and data evaluation software.

In addition to the instrumentation, LKB provides a range of 18 major kits plus a complete programme of high quality chemicals, supplies and accessories, backed by the full worldwide support of the market leader in electrophoresis and electrofocusing.

LKB—the electrophoresis experts.



trophoresis System do you also get...

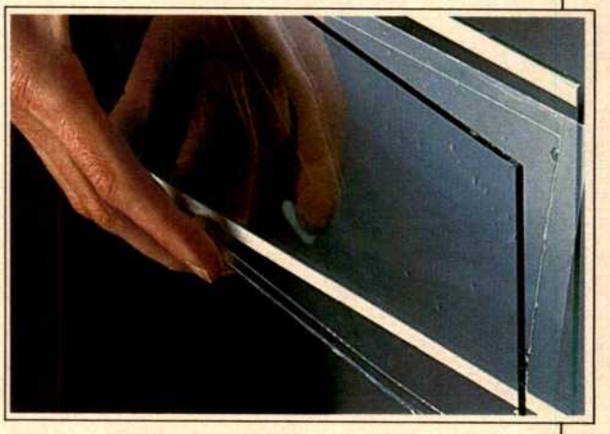
nalytical electrofocusing



AGAROSE GELS FOR MAXIMUM POROSITY

Another of the many options available to the user of the LKB Horizontal Electrophoresis System is the application of Agarose electrofocusing gels. LKB supplies extremely pure agaroses, carefully developed for electrofocusing, and the original capillary casting method simplifies the task of producing 0.5 mm gels on GelBond Film backing sheets. Agarose is non-toxic and easy to handle and the entire analysis procedure, including casting and running, is very rapid.

Separating the GelBond Film from the glass plate gives a 0.5 mm agarose gel, firmly attached to its support and ready to use ▶

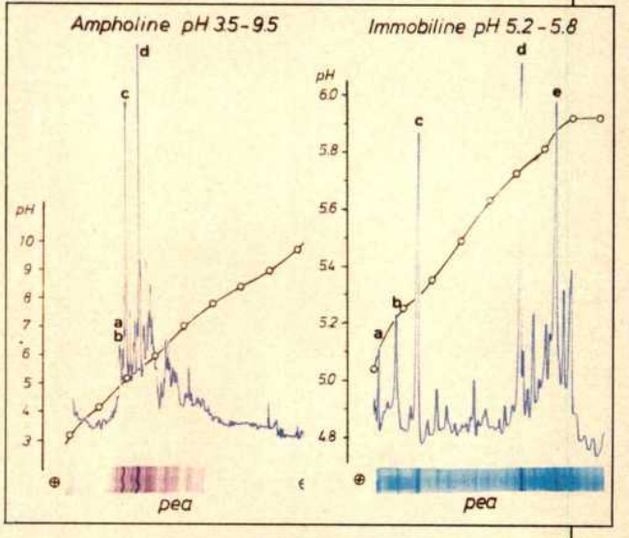




IMMOBILINE GELS FOR MAXIMUM RESOLUTION

The unique LKB Immobiline System is significantly different to traditional gels in that its pH gradient is an integral part of the gel. The system is designed to allow the user to select a pH gradient as wide as 3.0 pH units, or as narrow as 0.1 pH units, anywhere in the range 3.6 to 9.5. This tailoring of the gradient to suit specific applications permits extremely high resolution to be achieved with the LKB Horizontal Electrophoresis System. Stable and reproducible linear gradients having a slope as low as 0.01 pH units/cm are possible, allowing you to separate proteins whose pI values differ by as little as 0.001 pH units.

Comparison of traditional electrofocusing and LKB Immobiline System (courtesy of Dr A Görg et al, Technische Universität, München) ▶

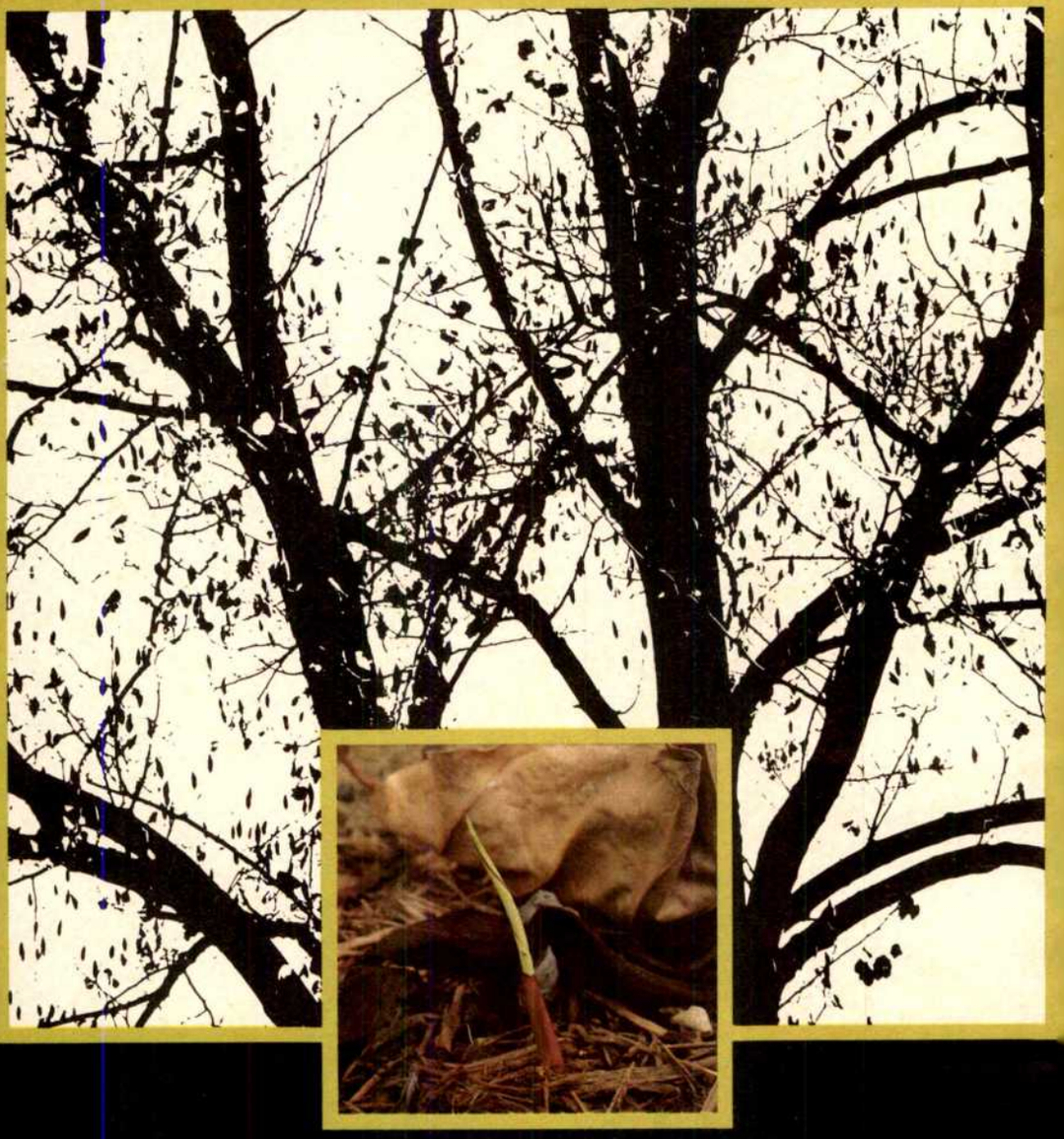




LKB
BROMMA

Head office:
LKB-Produkter AB,
Box 305, S-16126 Bromma, Sweden.
Tel. 08-98 00 40, telex 10492
Main US sales office:
LKB Instruments, Inc.
9319 Gaither Road, Gaithersburg,
Maryland 20877.
Tel. 301-963-3200,
telex 909870 (dom.), 64634 (intern.)
UK sales office:
LKB Instruments Ltd.,
232 Addington Road, S. Croydon,
Surrey CR2 8YD. England.
Tel. 01-657 8822, telex 264414
Other sales offices in:
Antwerp, Athens (for Middle East), Bangkok,
Copenhagen, Lucerne, Moscow, Munich,
Paris, Rome, Turku, Vienna, Zoetermeer

Circle No.40 on Reader Service Card.



an Emerging Technology

technology is emerging. Its seeds are in
d cell biology, microbiology, biochemistry and
. Rooted in the experience and knowledge of
isciplines, this new technology embraces ad-
ry area of life science research.
he search for a fuller understanding of the
of life, researchers are using tools and methods
many areas of science, lessons learned and
gh years - sometimes decades - of experience.
sted lessons form the core of an evolving
at will live through many generations, pro-
rs that otherwise would have been out of reach.
grated technology takes shape, a new company,
in experience, is emerging to serve it:

Life Technologies, Inc. At the company's GIBC
sion, we bring to the life scientist years of experti
respective fields, GIBCO in cell and tissue cultur
microbiology, BRL in molecular biology. Togetl
vide the firm, fertile ground on which science ca
most comprehensive selection of life science rese
products available.

To learn more about GIBCO/BRL and how
helping a powerful new technology grow, ask f
of the booklet, "GIBCO/BRL: Products for
an emerging technology."

GIBCO/

GIBCO/BRL is a division of LIFE T

nature

NATURE VOL. 307 2 FEBRUARY 1984

COVER



Human T lymphocytes, showing one sealed on to a patch electrode. This technique is used to study the function of potassium channels on pages 465 and 468.

OPINION

Snapshot of a new industry 399
Binary is divisive 400

NEWS

US defence research 401
Ariane launch delay
OTA biotechnology report 402
Technical innovation 404
China's research
Netherlands energy policy 405
It's an ill wind . . .
Polish science
Spare parts wanted
Genetic manipulation 406
European air pollution
Wolf winners 407
US space station
US material science

CORRESPONDENCE

Scientists at symposia/Creationism/ Page charges/etc. 408

NEWS AND VIEWS

New twist for anthropic principle
John Maddox 409

A test of ideas about mutualism
Robert M May 410
Last gape of the Tasmanian tiger
Jon Darius 411
The end of the message and beyond
Nick Proudfoot 412
Morphogenesis by fibroblast traction
Julian Lewis 413
Ahmad Bukhari 1943–1983
Neville Symonds 414
Multiple forms of mitochondrial DNA in higher plants
Thomas D Fox 415
Palaeobathymetry from sinking shells
Peter J Smith 416
IRAS circular 7

BOOK REVIEWS

The Higher Education System: Academic Organization in Cross-National Perspective *by B R Clark*
Eric Ashby 483
The Macmillan Dictionary of Archaeology *R D Whitehouse ed.*
Colin Platt 484
Certain Philosophical Questions: Newton's Trinity Notebook *by J E McGuire and M Tamny*
C W Kilmister 485
Weak Interactions of Leptons and Quarks *by E D Commins and P H Bucksbaum*
C H Llewellyn Smith 486
Principles of Fluorescence Spectroscopy *by J R Lakowicz*
F W J Teale

REVIEW ARTICLE

The molecular biology of immunoglobulin D
F R Blattner & P W Tucker 417

ARTICLES

Palaeoanthropological discoveries in the Middle Awash Valley, Ethiopia
J D Clark, B Asfaw, G Assefa, J W K Harris, H Kurashina, R C Walter, T D White & M A J Williams 423

Origin and evolution of the Mediterranean vegetation and climate in Europe
J-P Suc 429

Construction, expression and recognition of an H-2 molecule lacking its carboxyl terminus
C Murre, C S Reiss, C Bernabeu, L B Chen, S J Burakoff & J G Seidman 432

Tripartite structure of the *Brassica campestris* mitochondrial genome
J D Palmer & C R Shields 437

LETTERS TO NATURE

On the far-infrared excess of Vega
P M Harvey, B A Wilking & M Joy 441

$^{15}N/^{14}N$ ratio of dissolved N_2O in the eastern tropical Pacific Ocean
N Yoshida, A Hattori, T Saino, S Matsuo & E Wada 442

Rare earth element fluxes and geochemical budget in the eastern equatorial Pacific
K Murphy & J Dymond 444

Apparent changes in the climatic state of the deep North Atlantic Ocean
D Roemmich & C Wunsch 447

Suspended particulate organic material from hydrothermal vent waters at 21°N
P B Comita, R B Gagosian & P M Williams 450

Experimental manipulation of a contact guidance system in amphibian gastrulation by mechanical tension
N Nakatsuji & K E Johnson 453

Forskolin activation of adenylate cyclase *in vivo* stimulates nerve regeneration
S L Kilmer & R C Carlsen 455

Acceptors for botulinum neurotoxin reside on motor nerve terminals and mediate its internalization
J O Dolly, J Black, R S Williams & J Melling 457

L-Glutamate has higher affinity than other amino acids for [^{3}H]-D-AP5 binding sites in rat brain membranes
H J Olverman, A W Jones & J C Watkins 460

Magnesium gates glutamate-activated channels in mouse central neurones
L Nowak, P Bregestovski, P Ascher, A Herbet & A Prochiantz 462

Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis?
T E DeCoursey, K G Chandy, S Gupta & M D Cahalan 465

Contents continued overleaf

Nature (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Annual Index (mailed in February). Annual subscription for USA and Canada US $230 (for subscription prices elsewhere, see next page). USA and Canadian orders only to: *Nature*, Subscription Dept PO Box 1018, Manasquan, New Jersey 08736, USA. All other orders to *Nature*, Brunel Road Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY10010 and additional mailing offices. US Postmaster send form for 3579 to: *Nature*, 15 East 26 Street, New York, NY 10010. Published in Japan by Macmillan Shuppan K.K., 5th Floor Eikow Building, 1-10-9 Hongo, Bunkyo-ku, Tokyo, Japan. 1984 Macmillan Journals Ltd.

Specifically labelled ^{125}I-peptides

These peptides are purified by HPLC to provide

- increased sensitivity in RIA and receptor studies
- high specific activity
- more stable tracers
- increased consistency and reliability

Compatible antisera are also available

● **CCK-8 (SO_4), ^{125}I-labelled with Bolton and Hunter reagent**	IM.159
Antiserum	N.1591
● (3-[^{125}I]*iodotyrosyl*^{27})**ß-Endorphin**	IM.162
Antiserum	N.1621
● (3-[^{125}I]*iodotyrosyl*^{10})**Glucagon**	IM.160
Antiserum	N.1601
● (3-[^{125}I]*iodotyrosyl*^{3})**Neurotensin**	IM.163
Antiserum	N.1631
● (3-[^{125}I]*iodotyrosyl*^{11})**Somatostatin, Tyr^{11}**	IM.161
Antiserum	N.1611
● **Substance P, ^{125}I-labelled with Bolton and Hunter reagent**	IM.157
Antiserum	N.1571
● (3-[^{125}I]*iodotyrosyl*^{10})**VIP**	IM.158
Antiserum	N.1581

Please contact Amersham or your local representative for more details

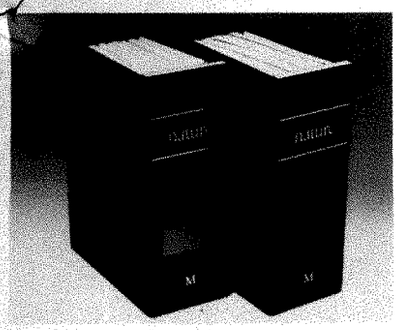
Amersham International plc
Amersham England HP7 9LL
telephone Little Chalfont (024 04) 4444
telex 83141 ACTIVA G

Amersham

Amersham Australia PTY Limited Sydney **Amersham Belgium SA/NV** Brussels **Amersham Buchler GmbH & Co. KG** Braunschweig W. Germany
Amersham Corporation Arlington Heights USA **Amersham Denmark ApS** Birkerød **Amersham France SA** Paris **Amersham Japan** Tokyo **Amersham Nederland BV** Utrecht

Circle No.50 on Reader Service Card.

Keep your copies of *Nature* protected in these tough black PVC binders



- Each binder is supplied with a set of wires for the easiest possible loading
- *Nature* logo blocked in gold on spine
- Transparent plastic pocket provided for your own reference
- Each binder takes two volumes of *Nature*; three binders will serve for a year
- Prices and address for orders are set out below

Annual Subscription Prices

UK & Irish Republic		£98
USA & Canada		US$230
Australia & NZ	Airspeed	£150
Continental Europe	Airspeed	£120
India	Airspeed	£115
Japan	Airspeed	Y85000
Rest of World*	Surface	£120
Rest of World*	Airmail	£180

**(not USA, Canada, Europe & Japan)*

Orders (with remittance) to:

USA & Canada
Nature
Subscription Dept
PO Box 1018
Manasquan
NJ 08736
USA

UK & Rest of World
Nature
Circulation Dept
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to:
Japan Publications Trading Company Ltd
2-1 Sarugaku-cho 1-chome
Chiyoda-ku, Tokyo, Japan
Tel: 03-292-3755

Personal subscription rates
These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
15 East 26 Street
New York,
NY 10010, USA
Tel: (212) 689-5900

UK & Europe
Felicity Parker, Nature
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

Credit card orders only (in USA):
Call toll-free: (800) 824-7888 (Operator 246)
In California: (800) 852-7777 (Operator 246)

Back issues
UK, £2.50; USA & Canada, US$6.00 (surface), US$9.00 (air); Rest of World, £3.00 (surface), £4.00 (air)

Binders
Single Binders: UK, £4.50; Rest of World, $10.00
Set(s) of 3 Binders; UK, £12.00; Rest of World, $25.00

Annual indexes (1971–1982)
UK, £5.00 each; Rest of World, $10.00

Nature First Issue Facsimile
UK, 75p; Rest of World (surface), $1.50; (air) $2.00

Nature in microform
For Information:
University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

K channels in T lymphocytes: a patch clamp study using monoclonal antibody adhesion
D R Matteson & C Deutsch **468**

Retinoblastoma — origin from a primitive neuroectodermal cell?
A P Kyritsis, M Tsokos, T J Triche & G J Chader **471**

A novel 6:10 chromosomal translocation in the murine plasmacytoma NS-1
R M Perlmutter, J L Klotz, D Pravtcheva, F Ruddle & L Hood **473**

Activation of N-*ras* in bone marrow cells from a patient with acute myeloblastic leukaemia
C Gambke, E Signer & C Moroni **476**

Three-dimensional structure of the light-harvesting chlorophyll *a*/*b*-protein complex
W Kühlbrandt **478**

Thermodynamics of the B–Z transition in superhelical DNA
M D Frank-Kamenetskii & A V Vologodskii **481**

MISCELLANY

100 years ago **415**
Announcements **487**
Employment: Students and industrial sponsorship
R Pearson **488**
New on the market **xv**

NATURE CLASSIFIED

Professional appointments — Research posts — Studentships — Fellowships — Conferences — Courses — Seminars — Symposia: Back pages

Nature in Brazil

● Because of restrictions on foreign exchange in Brazil, the purchase there of international journals has become virtually impossible.

Nature is therefore introducing a scheme whereby scientists may purchase a year's subscription to *Nature* for a colleague in Brazil at half the usual price. Those wishing to take advantage of this scheme should write to either:

Felicity Parker
Nature
Houndmills
Basingstoke
Hants RG21 2XS, UK

Mirta Soto
Nature Publishing Co.
15 East 26 Street
New York,
NY 10010, USA

enclosing a cheque for $90.00 (surface mail) or $135.00 (airmail) or the equivalent in any currency, together with their full name and address and those of the intended recipient. Telephone orders may be charged to international credit cards. *Nature* will meet the extra cost of despatching the journal to Brazil.

This offer will remain open until 31 December 1984. The similar scheme to help Polish scientists continues.

Next week in *Nature*:

- Oncogenic determination of sequence for epidermal growth factor?
- Gene amplification by differently placed genetic inserts
- Albedo feedback and Sahel drought
- Solar conversion at 10 per cent efficiency
- More mantle inhomogeneity
- Pulsating stars for seeking gravitational waves
- Philip Gummett on *Government and Research* by M. Kogan & M. Henkel
- Sir Fred Hoyle reviews S. Chandrasekhar's *Eddington: The Most Distinguished Astrophysicist of his Time*

GUIDE TO AUTHORS

Authors should be aware of the diversity of *Nature*'s readership and should strive to be as widely understood as possible.

Review articles should be accessible to the whole readership. Most are commissioned, but unsolicited reviews are welcome (in which case prior consultation with the office is desirable).

Scientific articles are research reports whose conclusions are of general interest or which represent substantial advances of understanding. The text should not exceed 3,000 words and six displayed items (figures plus tables). The article should include an abstract of about 50 words.

Letters to *Nature* are ordinarily 1,000 words long with no more than four displayed items. The first paragraph (not exceeding 150 words) should say what the letter is about, why the study it reports was undertaken and what the conclusions are.

Matters arising are brief comments (up to 500 words) on articles and letters recently published in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted.

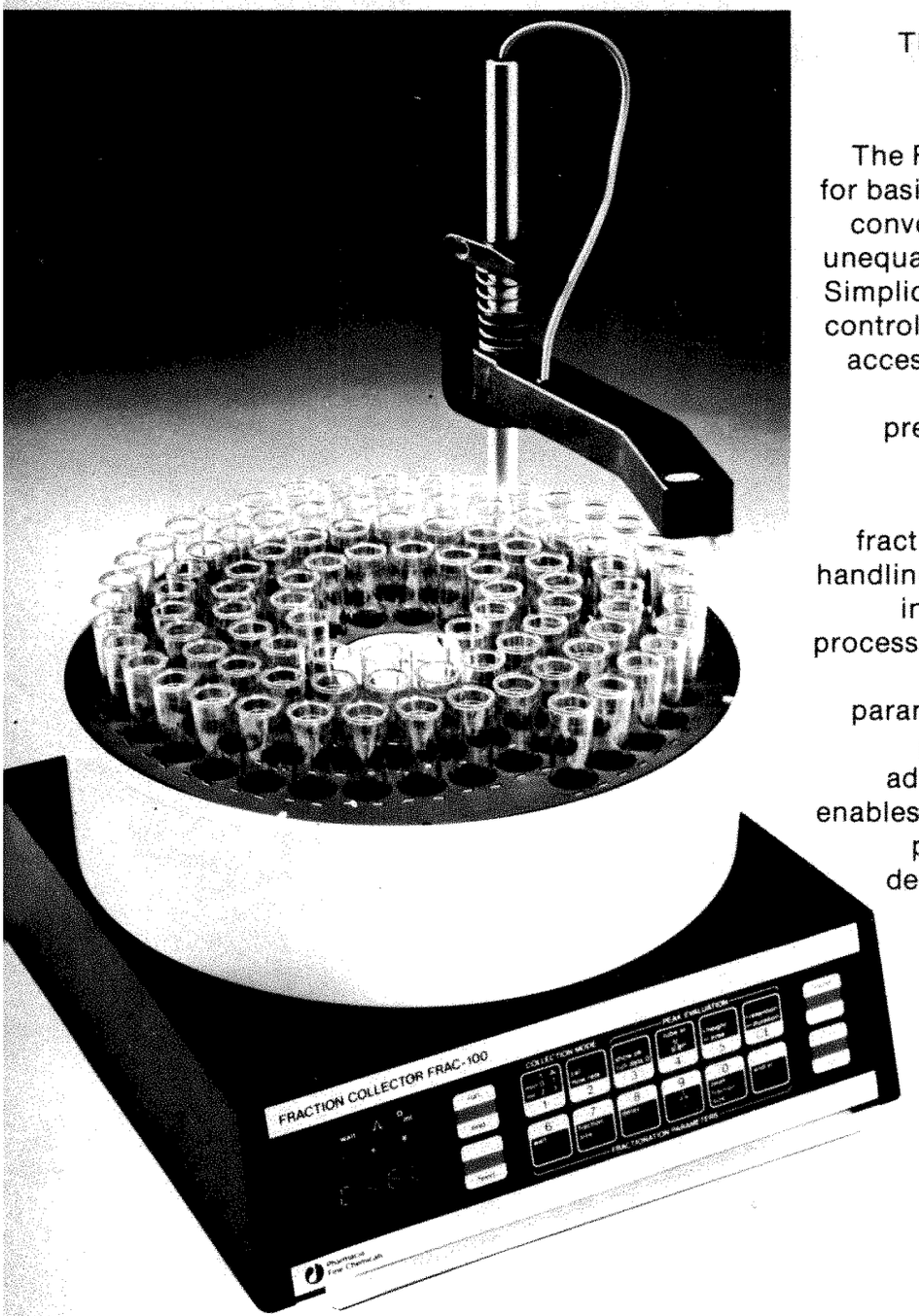
Manuscripts may be submitted either to London or Washington (decisions being made only in London). Manuscripts should be typed (double spacing) on one side of the paper only. Three copies are required, each accompanied by copies of lettered artwork. No title should exceed 80 characters in length. Reference lists, figure legends, etc. should be on separate sheets, all of which should be numbered. Abbreviations, symbols, units, etc. should be identified on one copy of the manuscript at their first appearance.

References should appear sequentially indicated by superscripts in the text and should be abbreviated according to the *World List of Scientific Periodicals*, fourth edition (Butterworth 1963–65). The first and last page numbers of each reference should be cited. References to books should clearly indicate the publisher and the date and place of publication. Unpublished articles should not be formally referred to unless accepted or submitted for publication, but may be mentioned in the text.

Each piece of artwork should be clearly marked with the author's name and the figure number. Original artwork should be unlettered. Suggestions for cover illustrations are welcome. Original artwork (and one copy of the manuscript) will be returned when manuscripts cannot be published.

Requests for permission to reproduce material from Nature *should be accompanied by a self-addressed (and, in the case of the United Kingdom and United States, stamped) envelope.*

The FRAC-100 *Fraction Collector*



The FRAC-100 does what any other small fraction collector does - collects fractions, PLUS .....

The FRAC-100 will fulfill all your needs for basic fractionation requirements. As a conventional collector the FRAC-100 is unequaled in performance and reliability. Simplicity in design (3 moving parts) and control (flexible fractionation parameters accessed by a multifunction key board) accomplish fractionation routines previously attainable only with large, expensive instruments.

The FRAC-100, as a sophisticated fraction collector presents unique data handling and integration capabilities. The input of a 0-10mV detector signal is processed by the FRAC-100 to report as a digital display such separation parameters as peak height, peak width, peak retention and peak area. The addition of a PSV-100 solenoid valve enables selective fractionation of only the peak material as judged by a slope detector defined by a programmable threshold value.

If what you're looking for is a small, simple, inexpensive fraction collector - the FRAC-100 is your best choice!

If what you're looking for is a small sophisticated collector with power and abilities far beyond anything presently available - the FRAC-100 is your only choice!

Pharmacia Fine Chemicals AB
Box 175
S-751 04 Uppsala 1
Sweden

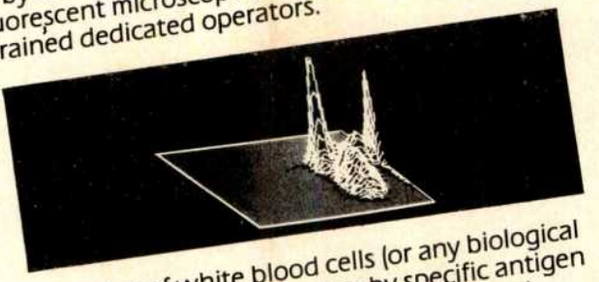
Pharmacia Fine Chemicals

Circle No. 14 on Reader Service Card.

Does your fluorescent analyser keep you in the dark?



New from Coulter. The EPICS® C clinical flow cytometer analyses and sorts cells routinely on the basis of number, size and fluorescence by use of programmable protocols.
No fluorescent microscope analyses, no darkened rooms, no highly trained dedicated operators.



● differentiation of white blood cells (or any biological cells) ● fluorescence determination by specific antigen characteristics ● range of compatible monoclonal antibodies available (e.g. for T & B cell ratio, T helper/T suppressor) ● physical sorting of populations into sub-populations for further study ● high specificity allows previously inseparable cells populations to be determined ● automatic operation allows short 'half life' fluorescent determinations to be made.

Epics® C

Automatic cell analyser and sorter at an 'analyser only' price

PIONEERING 1959 25 YEARS TECHNOLOGY 1984

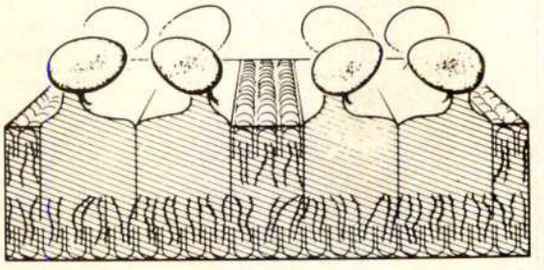
Coulter Electronics Limited,
Northwell Drive,
Luton, Beds., LU3 3RH.
Tel: (0582) 582442
Telex: 825074

Coulter Electronics®

Circle No.10 on Reader Service Card.

Attention all biophysicists, molecular biologists and biochemists!

Biomembrane Structure and Function



Edited by
DENNIS CHAPMAN
Professor of Biophysical Chemistry
Royal Free Hospital
School of Medicine, London

Volume 4 in the TOPICS IN MOLECULAR AND STRUCTURAL BIOLOGY Series
Series editors:
Professor Watson Fuller, Dr Stephen Neidle and Professor Tony Atkinson

The application of physical techniques – such as X-ray diffraction and Raman spectroscopy – has revolutionised much of our thinking on the structure and function of membranes. In this important new study, contributors of international standing review a topical selection of approaches to the elucidation of **biomembrane structure and function.** The emphasis, throughout, is on the in-depth analysis of recent experimental work.

Published January 1984 Price £50.00 432pp
ISBN 0 333 31869 2

Contents

Calorimetric studies of model and natural biomembranes – *Diana Bach*, The Weizman Institute of Science, Israel; X-ray diffraction and electron microscope studies of the molecular structure of biological membranes – *Lee Makowski and Jade Li*, Columbia University, USA; Raman spectroscopy of lipids and biomembranes – *Surendra P. Verma and Donald F.H. Wallach*, Tufts-New England Medical Centre, USA; Infrared spectroscopic studies of model and natural biomembranes – *Ralph L. Amey*, Occidental College, USA and *Dennis Chapman*; Rotational and lateral diffusion of membrane proteins as determined by laser techniques – *Winfried Hoffman*, NUKEM GmbH, E.Germany, and *Colin J. Restall*, Royal Free Hospital, London; Theoretical models of monolayers, bilayers and biological membranes – *David A. Pink*, St Francis Xavier University, Canada; The Ca^{2+} dependent ATPase of sarcoplasmic reticulum – *G. Inesi and M. Kurzmack*, University of Maryland, USA.

Please send orders and requests for information to:
Carolyn Leuw, Product Manager,
Scientific and Medical Division,
The Macmillan Press Ltd.,
Houndmills, Basingstoke, Hants RG21 2XS, England

SCIENTIFIC & MEDICAL | M | MACMILLAN PRESS LTD

Circle No.64 on Reader Service Card.

EDITORIAL OFFICES

London Office
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024

Washington Office
1134 National Press Building, DC 20045
Telephone: (202) 737-2355

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff: Alun Anderson, Tim Beardsley, Philip Campbell, Isobel Collins, Peter Gambles, Melanie Kee, Tim Lincoln, Naomi Molson, Sara Nash, Geoffrey North, Peta Pickering, Miranda Robertson, Robert Walgate, Charles Wenz, Nigel Williams, Guil Winchester

Washington Editor: Peter David
Washington Correspondent: Stephen Budiansky

Art and Design: Joan Godwin, Joel Chernin, Theresa George, Paul Norris, Marie Xeridat

Administration: Mary Sheehan, Jacqueline Cole, Ruth Cutts, Joanna Czechowska, Ruth Goldenberg, Sharon Isaacs and Penny Walker (Washington)

Publisher: Elizabeth Hughes

Marketing Director: Ray Barker

Promotion Manager: Felicity Parker

International Advertising Manager: Andy Sutherland
Features Advertising Manager: Marion Delaney

Group Classified Advertising Manager: John Barnes — Telephone: (01) 240 1101

Nature New York Office
15 East 26 Street, New York, NY 10010
Telephone: (212) 689-5900 Telex: 668497

General Manager: Gary Rekstad

American Advertising Manager: Henry Dale

Classified Advertising Manager: Miss Gene Fein

Circulation Manager: Mirta Soto

Canadian Display and Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1—(416) 690-2423

Display Advertising Representatives

New York: Nature Office
Chicago: Didier & Broderick, Inc.—(312) 498-4520
San Francisco: Jobson/Jordan/Harrison/Schulz, Inc.—(415) 392-6794
Los Angeles & Pasadena: Jobson/Jordan/Harrison/Schulz, Inc.—(213) 796-9200
Philadelphia: Daniel Adams Associates, Inc.—(215) 353-6191
Boston: CEL Associates—(617) 848-9306
Fort Lauderdale: Brinker & Brinker—(305) 771-0064
New Jersey: Mary Grunmeier—(201) 572 9018
Dalton, Pennsylvania: Donald E. Northup Associates—(717) 563 2572
West Germany and Austria: Franz Schrecklinger TMW Top Media Werbegesellschaft GmbH Frankfurt—(611) 726046
Holland and Belgium: Adele Struyck G. Arnold Teesing BV Amsterdam—(020) 2636 15
Switzerland: Werner Stähli Agentur Iff AG Schaffhausen—(053) 45821
Scandinavia: Andrew Karnig Andrew Karnig & Associates AB Stockholm—(08) 51 68 70
Japan: Mashy Yoshikawa Orient Echo Inc Tokyo—541-4923
Australia: Gus Bartel & Associates PO Box 1755, Southport, Queensland 4215 (075) 466 577

Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office
©1984 Macmillan Journals Ltd
Vol. 307 No. 5950 2 February 1984

nature

Snapshot of a new industry

The much-awaited report to the US Congress on the international biotechnology industry may be flawed but it deserves to be widely read, not only in the United States.

THE US Congress, which commissioned the report on the international biotechnology industry published last week, should beware of the beguiling trap of chauvinism as it thumbs through the huge document that has now emerged (see page 402). For the Office of Technology Assessment (OTA), in the course of its patient description of who where is doing what, has unaccountably peppered an otherwise sober report with declarations that the United States has a "commanding lead" (in the development of software for the use of nucleotide-sequence data-banks) or is the "strongest in the world" (in its "efforts to commercialize biotechnology"). Few will dispute the validity of these and other assessments in the same genre, which in any case have the tactical advantage of telling Congress what it wants to hear. (Bearers of bad tidings are not much welcomed anywhere.) Moreover, these opinions bear directly on the chief conclusion of the report, that while "continuation of the initial preeminence of American companies in the commercialization of new biotechnology is not assured, . . . Japan is likely to be the leading competitor . . ."

The flaw in this jump from the particular to the general is not, of course, that it may be mistaken, for it is far too soon for anybody to tell, but that it is simplistic. OTA, which likes to be known as Congress's own think-tank, has let slip a splendid opportunity to read its masters a lesson in the economics of technological innovation and has couched its otherwise helpful answer in the outdated language of the 1950s (or even the 1930s). Indeed, the constant harping on the theme of leadership, by representing the process of technological innovation as a battle between two Goliaths, obliterates the texture of what is going on. In such an interesting field, there will be opportunities for all and, eventually, a division of labour, Adam Smith style.

Biotechnology may or may not be as old as lithofracture as a tool-making technology, but certainly dates back to the use of bacteria for turning milk solids into cheese and the use for other purposes of the liquids produced by the natural fermentation of fruit. So much, the report acknowledges in passing. In recent decades, the industry has gone up (with the use of fermentation techniques for making solvents in the 1930s) and down (with the advent of cheaper feedstocks from oil and natural gas). The novelty, in the past decade, is that techniques of genetic manipulation are certain to bring within the scope of traditional biotechnology the production of materials hitherto produced in other ways by, for example, the organic synthesis of pharmaceuticals.

Disappointment

Nobody doubts that the consequences will be important. Why else would investors in the United States and elsewhere have been so eager, in the past five years, to help launch new companies in this field? But the experience of the past five years has also shown that the new biotechnology differs in no substantial way from other new technologies, the development of steam engines in the second half of the eighteenth century for example. The immediate course of development abounds with disappointment — steam engines blow up, materials such as cloned interferon turn out not to be wonder drugs but chemicals in search of a disease to cure — yet confidence in the long-term future persists. The difficulty, in such circumstances, is that the pattern of the future is almost literally unpredictable.

The OTA report itself points to many of the potential sources of uncertainty — the likelihood that new biotechnology companies in the United States will find it harder to re-finance themselves when their original capital is exhausted, the high chance that even if things go well, many of them will have to rely on the sales organizations of established companies to ensure that their new products reach their intended users and the simple truth that vast areas of the potential of the new biotechnology are almost entirely unexplored. (Genetically manipulated plants that produce their own insecticides, for example, are a splendid and no doubt attainable goal but cannot yet be bred.) Short-term uncertainty against the background of long-term confidence should be the motto for the next few years. The question of whether the United States is "ahead", or whether Japan is a greater "threat" than any other industrialized country, is both irrelevant and dangerously misleading.

Clamour

If the new biotechnology is as important as it now seems, it will be important for decades to come and not merely for the lifetimes of the patents now being clamorously taken out. Moreover, if the opportunities for innovation span even a fraction of the ground now in prospect, there will be work enough to keep the technical communities of three continents (and perhaps even five) busy for decades to come. For the immediate future, of course, OTA is right to draw the attention of Congress to the reasons for thinking that the United States is an especially favourable place in which to embark on these exciting adventures — the availability of venture capital, the flexibility of the patent system (less is said about the problems that beset it) and the supply of talent. But the report enormously undervalues the way in which, in the long run, the emerging industry will be shaped by the unforeseeable application of unidentifiable people's ingenuity to the solution of practical problems by means that will not always yield intellectual property (as the saying goes) that can be protected.

The opportunity OTA has lost is that of reminding the US Congress that at the beginning of an industry capable of transforming the pattern of what now passes for industry, predictions of the future based on the extrapolation of the present are unsafe. What if, for example, several years of the accumulation of budget deficits on the scale made public this week should force the United States to follow other spendthrift governments along the paths that have undermined other people's entrepreneurial spirit? Or if the arrangements that have been made between industrial companies and universities turn out to be more distracting for the academics involved than the report considers likely? The danger, for the US Congress, is that while the OTA report will bring a good deal of comfort, it may also induce complacency.

What will be made of the document elsewhere? One thing is certain — it will be widely read. Governments elsewhere, but especially in Western Europe, will pay close attention to the argument that United States companies active in this field must chiefly fear competition from Japan, not Europe. On one point, they must agree with OTA: the flexibility of the system whereby new companies in the United States can be launched with the help of risk capital channelled through specialist financial companies is enviable, as is the arrangement by means of which investors may put money into specific research and development projects under-

© 1984 Macmillan Journals Ltd

taken by biotechnology companies in such a way as to ensure that gains are taxed as capital gains, not as income. European governments should also take to heart OTA's argument that the ease with which new companies can spring into existence in the United States, confusing though it may be for the stock markets, is a powerful spur to copious innovation and, perhaps more important, its rapid exploitation. It is, however, important to acknowledge that in the past two years, in Britain and West Germany, venture capitalism has been growing even faster than biotechnology. Whether the same governments will sanctify this trend with tax advantages comparable with those enjoyed in the United States remains to be seen.

By themselves, however, these differences do not explain why Japan should be distinguished from Western Europe as the ground in which biotechnology is more likely to flourish. Indeed, Japan (like West Germany until very recently) differs radically from the United States in that venture capital plays only a minor part in the capital financing of industry. OTA's argument is that several Japanese companies have admirable expertise in the exploitation of fermentation technology (which is true), that Japan is a huge market for pharmaceuticals (which is likely to be important in the short run) and that the Ministry of International Trade and Industry in Tokyo has launched a cooperative programme in biotechnology (which is less significant than OTA appears to think). Readers of the report will, however, conclude that OTA is most of all impressed at the number of Japanese companies that have declared their determination to prosper in biotechnology — and which are likely to turn their ambitions into reality with the zeal that has marked the success of Japanese manufacturers of automobiles and of electronic consumer goods. That is an awesome prospect but not a proof that European companies will be also-rans.

For those who would predict the future, one missing element in these calculations is the character of the relationship between the newer biotechnology companies and the larger companies with ambitions in the same field. OTA is probably right to guess that partnerships between big and small companies that already exist will often develop into even stronger relationships, outright mergers included. For large companies, which may have been slow to respond to the opportunities presented by biotechnology in the past decade, often enjoy the luxury of being able to bide their time, relying on their financial resources to be able to move quickly when the time is ripe. And once committed to the development of a new product, established companies have an advantage that newly established companies cannot but envy — an established and sometimes efficient mechanism for putting what they seek to sell into the hands of customers. Especially now that it has become plain that the benefits of biotechnology are further away than at first seemed possible, the large pharmaceutical and chemical manufacturers now operating on an international basis have powerful advantages over most newly created companies. None of this implies that there is no place for the smaller companies, nor even than none of them will grow big; indeed, OTA is entirely right to emphasize the value of the smaller companies as spurs to innovation in the past few years. But the growing importance of the multinational manufacturers in the development of biotechnology is yet another reason why pure chauvinism is out of place.

In the circumstances, the positive recommendations put forward by OTA are probably less urgent than they seem. OTA would have the administration reverse the recent decline of support for basic biological research (for which there are other good reasons) and do something unspecified to strengthen "bioprocess engineering" (for which there are few precedents). In strictly parochial terms, indeed, the threat that if Congress should fail to provide extra resources in those fields, the United States may lose its "commanding lead" to Japan or some other nation may be effective. It will be interesting to see how that issue is decided. Meanwhile, the chief value of OTA's gigantic study is that it has provided a unique snapshot of a new and important industry at a critical epoch in its growth, no longer neonate, still far short of puberty. □

Binary is divisive

Britain's system of higher education suffers from too little diversity and from being split in two.

FOR how much longer can the British system of higher education be allowed to continue as if it were two separate systems, one consisting of institutions called universities financed by central government through the University Grants Committee and the other consisting of institutions called polytechnics whose bills are paid by local authorities (with money provided by the central government)? The question has been in the air ever since the polytechnics were designated as degree-giving establishments nearly twenty years ago. The argument then was that there would be virtues in operating a dual system of higher education, if only because the newly established polytechnics could be guided, or coerced, to take a direct interest in the vocational needs of their students. It was almost as if the late Mr Anthony Crosland, the then Secretary of State for Education and himself once a university teacher, egged on by his Oxbridge civil servants, had washed his hands of the universities and decided that a quite different system would better serve the social need of higher education. In the event, the binary system (Mr Crosland's name for it) has not been much of a success. While many polytechnics have stuck admirably to their brief, and have won respect for their teaching and research in the process, others (there are twenty-six altogether) have aped the universities in the breadth of the curricula on offer, or have embraced unproved and therefore mischievous courses (admittedly fashionable in the 1960s) with elaborate titles such as "independent study", or have merely become dull. And sadly, both among academics and would-be students, the polytechnics are less eagerly sought after than are the universities.

Now, though, there is a chance that this may be changed. Guilefully, the Department of Education and Science has persuaded the universities that the system they operate for administering applications to enter universities from school students should also be used to administer applications for entry to the polytechnics. The scheme (whose cost will be met by the government) will operate for a trial period of two years, but everybody concerned insists that students wishing to hedge their bets on securing a place somewhere in higher education will have to complete two separate application forms, the data on which will be sedulously kept separate. This is either a nonsense or the thin end of a wedge, one that leads to a unified procedure for entry into higher education of either kind.

And not before time. Not merely young people looking for places in higher education but those who eventually employ them are thoroughly confused by the present system, which is a disgraceful monument to the corrosiveness of academic snobbery. Both types of institutions allow young and not-so-young people to earn degrees, whose authenticity in the case of the polytechnics is vouched for by an independent council. Polytechnics, it is true, lack people called vice-chancellors, and have directors instead. Senior teachers are called "heads of department", not professors. And they have courses in hotel management and catering as well as in Latin and Greek. But could all British universities justly claim that they are themselves so very different?

If not, university academics should follow the only honourable course open to them, and clamour for the abolition of the binary system by means of the abolition of the boundary between its two unjustly unequal parts. The opportunity will arise when they come to reply to the questionnaire put out last autumn by the still-new chairman of the University Grants Committee, enquiring in a neutral fashion about university opinion on the binary system. The practical benefits are obvious — students will no longer be quite as mystified, while more efficient use will be made of scarce resources. More important, however, the British system of higher education would at one swoop acquire the diversity of institutions only now being forced to emerge by financial deprivation. □

0028-0836/84/050400-01$01.00

US defence research

Pentagon asks for stricter control of publication

Washington

THE Pentagon is seeking extensive new powers to change or block the publication of scientific papers written under Department of Defense (DoD) contracts. In a new policy directive (number 2040.2), DoD sets out a general policy for reviewing both basic and applied research carried out in academic settings.

Under the scheme, basic and applied research papers deemed not to be sensitive will have to be submitted to a DoD contract office at the same time as they are submitted for publication, although DoD will have no right to insist on changes or to block publication. Basic research characterized as sensitive will have to be submitted to DoD 60 days before it is submitted for publication. In these cases DoD will be allowed to suggest changes or recommend that publication should not go ahead, but the final decision will rest with the investigator.

Applied research papers which are sensitive will, however, have to be submitted to DoD 90 days before they are



submitted for publication, and DoD will be entitled to insist on changes in the manuscripts or to block publication altogether.

The new directive is part of an extensive Pentagon review of technology transfer which started in 1981 and is now nearing completion. As part of the review, six panels looked at research contracts, visa controls, emerging technologies, scientific conferences, publication and rules for exemption from the Freedom of Information Act. Their findings are being coordinated with those of a still secret interagency review of technology transfer started in 1982 after a National Academy of Sciences report called for an easing and simplification of existing controls on the publication of research with a bearing on national security.

The restrictions on publication proposed in the DoD directive are far stricter than those recommended by the academy's report, prepared under the direction of Dale Corson, former president of Cornell University. The main conclusion of the Corson panel was that too much secrecy in scientific communication would damage rather than strengthen US security because of its chilling effect on the scientific enterprise itself.

Warning that the effort to protect sensitive information was spread too thinly, the Corson panel proposed confining controls to a few "grey areas" where the technology was developing rapidly, had clear military applications and would, if released, give the Soviet Union a short-term military advantage it could not otherwise acquire.

DoD, however, wants to extend the scope of its controls. It suggests a looser definition of sensitive technology as that "perceived to have a military impact" and in which the Soviet Union is less advanced than the United States. Furthermore, DoD wants a new Militarily Significant Emergent Technologies Awareness List (METAL) to pinpoint frontier technologies that could be candidates for stricter controls.

DoD's break with the Corson recommendations has come in spite of a formal improvement in liaison with the universities. Twenty academics have been invited to join an academic advisory group for Comex, the committee on exchanges which recommends whether scientific visitors from unfriendly nations should be granted visas. A government/industry/university research round table has been established within the National Academy of Sciences but has not yet met. And a working group on export controls, attached to the DoD/University Forum, has given the academic community periodic progress reports on the Pentagon's internal review of scientific controls.

The complexity of the existing control mechanisms continues, however, to baffle both scientists and officials. For example, an extension course on materials science held recently at the University of California, Los Angeles (UCLA), was barred to foreign citizens. Michael Bley, UCLA's director of marketing, said some of the technologies to be discussed were on the US Munitions List. But he admitted that publicity for the course had not mentioned that citizens of the United Kingdom, Canada, Australia and New Zealand would have been able to attend under the terms of a technical cooperation agreement with the United States.

Publication, if it is ever declassified, of the interagency study on technology transfer may clarify the issue. The DoD study, however, gives little cause for optimism. From the point of view of the universities, its only welcome recommendation is the creation, for the first time, of a technology transfer appeals board within the Pentagon. Otherwise, its recommendations are far more conservative than Carson had wished.

Peter David

Ariane launch delay

Europe's hands clean

THE launch of Ariane, Europe's hope of an answer to the space shuttle, is suffering from greater and greater delays, just as the shuttle is getting into its stride.

For once, however, the problem is in the United States. Ford Aerospace in Palo Alto, California, is desperately trying to remedy a fault discovered in the Intelsat V (F8) series of communications satellites, one of which was due to have been launched by Ariane last November. Neither Ford nor the European Space Agency (ESA), administratively responsible for launching the satellite from its Kourou (French Guyana) base, will commit itself to a date more precise than "the end of February". Only two weeks ago, the canvassed launch date was mid-February.

The problem — interference on an L-band maritime communications system attached to Intelsat Vs from the fifth onwards — is "intermittent", says Ford. This may be taken as a code word for almost impossible to reproduce.

The Intelsat organization has leased these L-band systems to London-based Inmarsat, which deals solely in ship communications, and Intelsat will suffer penalties if the systems do not work. Equally, however, the organization faces penalty clauses in the contract with ESA, if launch is delayed too long. But, according to an ESA official, relations with Intelsat have not deteriorated to that stage yet: "we are not thinking about it", said the official.

According to ESA, if Intelsat V (F8) does get away by the end of February, there will still be five Ariane launches this year. But this compares with as many as eight foreseen last May and with ten scheduled shuttle launches. The next 18 or so satellites to be launched in the Ariane series, some in multiple launches with the more powerful Ariane-3 launcher, are all communications satellites, the first novelty coming with the high-resolution French SPOT remote sensing satellite, now due to be launched some time in mid-1985.

Robert Walgate

© 1984 Macmillan Journals Ltd

OTA biotechnology report

Congress told US ahead, Japan second, rest nowhere

Washington

PREEMINENCE in the life sciences, abundant venture capital and an entrepreneurial spirit have combined to give the United States a commanding lead in the commercial application of new biotechnological techniques, although Japan will pose a growing challenge. That is the conclusion of a 612-page study of the international biotechnology industry published last week by the Office of Technology Assessment (OTA), Congress's science think tank.

The report, *Commercial Biotechnology: An International Analysis,* predicts that within the next 5–10 years a range of new biotechnological products — including pharmaceuticals, animal vaccines and speciality chemicals — will reach the market. Europe is lagging behind the United States and Japan but a few big chemical and pharmaceutical companies in Britain, France, West Germany and Switzerland may compete strongly in the manufacture of some products.

OTA ascribes the US lead to a number of factors, notably its uniquely favourable tax climate. In 1983 alone, the private sector invested more than $1,000 million in the commercialization of biotechnology in the United States, and since 1976 more than 100 new biotechnology companies have been started up with private venture capital. Underpinning this effort is an enormous investment by the federal government in basic research in biotechnology — some $500 million a year compared with about $60 million each by Japan, Britain, France and West Germany.

Ready access to venture capital has given the biotechnology industry in the United States a different structure from that of other countries. While its main competitors have depended for the commercialization of biotechnology on established companies, the US effort has been strengthened by a profusion of embryonic companies that have sharpened competition and hastened the spread of expertise from the universities.

But the US lead is not unassailable. OTA believes Japan could catch up if the United States continues to prune funds for basic research in the life sciences and to neglect applied research and bioprocess engineering — the skills that take novel production techniques the laboratory to the factory. Japan has more bioprocess engineers than the United States and spends a bigger slice of its science budget on the solution of applied research problems. Its knack of beating other countries in the race to apply their own basic research could, says OTA, give Japan a bigger share of the biotechnology market than the United States.

What accounts for the United States' present lead? The report says the three most important factors in the competition to commercialize biotechnology are the financial and tax incentives provided by governments, the amount they invest in research and the degree to which they ensure that there are enough well trained experts. In all three areas the United States scores higher marks than its competitors but it cannot afford to be complacent.

The big postwar investment in the basic life sciences has given the United States a competitive edge in the supply of molecular biologists and immunologists, but there may not be enough plant molecular biologists and scale-up experts. Japan, Britain and West Germany, unlike the United States, have invested steadily in generic applied microbiology and bioprocess engineering, areas which will become increasingly important as the industry turns from its present fascination with research and development towards the manufacture of products.

OTA also adds a warning that the dynamism of the newly created biotechnology companies cannot be taken for granted as the industry matures. The existing division of labour between the small companies that have pushed ahead fastest in research and the established companies that have concentrated on marketing and production may break down as the larger companies do more of their own research. And many of the new companies may be unable to raise the money they will need in 5–10 years to move from research to production.

OTA expects the real test of US competitiveness to come when large-scale production begins and bioprocess problems must be faced, initially in pharmaceutical markets. Here the United States will face impressive competition from both Japan and Europe. OTA notes that Britain's major pharmaceutical companies — such as ICI, Glaxo and Wellcome — have a great deal of expertise in bioprocessing and that Britain conducts some of the strongest basic research in interdisciplinary plant sciences.

Japanese companies, spurred by government "targeting" of biotechnology, have launched a new drive to enter international pharmaceutical markets. Japanese companies are already world leaders in large-scale plant tissue culture, and the Ministry of International Trade and Industry (MITI) has identified secondary compound synthesis from plants as a major area for commercialization. Unlike the United States, Japanese industry is investing heavily in research on speciality chemical production, an area where Japan is already prominent. **Peter David**

Europe

OTA's slight resented

THE Office of Technology Assessment (OTA) report on world biotechnology seriously underestimates Europe's potential, specialists at the European Commission in Brussels were saying last week. "The report puts Europe firmly in third place, with Japan the only competition for the United States", but this overlooks the quality of basic research in Europe according to one official.

Another critic, Dr Mark Cantley, who for two years headed a team investigating the future of biotechnology in Europe as part of the Commission's FAST (Forecasting and Assessment in Science and Technology) investigation, and who is listed as a contributor to the report, says that the last version of the OTA report that he saw was "a relatively messy document", uneven in quality.

A sense of umbrage about the report has been building up in Europe since nearly a year ago, when drafts of the final version first became available. It seems that many European governments were able to persuade OTA to incorporate last minute assessments of the state of biotechnology in their countries. The difficulty seems to have been OTA's reliance on published documents and reports from national contributors rather than on first-hand investigation.

Cantley nevertheless says that OTA is correct in its political judgement of the "difficulty of getting our act together". He illustrates this with the problem of isoglucose in Europe, where a cheap enzymatic method for producing the sugar was effectively vetoed by the sugar-beet lobby. One of Cantley's hopes for the future is that it may be possible to bring about a better integration of agricultural and industrial policy so as to remove impediments to innovation in the agricultural field.

One of Cantley's colleagues, Ken Seargent, noting OTA's dismissivenes of Europe, nevertheless insists that "it need not be like that". He cited both the potential of research in the natural sciences and practical successes such as the pyrethrin analogue insecticide developed in Britain.

Meanwhile, in Brussels, officials were hoping earlier this week that the Commission would agree on Wednesday 2 February to establish a "concertation unit" in biotechnology as a catalyst for more European cooperation in the field. Research commissioner Etienne Davignon is thought to consider this step so important that he will find the money (roughly $1 million a year) from within his existing budget, thus avoiding reference to the strife-torn Council of Ministers.

Some in Brussels even consider the new unit as the foundation stone of a European

0028-0836/84/050402-01$01.00

policy on biotechnology as ambitious as the information technology policy embodied in the Esprit programme still awaiting political approval and financial blessing. In the meantime, however, the concertation unit would establish and maintain a network of experts, strengthening the present arrangements for coordination on biotechnology among the ten member states. **Robert Walgate**

Finance

Small companies star

ONE theme running through the Office of Technology Assessment (OTA) report is the importance to US biotechnology of the newly formed specialist companies of which more than 200 are listed in the report. OTA says that these appear to be more productive of research-based innovation than established companies when compared person for person, and that they have a tendency to bring research results to the market more quickly than better established companies. The report notes, however, that newly formed companies, launched with private funds or by means of a public offering, may find the problems of refinancing more difficult when their initial capital is used up, if only because investors in new companies necessarily look for good returns. This opinion appears to have been written before the onset of the fall in the value of biotechnology stocks last autumn.

In an analysis of 18 US biotechnology companies whose shares are publicly traded, OTA points out that only three (Cetus, Genentech and International Genetic Engineering) had positive earnings in the latest complete financial year and that two of these were in that happy position because of interest earned from funds obtained in public offerings. OTA notes that the high cost of financing research programmes has persuaded many companies to finance their operations through research contracts with larger companies or to recruit capital by means of limited partnerships in respect of research and development projects. For similar reasons many companies are giving priority to the development of products that will have an immediate market, such as monoclonal antibodies for the diagnosis of disease and veterinary products and feed additives for livestock.

In spite of the publicity given to biotechnology, OTA points out that venture capital is still flowing more strongly into the fields of communications, computers and electronics, which consumed nearly 60 per cent of investments in 1982. One striking feature of the pattern of investment in the past few years has, however, been the willingness of established US companies to invest in their smaller brethren. In 1982, the total investment in new biotechnology companies from this source is estimated to have been $120 million but to have fallen to $40 million in 1983, chiefly because of the increasing attention paid by the larger companies to strengthening their own in-house activities.

Looking ahead, OTA says that new companies may ultimately suffer from these arrangements because of the low profit margins to be expected from patent royalties, the chance that research contracts will not be renewed and the willingness of the larger partners in research contracts to devote resources to the exploitation and marketing of new products. For these reasons, OTA concludes, successful biotechnology companies will in due course find it necessary to engage in the whole range of activities bearing on the exploitation of their innovation.

On the links between universities and industrial companies, new or well established, OTA notes that there are advantages for both partners as well for the "national innovative process". At the same time, it points out that universities cannot expect that royalty income from patents will ever be a substantial part of their current costs. On the basis of a questionnaire sent out to university administrators, teachers and students, OTA comes to the conclusion that the reduction of federal funds for universities has been a spur, that few difficulties have arisen in the conduct of these relationships, and that 85 per cent of respondents considered that links with industry had not damaged university research. □

West Germany

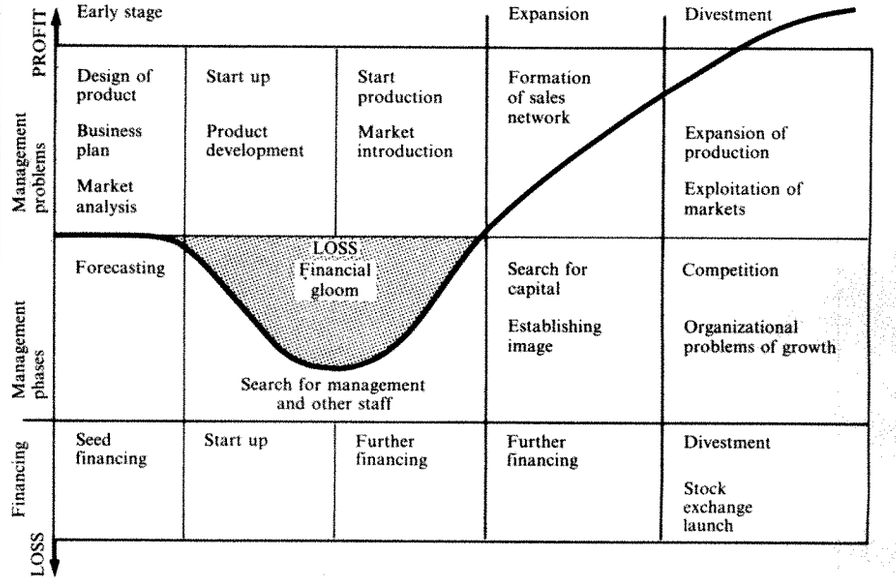
More adventure from capital

OBSTACLES to technology transfer and the formation of new companies in West Germany could be swiftly removed if a motion tabled in the Bundestag by two parties of the government coalition (the Christian Democratic Union, CDU, and the Free Democratic Party, FDP) is adopted. The need to improve the position of small and medium-sized companies in the Federal Republic is confirmed by the dim view of European investment in biotechnology taken by the US Office of Technology Assessment (OTA) (see page 402).

Although the stock market recently reached an all-time high and West Germany is still a leading world exporter, its share of world trade has fallen. Except for a few traditional areas such as chemicals, automobiles and electronics, the country has become an importer of high technology. More companies are going bankrupt than are starting up whence the interest in the search for means to encourage the formation of new high technology companies.

A major part of the problem is recognized to be the dependence of West German industry on loan capital rather than equity (risk) capital, which at present accounts for only 20 per cent of the capital employed by public companies, with a mere 5 per cent representing savings by members of the public. The banks, the main source of capital and the only insti-

Profile of a company start-up by venture capital



VENTURE capitalism combines financing with management and technical expertise. The investors take an active role in the development of the company. Misjudgement, takeover, merger or loss of crucial staff are a few "risks" that would give a different profile. A successful investor can hope to see this picture emerge for one third of his ventures; one third would level off to a low profit; one third would be written off. A reasonable time would be five years. □

© 1984 Macmillan Journals Ltd

tutions allowed to launch companies on the stock market, have been reluctant to support ideas and technological expertise alone, preferring more tangible bricks-and-mortar propositions. In addition, high taxes and charges discourage companies from flotation on the stock market and make equity capital more expensive than bank finance. It is estimated that this has created a DM100,000 (£25,000 million) "equity gap". In 1984, some 600 new companies will probably be launched on the US stock exchanges, whereas in West Germany, if the present situation continues, there will probably be no more than 20 new arrivals.

The lack of venture capital, the power behind the technology transfer represented by Silicon Valley and US biotechnology, is acute in West Germany. Yet in the United States since 1970, venture capitalists in 600 firms have invested a cumulative total of more than $8,000 million and have, in the process, created more than 600,000 jobs. Fifteen per cent of this capital has come from abroad and an optimistic estimate is that some DM 100,000 million could be available in West Germany if the right investment framework were created. Citicorp, experienced venture capitalist of the New York Citibank, is planning to invest DM420 million in Europe, including DM 140 million in West Germany.

Home-grown steps in this direction have also now become apparent. Apart from the Wagnisfinanzierungs GmbH, founded by a grouping of 29 banks, the most significant venture fund in West Germany has been started by Siemens under the name Techno Venture. The fund is capitalized at DM130 million, of which Siemens has contributed DM20 million and DM70 million has come from within West Germany. Private groups administer other funds, a small community has launched a fund through its local savings bank and the city of Berlin has begun a DM10 million fund. Minister of Research and Technology, Dr Heinz Riesenhuber, himself a successful businessman has advocated that 0.1 per cent of institutional funds should be used as venture capital.

Dr Riesenhuber emphasizes the need to create the right framework for research, technology transfer and commercialization, rather than direct state support and direction.

In the United States last year, it is estimated that 32 per cent of venture capital funds were contributed by pension funds, 32 per cent by insurance companies and 21 per cent by industry and foundations. Other financial routes such as tax-favoured research and development partnerships also exist to channel private funds into US industrial growth points. The OTA report concludes that "the unique complementarities between established and new firms, the well-developed science base, the availability of finances and an entrepreneurial spirit have been important in giving the United States its present competitive advantage in the commercialization of biotechnology . . . the (European countries) lag behind because they generally do not promote risk-taking, either industrially or in their government policies." The CDU/FDP motion is one step towards correcting this.

Sarah Tooze

Technical innovation

Plea for UK tax-breaks

THE British treatment of scientific research and patenting costs for taxation purposes is anomalous and may be inhibiting research and innovation in Britain, according to a report published last week by the Technical Change Centre. The report, written by a former senior economic adviser at the Inland Revenue, concludes that there is a powerful case for a more favourable tax treatment of research, which would be helpful to progressive industrial companies and would cost no more than £80 million in its first year.

The Technical Change Centre, which is supported by the Leverhulme Foundation, the Science and Engineering Research Council and the Economic and Social Research Council, produced studies on a variety of subjects, linked by the common theme of technical change. Its examination of the tax treatment of intellectual property in Britain finds that Britain gives highly favourable treatment to capital expenditure on plant and machinery compared with that on patents and know-how. As things stand, capital expenditure on patents in Britain is written off over 17 years and expenditure on know-how over 6 years. The specific proposal examined is that expenditure should be written off as it is incurred, like that on plant and machinery. This would, it is argued, restore some of the advantages once enjoyed by research that have now all but disappeared.

The report considers a number of possible objections but does not find any of them conclusive. Some, like the probable need for more civil servants in the Patent Office to cope with the likely increased number of patent applications, are dismissed as only "debating points", while others, such as the objection that companies might make increased use of patents as marketing gimmicks, are considered to be small risks.

On the positive side, it is concluded that the total cost of the scheme would be justified to the public by its immediate benefit to the more technologically progressive firms that are thought to be essential for Britain's future economic prosperity. While the cash flow of industry as a whole would not be affected to any significant degree, there would be a strong incentive to licensees to acquire patents if their costs could be written off immediately.

Tim Beardsley

China's research

Call to go back to basics

CHINA'S minister in charge of the State Scientific and Technological Commission, Mr Fang Yi, has spoken out firmly in favour of basic research as the foundation of the future scientific and technological development of the country. Addressing the fifth session of the scientific council of the Chinese Academy of Sciences recently, Mr Fang Yi noted that because major technological advances will become more and more dependent on breakthroughs in basic research, "some foreign countries" will be less willing to publicize their basic research in the open literature. China should therefore pay ever more attention to fields of basic research that will "open new paths" for technological development.

Mr Fang Yi's remarks differed considerably in emphasis from other recent official statements which put greater stress on the importance of applied research for the development of the Chinese economy, although he did note that particular emphasis should be given to "those fields of basic research which could have significant effects on the long-range development of the national economy". Current guidelines specify these fields as information science and technology, biotechnology, lasers, nuclear technology, computers and marine engineering.

As far as "big science" is concerned, with its demands for "enormous human, material and financial resources", Mr Fang Yi said that China's "limited conditions" imposed a need to be selective and to carry out only a few projects "according to our capabilities". Once a project is approved, however, scientists should be left to work in peace, since basic research demands "long-term efforts" and a tranquil atmosphere in which scientists can work in a "steady and sustained manner".

Mr Fang Yi's remarks seem intended as a not-too-veiled criticism of the attitude which still pervails in China as an aftermath of the cultural revolution and which is highly critical of the value to the economy of "intellectual" work. (Cases have been reported during the past year of misguided party activists criticizing the payment of bonuses of a few hundred yen to engineers and designers whose innovations have produced a very much larger financial gain.)

At the same time, even the Chinese Academy of Sciences will not have an entirely free hand. It must, Mr Fang Yi said, "show an adequate division of work and foster close cooperation with industrial departments, universities, national defence research departments and local research centres". In the applied sector, the Academy of Sciences must carry out research "more farsightedly, sooner and more intensively" than these other bodies.

Vera Rich

0028-0836/84/050404-01$01.00

Netherlands energy policy

More wind in prospect

Waalre, The Netherlands

AFTER two years, during which some 42,000 people took part in discussions throughout the country, great public debate in the Netherlands on energy policy has finally ended. But for the politicians, for some of whom the debate has conveniently postponed difficult decisions, the argument is only now beginning.

Predictably, the consensus reached in the final report on the debate favours much greater efforts in conservation and greater use of renewable energy. There is a need for the continued use of coal, the report concedes, but it comes out against the idea of any new nuclear power stations.

About Dfl 28 million (£6.3 million) has been spent on the two-year consultation exercise, of which Dfl 8.5 million went on 63 information projects (brochures, exhibitions and so on) and 14 research projects (energy analysis of nuclear power, for instance). A nine-member steering committee was set up in July 1981 under the chairmanship of the then minister for higher education and science policy, M. de Braauw. The committee had a scientific staff of 14 and an administrative staff of 16.

The idea for a public debate on energy came originally from the largest of the Protestant churches, with support from the largest labour union. In 1980, a parliamentary decision was made to embark on the public debate, providing a forum for the heated discussions that followed the abandonment in 1976 of a plan to build three nuclear power plants. Ironically, that plan had been put forward by Ruud Lubbers, then minister for economic affairs, who is now prime minister.

The first reactions to the report from the electricity producers are hostile. Public feeling against new power plants using fossil fuels is sustained by the knowledge that at present the Netherlands has a 50 per cent surplus of electricity generating capacity (the total is now 16,000 MW). After 1992, however, new capacity will probably be needed to replace obsolete plant. The report calculates that a "decentralized capacity" of 8,000 MW is feasible — 2,500 MW as wind power and 5,500 MW as combined heat/power in industry and city heating systems. In the past few weeks the electricity producers have been lobbying for more nuclear power, but to little avail.

In condemning nuclear power, the report stressed the problem of disposal of nuclear waste in a country as densely populated as the Netherlands. Supporters of nuclear power have concentrated on the high cost of electricity in the Netherlands compared with that in neighbouring countries such as West Germany and France. The steering committee claims, however that costs in France are kept artificially low by large government subsidies.

Environmentalist groups have naturally welcomed the report's conclusions, but whether any action is taken will depend on the politicians. And as the present policies of the three main political parties are not in line with the outcome of the public discussion, it may be a long time before Dutch energy plans for the 1990s are known.

Casper Schuuring

It's an ill wind. . .

THE following exchange took place in the British House of Lords on 23 January. Lords Paget and Cledwyn are members of the Labour Party, Lord Trefgarne is an under secretary of state in the Ministry of Defence.

Lord Paget of Northampton: My Lords, does not the noble Lord the Minister feel that all these questions are along the wrong lines? *Homo sapiens* has launched upon a career in which he is numerically doubling himself every 30 or 40 years. He is plainly in need of a massive cull. Nuclear weapons will make possible that massive cull — and, even more, they have the special factor of muddling up the genes so that from the new crop, something rather better than the present lot may emerge. Ought we not look to our good fortune?

Lord Trefgarne: My Lords, the originality of the noble Lord's views never ceases to impress me.

Lord Cledwyn of Penrhos: My Lords, is the noble Lord the Minister aware that what my noble friend has just said is not Labour Party policy? □

Polish science

Academy's position compromised

THE Polish Academy of Sciences has experienced some difficulty in the election of a new president, and many academy members see this as an indication of increased government and party supervision of the academy. Unlike most socialist countries, Poland has no effective analogue of the Soviet State Committee for Science and Technology, so the academy plays a major role not only in basic science but also in the coordination of applied research. The academic secretary of the academy holds ministerial rank, and reports directly to the prime minister. During the liberalization of 1980–81, there was pressure within the academy to make the academic secretary responsible to the academy members.

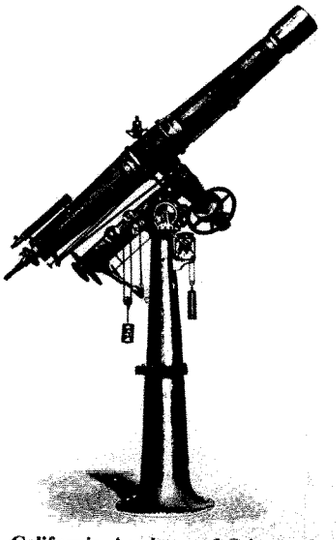
The promised new legislation on the academy is said still to be in the preparation stage, although it will almost certainly differ considerably from the hopes of the Solidarity period. In the meantime, Dr Aleksander Gieysztor, a historian, who was elected president in the early days of Solidarity, came to the end of his three year appointment, and was due to retire or stand for re-election in December. Party officials responsible for scientific matters thought Dr Gieysztor's reelection would be a hindrance to closer ties between the Polish academy and the academies of other "friendly" (that is socialist) countries, and he was urged not to stand for re-election.

The party's own choice for president was Dr Leonard Sosnowski, a solid state physicist, who is personally popular with many academy members. In the first round of academy elections in December, however, Dr Sosnowski was roundly defeated in the secret ballot. It seems likely that some voted against him on the grounds that they did not wish to be "manipulated" by the party, while others felt that, without casting any aspersions on Dr Sosnowski himself, it was unwise on principle to elect as president a party member, because this might give rise to a conflict of interests if the academy found itself opposed to party policy.

The second round of voting last week, however, resulted in a clear winner, Dr Jan Karol Kostrzewski, an epidemiologist and a vice president of the academy, of whom little appears to be known — except that he is not a party member.

Vera Rich

Spare parts wanted



THE California Academy of Sciences in San Francisco is appealing for help in its search for spare parts for the 6.4 inch telescope installed in 1879 at what was then the city's first observatory. Access to a similar telescope would allow the missing parts to be replicated.

The instrument was constructed by the Fauth Company and the mounting may even have been the first of that company's model 72 to have been built. Those who know of the whereabouts of a telescope of this construction are asked please to contact Allan Wilson at the California Academy, Golden Gate Park, San Francisco, California 94118. Collect calls will be taken. □

© 1984 Macmillan Journals Ltd

Genetic manipulation

British watchdog reconstituted

BRITAIN's Genetic Manipulation Advisory Group, known as GMAG (pronounced Gee-mag with the first syllable as in "genome"), met for the last time last week. The group is to be replaced by a new body, the Advisory Committee on Genetic Manipulation (ACGM), which will give advice on safety aspects of recombinant DNA techniques to the Health and Safety Commission, an independent body charged with devising policy on occupational safety for implementation by the Health and Safety Executive.

The formation of ACGM was announced in the House of Commons by Mr Peter Brooke, Under Secretary of State in the Department of Education and Science, under whose aegis GMAG operated. Like GMAG, the new body will have representatives of employers' and employees' organizations as well as technical experts. Unlike GMAG, however, ACGM will have no representatives of "the public interest". The new committee, whose membership has not yet been announced, will be concerned with policy matters, leaving the scrutiny of individual experiments to officials.

There has been a growing consensus that work involving genetic manipulation holds fewer hazards than was at one time feared, and so GMAG's role has become correspondingly less important. It would have been disbanded before now but for the delay in setting up a parallel body, the Advisory Committee on Dangerous Pathogens, itself caused chiefly by an outbreak of smallpox infection at the University of Birmingham in 1978. The government seems to have decided that it would be politically inappropriate to do away with GMAG while the pathogens committee was being reorganized.

ACGM will take more of a back seat than did GMAG. GMAG members, many of whom will serve on the new committee, have, however, been told that the new committee will be free to initiate action and to plan its own work. The decision to hand over the day-to-day business of keeping records of recombinant DNA research to the Health and Safety Executive was fully approved by GMAG members, although some working scientists doubt whether the change will make their lives easier. Most say they were content with the way in which GMAG carried out its duties in recent years, since the levels of containment required for most work have been reduced and notifications of routine work can now be made retrospectively. In future, scientists will have to deal with officials of the Health and Safety Executive who may be less easy to please than GMAG.

The new committee has been given by GMAG a list of several urgent topics for discussion, including commercial confidentiality, the release of genetically engineered organisms into the environment and work with oncogenes. Despite industrial scepticism at the outset, GMAG seems to have been able to assure commercial companies of confidentiality; now, doubts have arisen whether the constitution of the Health and Safety Executive, which includes provision for the disclosure to work-people of companies' plans, will allow this guarantee to be continued.

British opinion on the release of genetically-engineered organisms into the environment, recently a source of controversy in the United States (*see Nature* **305**, 564; 1983), is likely first to be tested by research at the John Innes Institute in Norwich. Work with *Rhizobium* bacteria, which fix nitrogen in legumes, holds the promise of allowing the host specificity of different species to be altered, when the efficiency of nitrogen fixation might also be increased. Before approving field trials, ACGM will have to be sure that an experimental organism could not compete with wild types or, alternatively, could be destroyed if necessary. One fear is that host growth could be inhibited: disablement of legumes with a selected strain of *Rhizobium* has already been demonstrated and an engineered strain could conceivably be an effective pathogen. And the committee is asked to note the growing evidence that some human oncogenes linked to particular promoters may function like animal tumour viruses.

While nobody doubts the competence of the new committee to tackle these issues, another question remains: who will be responsible for considering ethical problems that may arise with genetic manipulation techniques? The government's Warnock committee, which is expected to report in the summer, is considering these questions as they relate to human fertility, but some members of GMAG want to see more permanent provision made. The issue is so far unresolved. **Tim Beardsley**

European air pollution

UK inspector protests at Brussels

SHOULD air pollution controls in Britain be tightened in order to combat acid precipitation? The latest body to enter the debate is the Industrial Air Pollution Inspectorate. In the inspectorate's annual report published this week*, the chief inspector, Dr Leslie Reed, inveighs against the use in legislation of emission limits such as those planned in two European Commission proposals for Community directives. Dr Reed will be giving evidence on the European air pollution proposals next week to the House of Lords' European Communities committee.

The cornerstone of British air pollution control since the first Alkali Act was passed in 1863 has been the statutory requirement that operators of scheduled plant should employ the "best practicable means" to control emissions. The chief industrial air pollution inspector, who was until recently known as the chief alkali inspector, has in practice drawn up presumptive emission limits — specified as milligrams of pollutant per cubic metre of air emitted — but these are used to indicate only whether an operator is making a reasonable effort and have no force in law. Dr Reed's defence of the system is that presumptive limits can be changed rapidly as technological advances allow and that the actual emissions allowed may be altered to suit local circumstances.

Britain has already objected at Brussels to the section of a proposed framework directive on air pollution control that calls for the introduction of Community-wide emission limits agreed by a two-thirds majority. The European Communities' Council of Ministers will meet in March to try and find a way round the impasse. A second proposed directive on fixed emission limits for major combustion plant, which also specifies targets for swingeing cuts in total national emissions, is likely to be opposed by Britain. This proposal is being pushed hard by West Germany which fears that transboundary pollution is contributing to mounting damage to its forests.

But Dr Reed fears that adopting these directives will introduce another tier of authority in Britain for the sake of "one or two European countries which have inadequate controls of their own". Dr Reed also has doubts about a third European directive on air quality standards for nitrogen dioxide. While accepting the principle, Britain should, he says, resist demands for standards that would require costly measures by industry to ensure compliance but which are based on inadequate evidence of need.

Acid rain was the subject of a major speech recently by Mr Ian MacGregor, chairman of the National Coal Board, who said that "after we have crippled some of our basic industries we could then find we had not solved the problem". He pointed out that volcanic activity may emit huge quantities of sulphuric acid into the atmosphere and that some of the reported damage to conifers was unlikely to be due to sulphur dioxide pollution because the trees bore healthy growths of lichen. He concluded that short-term action, such as liming affected waters, should be applied vigorously until science is able to establish the facts. **Tim Beardsley**

*Industrial Air Pollution: Health and Safety 1982 (Health and Safety Executive).

0028-0836/84/050406-01$01.00

Wolf winners

Rehovot

THE winners of the 1983–84 Wolf Foundation Prizes in the sciences have been announced in Herzliya, Israel. The Wolf Foundation was set up in 1976 with an endowment fund of $10 million and the prizes, worth $100,000 each, have become known unofficially as the Israeli Nobel prizes. The prizes are awarded for chemistry, physics, mathematics, agriculture, medicine and the arts, but this year there will be no prize for medicine. (The winner of the arts (architecture) prize has yet to be announced.)

The chemistry prize is shared by three US university professors, Herbert S. Gutowsky of the University of Illinois, Herden M. McConnell of Stanford University and John S. Waugh of Massachusetts Institute of Technology, for the "singular contribution" of magnetic resonance spectroscopy to the theory, structure and dynamics of molecules.

The physics prize is shared by Professor Erwin L. Hahn of the University of California at Berkeley, Dr Theodore H.



Sir Peter Hirsch — a share in the physics prize

Maiman of TRW Inc., Los Angeles, and Sir Peter Hirsch, chairman of the United Kingdom Atomic Energy Authority and professor of metallurgy at the University of Oxford. They are honoured for their work in discovering nuclear spin echoes, for the realization of the first operating laser, and for the development of the scanning electron microscope respectively.

The mathematics prize is shared by Professor Shiing S. Chern of the University of California at Berkeley and Professor Paul Erdos of the Mathematics Institute at the Hungarian Academy of Sciences in Budapest. Both are described as "veteran mathematical giants". The agriculture prize goes to Professor Don Kirkham of Iowa State University and Professor Cornelis T. de Wit of the Agricultural University of Wageningen in the Netherlands, both of whom have made "innovative contributions" to the quantitative understanding of soil/water and other environmental interactions influencing crop growth and yield. The awards will be made in Israel in May. Nechemia Meyers

US space station

NASA seeks partners, purpose

Washington

WITH presidential approval for a space station tucked firmly under his belt, James Beggs, administrator of the National Aeronautics and Space Administration (NASA), is about to tour world capitals in search of international partners for the new venture. And because NASA has still only the sketchiest notion of what it wants from a space station, countries willing to make an early investment can expect a big say in the final design.

During the past two years, while NASA has been arguing the case for a space station, tentative discussions have taken place with the European Space Agency, a number of individual European nations, Canada and Japan. But until last week, when President Reagan gave the space station formal approval in his state of the union message, NASA had been unable to negotiate in earnest.

Beggs is now in an unusually strong position. His status during his overseas tour will be as President Reagan's personal emissary. Meanwhile, one of the principal impediments to foreign collaboration — the involvement of the Pentagon — has been removed. The Department of Defense has so far refused to find a military argument for supporting the new venture. As a result, the space station is being justified by the administration solely on scientific and commercial grounds, and there will be no Pentagon contribution to the $8,000 million development budget.

For overseas partners, however, the most attractive feature of NASA's space station is that it has not yet been designed. Beggs intends to wait for at least another two years before completing plans for the station and beginning hardware development. After the state of the union message last week, NASA was careful to keep the technical specifications of the space station as vague as possible.

All that NASA is saying for the moment is that the station will provide for a permanently manned base in a low inclination orbit. In addition to living quarters for a crew of six to eight, the station will house data-processing facilities and a docking hub for the shuttle. Pressurized modules for scientists and astronauts will be attached to the station, as well as unpressurized free-flying platforms capable of performing a variety of functions.

The purpose of the space station is, so far, almost as vague as its design. Possible roles listed by NASA range from the relatively mundane, such as the tending and repair of satellites and space telescopes, to the frankly exotic. In the long run, NASA maintains, the space station could become the first step towards the establishment of a manned lunar base, or a manned mission to Mars.

Despite its glee at having at last won its battle for a space station, NASA was careful last week to mollify some of the groups that had opposed it. The space science board of the National Academy of Sciences concluded last year that developing a manned space station could not be justified on scientific grounds alone. NASA is now promising to work closely with the space science community and to do its best to ensure that space science funds are not raided to provide funds for development of the space station.

Peter David

US materials science

Setback for Keyworth

Washington

AFTER a year of trouble for his plan to establish a National Center for Advanced Materials (NCAM) at Lawrence Berkeley Laboratory (see *Nature* **301**, 456; 1982), presidential science adviser George Keyworth has bowed to the inevitable and asked the National Academy of Sciences to study priorities for "major facilities" for materials science.

The original NCAM plan met with harsh criticism from many materials scientists who complained that the proposal had been sprung on them without warning and without the benefit of peer review. Responding to these criticisms (and perhaps sensing a budget item that could be raided for several pork-barrel projects), Congress last spring cut the $26 million that the President had proposed for NCAM in his budget for fiscal year 1984 to a mere $3 million and ordered that no more should be spent until a scientific review of the project was completed.

A Department of Energy panel headed by Albert Narath of Sandia National Laboratories was appointed to carry out this review; last fall it declared that the centrepiece of the NCAM proposal, a new advanced synchrotron light source intended as a national facility should not be locked up in a laboratory dedicated to materials research. NCAM has since become mere CAM, and Keyworth's efforts to turn the spotlight on materials science have been pushed back to square one.

The National Academy panel, headed by Frederick Seitz, president of Rockefeller University, New York until 1978, will be considering facilities with an initial cost of $5–10 million or more, including synchrotron and neutron sources. The panel will report next summer, in time for its recommendations to be considered for inclusion in the then President's budget for the fiscal year 1985–86. **Stephen Budiansky**

© 1984 Macmillan Journals Ltd

Bad manners, USA

SIR — I recently had the pleasure of participating at the XVth International Congress of Genetics in New Delhi. I would like to congratulate my Indian colleagues for their successful effort. The congress was well organized, the atmosphere was friendly and congenial and the subjects covered were interesting and diverse. It was one of the best international congresses in the past few years.

However, I feel bound to apologize to the Indian organizers for the rude, inexcusable and unacceptable behaviour of a number of my American colleagues who never appeared at plenary sessions or symposia (as chairmen or as speakers). Their names were called without answer. Obviously their commitment to a "developing" country's meeting was not binding. Furthermore, quite a few failed to send a summary of the paper they were presenting at a symposium. Maybe they do not know that Indian students and young scientists are more eager to read than their counterparts in the United States.

ANNAMARIA TORRIANI-GORINI
Department of Biology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139, USA

Israel squeezed out

SIR — Israeli scientists will not be able to participate in the 7th International Biotechnology Symposium to be held in New Delhi, India, 19–24 February 1984.

According to the organizers, scientists from countries without an Indian Mission will obtain entry permits/visas if the organizers are contacted at least six months before the symposium. Since July 1983, I have been trying to obtain information on how to apply for a visa, but to no avail. This symposium is sponsored by international organizations such as the International Committee on Economic and Applied Microbiology and the International Union of Pure and Applied Chemistry and should therefore be open to scientists from all countries of the world.

I call upon fellow scientists to boycott this meeting to show their solidarity and to protest to the organizers and the Indian Government that scientists from all countries cannot participate in the New Delhi meeting. The Indian Government's attitude should also be kept in mind when selecting sites for future international meetings.

J. STEFAN ROKEM
Department of Applied Microbiology,
Hebrew University of Jerusalem, Israel

Creationist view

SIR — Professor Marsden overlooks some important points and makes some logical errors in his recent commentary on the history and sociology of the creation/evolution debate[1]. In his search for a hidden agenda in creationism, he overlooks the legitimate scientific case for creation, and he does not deal with possible philosophical, political, and economic motivations of the other group which seeks to polarize the debate — the evolutionary humanists[2].

He complains about the polarization of the issue as if a middle ground of progressive creationism, based on directed macro-evolution and an old Earth, is supported by scientific data. Professor Marsden has misunderstood radiometric dating and the geological time scale as simple scientific facts (SSFs), just like Newton's third law or the fact that a granite contains potash, feldspar and quartz.

If there were SSFs which indicated that the Earth is old and precluded *fiat* creation, then his point would be well taken. But the methods of evolutionary/uniformitarian geochronology, historical geology and palaeoenvironmental analysis are not SSFs, but rather complex scientific models (CSMs) involving many assumptions, some of which are demonstrably wrong, such as pleochroic haloes in granitic rocks, which are held to prove the constancy of radioactive decay rates through Earth history. Some of these data which argue against the doctrine of uniformity have even been published in *Nature*[3]. The historical assertions that the Cambrian began about 600 million years ago and that the Scopes trial began in 1925 are not equivalent concepts and, as such, should not be taught in the same breath to unwitting students.

Professor Marsden is also wrong in his thesis that we creationists are only a product of some "Post-Civil War cultural crisis". Creationists are convinced by coercive scientific data[4], not solely by conservative theology, demography or profession.

As a fundamentalist, southerner and geologist, I resent several accusations made by Marsden and the implication that a conservative exegesis of *Genesis 9* can support slavery, and that this is part of southern creationists' motivation. I also question his assertion that many southern anti-evolutionists are made because of "the relatively low levels of education of many Southern Bible-believers". I am a creationist because the basic facts of geology (and the other sciences) are consistent with *fiat* creation and several thousand years of Earth history dominated, during brief periods, by processes of catastrophic rate, on a continental scale and of high intensity[5].

DAVID R. MCQUEEN
Geology Section,
Institute for Creation Research,
El Cajon,
California 92021, USA

1. Marsden, G.M. *Nature* **306**, 528–532 (1983).
2. Dunphy, J. *The Humanist* **43**, 26 (1983).
3. Gentry, R.V. *Nature* **244**, 282 (1973).
4. Morris, H.M. & Parker, G.E. *What is Creation Science?* (San Diego, CLP, 1982).
5. Ager, D.V. *The Nature of the Stratigraphical Record* (Wiley, New York, 1981).

Page charge problem

SIR — In scientific publishing a symbiotic equilibrium had been established in which referees evaluate, without payment, papers submitted for publication, in the knowledge that refereeing and publication of papers they themselves submit will be carried out without cost to them. Unfortunately, this equilibrium has been disturbed by a considerable number of (I think exclusively) US journals which now require the prospective author to pay for publication. Many people are therefore being asked to contribute to the publication of journals both by giving free service as referees and also by paying "page charges".

I understand that provision is made on many US research grants for the cost of publication. This is not so in Europe, and it is a practice which, in effect, discriminates against non-US scientists wishing to publish in US journals. Whilst the practice eases the problem for US scientists, the principle of working for, and paying to publish in, the journal seems wrong.

None of us expects or wants payment for refereeing manuscripts. The situation as it existed for many years (and still exists in Europe) was a mutual-help system to which we contributed and from which we benefited. But many scientists and their financiers are unhappy at the extra financial load imposed by some journals.

As a partial solution I propose that referees should ask for page-charge credits which could, wholly or in part, be used to offset the cost of their next publication. In this way no money would change hands and work done by the reviewer would simply result in the amelioration of an added financial burden upon research institutions. The best arrangement, of course, would be to return to the original equilibrium where we paid, and were paid, nothing.

R. SCHOFIELD
Paterson Laboratories,
Christie Hospital &
Holt Radium Institute,
Manchester M20 9BX, UK

Sea cow born again?

SIR — If Steller's sea cow was first discovered in 1759 (as Vera Rich claims in *Nature* **306**, 415; 1983) how did the mining engineer, Retr Yakovlev know it was the "Kapustnik" and fear its extinction in 1755?

R. PARR
Miterdale Farm, Arrington, Royston,
Herts. SG8 0AE, UK

●Vera Rich responds:

Rhytina stelleri
Became extinct early,
After brief fame begun
In 1741.

(1759 was a printer's error).

0028-0836/84/050408-01$01.00

New twist for anthropic principle

The originator of the doctrine that our observations of the Universe are biased because they are made by us now concludes that there have been at most two critical steps in biological evolution.

THE anthropic principle is Dr Brandon Carter's choice of a philosophical middle ground between what he calls the "primitive anthropocentrism" of the pre-Copernicans and "its equally unjustifiable antithesis" of extreme representations of the relativistic cosmological principle, the assertion that, "apart from local inhomogeneities", no place (or time) in the Universe can be privileged in any way. When Carter introduced his principle a decade ago (*IAU Symp.* **63**, 291; 1974), his objective was modestly to point out that the mere fact that our observations of the Universe are made by people who are the products of some 3,000 million years of biological evolution implies an inescapable bias. Carter's latest version of the argument, now published as part of the report of last year's meeting at the Royal Society on the constants of physics (*Phil. Trans. R. Soc.* A **310**, 347-363; 1983) is more radical, and a challenge to biologists as well as astronomers.

The reasons why the anthropic principle should figure in a symposium on the constants of physics are not as surprising as they may seem. The luminosity of a star of given mass, and thus the speed of its evolution from a young star to, say, a red giant, depends linearly on the value of the gravitational constant. If this value were only a little greater than it is now thought to be, the Sun would have run through its evolution faster, so that biological evolution might have got no further than, say, the dinosaurs before the Earth was engulfed by a red giant. And a smaller gravitational constant might never have allowed for the particular circumstances that led to the emergence of living things in the first place. So there is a chance of learning something about the value of the gravitational constant simply by exploiting the circumstance that its evaluation depends on observations made by people. Carter now says that if he had known that the name "anthropic principle" would be so widely used, he would have looked harder for a different way of referring to the bias caused by self-selection.

In passing, Carter directs well-aimed darts at two fashionable preoccupations, speculation about extraterrestrial life and Dirac's large-numbers hypothesis, each of which he says is sustained by neglect of the anthropic principle. Telescoped, the argument is this. The search for extraterrestrial life is based on the hypothesis that the emergence of living things is likely whenever geophysical circumstances are favourable, and thus on a denial of the contradictory hypothesis that the emergence of life is improbable. But, says Carter, the only evidence bearing on the question is the observation (by ourselves) that life has indeed emerged on the surface of the Earth, whence there is no way of distinguishing between the two hypotheses.

Dirac's large-numbers hypothesis is logically identical. Dirac at the outset argued that coincidence between the age of the Universe (strictly, the Hubble time) and the gravitational constant is so remarkable that one must expect the gravitational constant to decrease with time so as to preserve the coincidence. (Numerically, the Hubble time is 10^{58} in units in which the velocity of light, Planck's constant and the gravitational coupling strength are determined by the three-halves power of the square of the proton mass, of the order of 10^{38} in the same units.) Carter's view is that the coincidence is not a coincidence at all, but a consequence of the selection bias occasioned because the observations are necessarily made by people whose existence requires a Hubble time large enough so that some stars can have completed their evolution and have generated the chemical elements of which people are made, but not so long that all stars have run their course.

This is the stuff of lunch-room arguments about the anthropic principle in the past decade. Carter's new departure begins with another coincidence — that between the time-span of biological evolution on the surface of the Earth (say, 0.4×10^{10} years) and the length of time the Sun is likely to spend as a hydrogen-burning star (say 10^{10} years) before becoming a red giant with dimensions engulfing the Earth's orbit.

What connection can there be between two such apparently independent quantities as the timescale of biological evolution, presumably determined by the complexity of living organisms, and the timescale of stellar evolution, determined (in Carter's words) by "the weakness of gravitation"? None, he says. The apparent coincidence is merely a consequence of the "habitual mistake" of overlooking the anthropic principle.

The most plausible way of accounting for it is to suppose that the timescale of biological evolution, itself a Markovian process (one thing leading to another) whose course must be uncertain, is on the average much longer than the time allowed by the lifespan of the Sun as a hydrogen-burning star. So the observation, after a mere 4,000 million years, that there are sentient beings able to contemplate their own emergence is a sign that, in the event, the course of evolution on the Earth has been exceptionally short.

Carter takes this argument one argumentative step further. Suppose, he says, that the evolution of sentient beings depends on n critical steps (defined as unlikely evolutionary steps, which are thus "slow", compared with the majority of evolutionary steps). Then it is possible to show that the chance of all these n steps being completed by time t is proportional to t^n and also to show that evolution (the Sun's time as a hydrogen-burning star or T_0) exceeds that occupied by evolution so far by a quantity of the order of $(1/n)t$. But this time is itself of the order of T_0, whence Carter concludes that n must be a whole number greater than zero and less than or equal to two — in short, either 1 or 2. Carter insists that the general character of this result does not depend on the details of the calculation but acknowledges that it poses a dilemma: how can the fossil record be squared with the conclusion that there have been at most two critical steps in evolution so far? His own preference is to face the dilemma head on; he recommends the evolution of the genetic code and the organization of the nervous system as candidates for the two critical steps.

What weight should be given to this conclusion? In his paper, Carter does not seek to mollify his critics, dismissing the attention being paid to the "inflationary universe" in all its varieties as an attempt to resurrect the "beguiling notion" of the perfect cosmological principle, and complaining that those who hold that only falsifiable hypotheses are admissible are in effect saying that science has accomplished nothing so far.

The Popper school will be further offended that Carter not merely uses the verb "to induce" but that he openly uses the language of Bayesian probability, the calculus of informed guesswork as some consider it. Even his friends may say that he has too lightly discarded the possibility that his coincidence may be explained if evolution is a rapid process which happens on the Earth to have been slow. But Carter is plainly not afraid to stir up trouble and has also provided a tangible example of how the anthropic principle may be made quantitative.

John Maddox

© 1984 Macmillan Journals Ltd

Ecology

A test of ideas about mutualism

from Robert M. May

THE past few years have seen some sharp debate over the extent to which competitive interactions are of importance in determining the structure of plant and animal communities[1,2]. In this debate, it is sometimes claimed that the 1960s and 1970s were an era in which researchers regarded competition as essentially the only force moulding community structure. Such charges are clearly exaggerations, as these two decades equally saw an upsurge in empirical and theoretical work on predator–prey and parasitoid–host interactions. Any contemporary ecology text, American or British, reflects this attention given both to competitive and to predator–prey associations. What is true, however, is that mutualism has remained relatively neglected — in field, laboratory, theory and textbooks — until recently.

Why this relative lack of attention to mutualism? My view is that it is partly because tight mutualistic associations between clearly distinct species (as opposed, for example, to entities like lichens, where algae and fungi have fused into something akin to a single organism) are less conspicuous in temperate regions than are competitive or predator–prey associations; only relatively recently has there been much ecological research in the tropics where mutualistic interactions are conspicuous elements of community organization. The idea that obligate mutualisms may be dynamically fragile (and consequently more likely to be found in the environmentally stable tropics) essentially goes back to Darwin and Wallace, who both noted that obligate associations between plants and pollinators are not easily transported to islands. These ideas are explored in more detail elsewhere[3,4].

D.S. Wilson has advanced an interesting alternative explanation for this relative neglect of mutualism[5]. He argues that the controversies about group selection in the 1960s led to a sharper recognition — crystallized in Williams's important book[6] on *Adaptation and Natural Selection* (1966) — that conventional Darwinian selection acts on individual organisms, not on groups or species as such. Much of the earlier discussion of mutualism, however, made implicit or explicit appeal to adaptations that benefited groups rather than individuals. Wilson notes that many older textbooks, such as the influential *Principles of Animal Ecology* (1949) by Allee *et al.*[7], had treated mutualism as a dominant interaction in well-ordered communities; but the explanation was in terms of the adaptation of species rather than individuals. Williams argued that mutualism can evolve, but only if conditions are such that it is advantageous for the individual involved, and he went on to observe[6] (p.247) "really good examples of mutualism are relatively rare, and it must be that these necessary [conditions] seldom arise".

Contemporary theory, as developed by Wilson[8,9] and Wade[10] (and reviewed by Uyenoyama and Feldman[11] and others[12]), considers more explicitly the effects that group size may have on the evolution of mutualistic associations. Suppose a species contains both mutualistic and nonmutualistic types: the mutualist benefits another species in its vicinity, but at a cost, c, to itself; the enhanced presence of the other species reciprocally confers a benefit, b, on all N individuals (mutualists and nonmutualists alike) in the group of which the mutualist is a member. It is clear that the proportion of mutualists must decrease in any group containing both types, because they alone bear a cost while the entire group benefits: this constitutes an evolutionary force against mutualism. But groups with many mutualists will, by virtue of the benefits conferred, produce more offspring than those with few mutualists: this constitutes an evolutionary force favouring mutualism. Thus these models suggest that[5] "the evolution of mutualism is a balance between the opposing forces of relative fitness within groups (individual selection) and the differential productivity of groups (group selection)"; The outcome will depend on the costs c, benefits b and — most important — on the degree of variability in group sizes (which variability will, under most statistical assumptions, be more pronounced if the average group size N is relatively small): mutualism will be favoured by small c, large b and small N.

Unfortunately, experimental tests of these ideas are difficult. For one thing, the parameters c and b can be hard to define, much less measure, in field settings. For another, the effective group size N may be smaller than it appears, either because apparently large groups actually derive from a small number of colonists, or because the population structure results in very large variation in size among groups (promoting the evolution of mutualism).

Wilson has fastened on the association between burying beetles and the mites they carry around as a system that may be particularly amenable to field and laboratory exploration of these questions about mutualism[5]. The associations he has been studying include five sympatric species of burying beetles of the genus *Nicrophorus*. These creatures are carrion feeders. They raise their broods on small carcasses (mainly dead rodents); the carcass is first buried, and the beetles then remove its hair and roll it into a ball. This remarkable sequence of actions is carried out by a pair of beetles, after which the male leaves while the female remains until her brood has completed its larval development. In northern Michigan, carried around on these beetles are some 15 species of mites from four families and at least one species of nematode. These 'phoretic' mites disperse to new habitats by attaching to the beetles and then abandoning their carriers at the new habitat to follow a free-living existence; the larval mites develop on buried carcasses, side by side with the beetle larvae that represent the mites' next 'aeroplane'. When mites leave their beetle host to take up residence on a buried carcass, they search out and destroy (by piercing with their chelicerae) fly eggs that may be present; thus the mites may benefit the beetles by reducing competition for carcasses by flies. Finally, Wilson observes that mixing of adult mite populations takes place at large unburiable carcasses where large numbers of beetles congregate to feed.

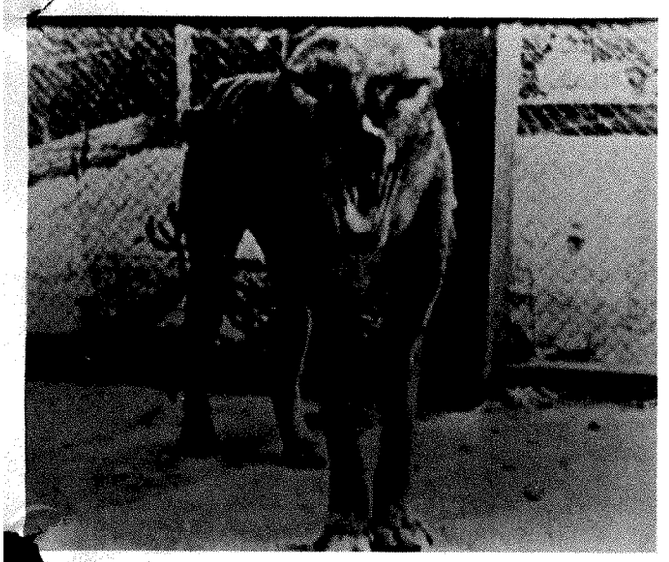
In short, these associations between phoretic mites and burying beetles exhibit the conjunction of characteristics envisioned in the models described above: on the one hand, local 'trait groups' of different sizes, in which mutualistic effects may be present, are found on individual buried carcasses; on the other hand, there is mixing of groups at the large carcasses where adult beetles feed communally.

Wilson focuses mainly on field and laboratory studies of the association between the mite *Poecilochirus necrophori* and the beetle *N. tomentosus*. He shows that such beetles carry on average about 12 *P. necrophori*, which (given that carcasses are buried by a male and female pair of beetles) makes the average group size rather large, $N \sim 24$; Wilson also shows there is much variability in the size of these groups. A complicated series of experiments can be summarized as broadly leading to the conclusion that the mites' habit of piercing the eggs of flies strongly enhances the breeding success of the carrier beetle species: in field studies, *N. tomentosus* raised broods of average weight 0.092 g from 26 of 30 buried carcasses when *P. necrophori* was present, while broods of average weight 0.088 g were raised from 15 of 28 buried carcasses in the absence of *P. necrophori*; this overall twofold difference in reproductive success is statistically highly significant.

Wilson discusses the possibility that the mites may pierce eggs purely to feed on them, so that the beneficial effects to the beetles are incidental rather than the outcome of a co-evolved mutualism. Were this the case, it would be expected that mite fecundity would be correlated, to some extent, with the presence of fly eggs. But a variety of laboratory and field experiments shows no correlation between mite fecundity and abundance of fly eggs; even the total absence of eggs has no discernible effect, as the mites reproduce well on the carcass itself. Costs associated with the egg-piercing behaviour are harder to document

0028-0836/84/050410-01$01.00

Last gape of the Tasmanian tiger





THAT most curious of marsupials, the thylacine or 'Tasmanian tiger', is commonly assumed by zoologists to be extinct. The last known specimen died in captivity in Beaumaris Zoo, Hobart on 7 September 1936 after a deadly campaign led by Tasmanian sheep-farmers over the preceding half-century. Anecdotal reports of more recent encounters notwithstanding, no solid evidence has been forthcoming since then despite several major expeditions and many forays by wildlife enthusiasts.

Thylacinus cynocephalus — literally 'pouched dog with a wolf head' — managed to combine a lupine visage with a tiger's stripes, a snake's gape, a kangaroo's tail and a unique rear-opening pouch (the thylacine loped about on all fours and would otherwise have risked injury to its young from rough undergrowth). From nose to tip of tapering tail the thylacine measured up to 2 metres, in height 55 cm and in weight 20 kg, thus qualifying as the largest carnivorous marsupial. Although its tawny fur and other external characteristics can be seen in pelts preserved at the British Museum (Natural History) and elsewhere, the astonishing capacity to open its jaws to an angle of over 120° is best appreciated on this unique surviving photograph, the last recorded gape of the Hobart specimen *circa* 1935. The two frames are reproduced from an 8-mm film by courtesy of the State Library of Tasmania.

The latest in a long line of claimed sightings was made in 1982 in northwestern Tasmania by a ranger of the National Parks and Wildlife Service, according to just released press reports. The news had been kept secret to deter hunters and sightseers, but the deployment of trip cameras and sand traps over the last eighteen months yielded neither photographs nor spoor. □

This is the first in a series of historic scientific photographs which will be appearing in News and Views. *They are selected from* Beyond Vision *by Jon Darius, to be published in April by Oxford University Press.*

(and if there are no costs, then in the above models mutualism can of course evolve in groups of arbitrary size). Wilson suggests two kinds of cost, which he hopes to quantify in future experiments. First, since the mites' diligence in piercing every egg they can find apparently pays no dividend in enhancing reproductive success, it represents a departure from optimal foraging; this is likely to carry some cost, even in the mites' food-rich environment. Second, the habit of *P. necrophori* of leaving its beetle carrier upon discovery of a carcass incurs the substantial risk of the carcass being appropriated by some other species of animal. As Wilson observes[5], "A mite that abandons its carrier only to have it fly away has made a poor choice, and I do occasionally observe a few *P. necrophori* wandering forlornly around a half-built burial chamber that has been robbed by a mammal. Yet the carrier must be abandoned to pierce fly eggs. Assuming that the beetle does not force abandonment, this may represent the greatest potential cost of the mutualistic behavior".

As Wilson emphasizes[5], this research programme is still in its formative stages. The existing work does a lot towards quantifying the benefits and the population structure of one particular mite–beetle association, but there is a need for more information (particularly about the costs). Beyond this, it can more generally be remarked that burying beetles live in a very competitive environment, where their relatively slowly developing larvae (the eggs hatch several days after the carcass is buried, and larvae take 10 to 20 days to complete their development) must contend both with faster developing flies (where the span from egg to adult fly takes seven days or less) and with the problem of 'spoilage' of the carcass by microbial activities. The mite *P. necrophori* is just one member of a diverse phoretic community; *N. orbicollis* species in northern Michigan, for example, typically carry seven species of mites and at least one nematode species. Wilson suggests that, as most of the mites filter microorganisms, these mite–beetle associations may represent elaborately co-evolved mutualisms (with prevention of spoilage of the buried carcass being an important component), in spite of average group sizes exceeding 100 mites per beetle. He concludes that[5] "it seems possible that the beetles and their phoretic associates have congealed into a multispecies mutualistic network that systematically alters the local environment in ways that cause its own perpetuation. If so, then the structure of these miniature communities more closely resembles the view of Allee *et al.*[7] than more modern ecological theories". In its larger aspects, Wilson's experimental study will of necessity unfold slowly. □

Robert M. May is Class of 1877 Professor of Zoology at Princeton University, Princeton, New Jersey 08544.

1. Lewin, R. *Science* **221**, 636; 737 (1983).
2. Harvey, P. & Silvertown, J. *New Scient.* **99**, 760 (1983).
3. May, R.M. (ed.) *Theoretical Ecology, Second Edition* Ch.5 (Blackwell, Oxford, 1981).
4. May, R.M. *Nature* **296**, 803 (1982).
5. Wilson, D.S. *Am. Nat.* **121**, 851 (1983).
6. Williams, G.C. *Adaptation and Natural Selection* (Princeton University Press, 1966).
7. Allee, W.C., Emerson, A.E., Park, O., Park, T. & Schmidt, K.P. *Principles of Animal Ecology* (Saunders, Philadelphia, 1949).
8. Wilson, D.S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 143 (1975).
9. Wilson, D.S. *The Natural Selection of Populations and Communities* (Benjamin, Menlo Park, 1980).
10. Wade, M.J. *Q. Rev. Biol.* **53**, 101 (1978).
11. Uyenoyama, M. & Feldman, M.W. *Theor. Pop. Biol.* **17**, 380 (1980).
12. May, R.M. *Nature* **254**, 485 (1975).

© 1984 Macmillan Journals Ltd

Gene expression

The end of the message and beyond

from Nick Proudfoot

SINCE last reviewed in these columns a little over a year ago[1], several major steps forward have been made in understanding how the 3′ ends of eukaryotic mRNA are formed. First and foremost, histone genes have joined other eukaryotic RNA polymerase II genes in generating their 3′ ends not by simple termination of transcription, but by termination followed by post-transcriptional cleavage. The activity responsible for this process has been nearly purified and looks very much like a small nuclear riboprotein or 'snurp', not the U1 snurp responsible for intron splicing, but another as yet unidentified one. Second, mutation of the AAUAAA conserved sequence found at the 3′ ends of poly(A) containing mRNAs has been shown to cause elongation of the mRNA transcript[2]. This formally proves that the AAUAAA sequence is required for mRNA 3′ end formation, and strongly suggests that the sequence is a signal for endonucleolitic cleavage. Finally, the feeling that RNA polymerase II must have a precise termination process, rather than just falling off the gene template at random, has been virtually proven, at least for the mouse β major globin gene.

Max Birnstiel's laboratory in Zurich has been working on the transcription of sea urchin histone genes by injecting them, together or separately, into *Xenopus* oocytes[3,4]. Not surprisingly, several of the activities necessary for the generation of sea urchin histone mRNA are absent in oocytes. Birnstiel's group therefore tried co-injecting sea urchin extracts along with the histone genes to complement the oocyte transcriptional apparatus[5] and found that with the extracts, histone gene H3 does generate mRNA that terminates at the correct 3′ position, while without the extracts it does not. Purification of the activity using this complementation factor assay[6] has now revealed that it is a nuclear RNA–protein complex known affectionately as a 'snurp'. Indeed, even a 60-nucleotide RNA fraction free of protein stimulates the production of correct histone mRNA 3′ends when injected along with the histone gene into oocytes — presumably oocytes can package the RNA into a snurp[7]. Although this group's experiments strongly imply that histone mRNA 3′ ends are generated by endonucleolitic cleavage, the final proof has come from other work.

Krieg and Melton of Harvard University[8] took a different histone gene, placed it under the influence of a bacteriophage (SP6) promoter[9], and synthesized *in vitro* transcripts extending beyond the 3′ end of the mRNA. When these RNAs were injected into oocytes, correct histone mRNA 3′ ends were created, proving that a precise RNA endonuclease activity is present in oocytes, capable of producing histone mRNA 3′ ends. Similar results have been obtained by Birnstiel's group (unpublished data) and by Carl Parker at Caltech (personal communication). Presumably the 'histone snurp', either single-handed or with the help of other activities, cleaves the histone mRNA primary transcript at the mRNA 3′ end position.

Birnstiel's group has now gone on to define rather precisely what these activities recognize in the primary RNA transcript[10]. Clearly, the hairpin at the histone mRNA 3′ end is all-important, although site-directed mutagenesis shows it does not matter exactly what sequence the hairpin has, as long as base-pairing is maximized. Also, the immediate 3′ flanking sequence to the hairpin structure appears to be necessary to form the mRNA 3′ end. In other words, the recognition site for cleavage is fairly complex — and that will make the precise biochemistry of the RNA–protein interactions hard to unravel.

Understanding of the processes involved in the formation of poly(A)-containing mRNA 3′ ends has similarly progressed. The conserved sequence AAUAAA is clearly important as two site-directed mutagenesis experiments show. In one case[11], the adenovirus E1A poly(A) site was mutated to AAGAAA, causing all E1A transcripts to extend into the next E1B gene as cleavage at the E1A mRNA 3′ end was prevented. In the second case[12], experiments on a new type of non-deletion α-thalassaemia discovered in Saudi Arabia (Saudi α-thal) have shown that the human α2 gene contains a single base change in the poly(A) site, AAUAAG, that again results in the production of α-globin mRNA with 3′ ends extending way beyond the normal 3′ end. These transcripts are unstable in human reticulocytes so insufficient globin is produced and the α-thalassaemia phenotype is seen.

Clearly, these two studies strongly suggest that AAUAAA forms part of a recognition signal for the endonucleolitic cleavage necessary to generate a correct mRNA 3′ end. A recent theoretical study by Susan Berget[13] neatly lends support to this presumption. She argues that the AAUAAA sequence, together with another less convincing sequence homology CApyUG, usually found close to the site of poly(A) addition[14], can base-pair reasonably well to a small nuclear RNA called U4. She assumes, of course, that cleavage at the AAUAAA sequence is yet another snurp-mediated process.

Considerable progress has also been made in understanding the first snurp to be talked about, U1. Since the original sequence homology arguments[15,16] that suggested U1 could base-pair with intron donor and acceptor sites[17,18] and thereby facilitate intron splicing, several more direct experiments have been carried out. Work in Joan Steitz's laboratory, where the term 'snurp' was first coined[19], has demonstrated the direct interaction of U1 with an intron donor site[20]. Also, very recently, *in vitro* splicing of adenovirus introns has been convincingly demonstrated in HeLa cell extracts[21,22]. Using this *in vitro* splicing system, Phillip Sharp's laboratory, in collaboration with Joan Steitz, has demonstrated the inhibition of *in vitro* splicing by antibodies directed against U1 snurps[23]. Further evidence comes from Hernandez and Keller[22] who have partially purified one 'splicing' component of the *in vitro* splicing extract and shown that this fraction co-purifies with snurps.

Finally, yeast introns have been shown to contain their own U1-like sequence actually as part of the intron sequence close to the 3′ acceptor site[24,25]. If it performs the right contortions the sequence may be able to interact with the donor site and mediate intron excision. It would thus seem that there is one general mechanism for the post-transcriptional processing of RNA polymerase II gene transcripts: each cleavage event, be it the removal of an intron or the formation of a mRNA 3′ end, is initiated by specific sequence recognition between the gene transcript and a small nuclear RNA. Activities associated with this RNA (that is, the snurp proteins) then cleave the gene transcript and are followed by RNA ligation in the case of intron removal, or by polyadenylation in the case of mRNA 3′ end formation.

Returning to the 3′ end of the mRNA, if, as described above, both histone and poly-(A)-containing mRNAs generate their 3′ ends by RNA cleavage, then where does RNA transcription actually stop? Specific RNA termination sites have been demonstrated in bacteria, so we might expect eukaryotes to have them too — and indeed this has now been shown to be the case, at least for the mouse β major globin gene. Jim Darnell and his colleagues at the Rockefeller University, New York, have precisely pinpointed a position about 1 kilobase beyond the end of the gene, beyond which no RNA transcripts can be detected[26,27]. They used a tissue culture cell line — the mouse erythroleukaemic Friend cell — that can be induced by various chemicals such as dimethyl sulphoxide to express high levels of globin mRNA and protein. By pulse-labelling the cells with [α-^{32}P]UTP, newly synthesized precursor RNAs were ^{32}P-labelled. Nuclear RNA was then purified and hybridized to single-strand 3′ flanking region DNA fragments

0028-0836/84/050412-01$01.00

cloned into the single-strand bacteriophage vector M13. The RNA–DNA hybrids obtained were next treated with ribonuclease and the protected RNAs fractionated on gels. One 3′ flanking region M13 clone protected RNA for only half of its length. The 3′ flanking region M13 clones taken from in front of this clone protected RNAs for their whole length, whilst the 3′ flanking region M13 clones from beyond the clone produced no protected RNA. These results clearly show the presence of a discrete RNA 3′ end well beyond the end of the mRNA. It seems likely that Darnell's experiments have identified a eukaryotic termination site. If such a site exists in the mouse β major gene, then it seems plausible that many, if not all, eukaryotic genes have termination sites.

Why are the termination of transcription and the formation of mRNA 3′ ends separate processes? Well, what at first sight appears an unnecessary complexity may perhaps give an additional control over gene transcription. We may find that specific factors modulate the efficiency of trancriptional termination. If termination were prevented, then either the primary transcript could be rendered even more unstable, so that less mature mRNA could be produced from it before it turned over, or alternatively, transcription might read through into the next gene and disrupt its normal transcription. Obviously, these possibilities are only speculation. But the view that all the controls that modulate eukaryotic gene expression centre on the promoter may well prove too narrow. □

Nick Proudfoot is in the Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE.

1. Proudfoot, N.J. 2 *Nature* **298**, 516 (1982).
2. Proudfoot, N.J. & Brownlee, G.G. *Nature* **263**, 211 (1976).
3. Hentschel, C.C. & Birnstiel, M.L. *Cell* **25**, 301 (1981).
4. Hentschel, C.C., Probst, E. & Birnstiel, M.L. *Nature* **288**, 100 (1980).
5. Stunnenburg, H.G. & Birnstiel, M.L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6201 (1982).
6. Galli, G. *et al.* **34**, 823 (1983).
7. De Robertis, E. M. *Cell* **32**, 1021 (1983).
8. Krieg, P.A. & Melton, D.A. *Nature*, (in the press).
9. Green, M.R., Maniatis, T. & Melton, D.A. *Cell* **32**, 681 (1983).
10. Birchmeier, C., Folk, W. & Birnstiel, M.L. *Cell* **35**, 433 (1983).
11. Montell, C., Fisher, E.F., Caruthers, M.H. & Berk, A.J. *Nature* **305**, 600 (1983).
12. Higgs, D.R. *et al. Nature* **306**, 398 (1983).
13. Berget, S. *Nature* (in the press).
14. Benoist, C., O'Hare, K., Breathnach, R. & Chambon, P. *Nucleic Acids Res.* **8**, 127 (1980).
15. Breathnach, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 53 (1978).
16. Mount, S.M. *Nucleic Acids Res.* **10**, 459 (1982).
17. Lerner, M.R., Boyle, J.A. Mount, S.M., Wolin, S.L. & Steitz, J.A. *Nature* **283**, 220 (1980).
18. Rodgers, J. & Wall, R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1877 (1980).
19. Lerner, M.R. & Steitz, J.A. *Cell* **25**, 298 (1981).
20. Mount, S.M. *et al. Cell* **33**, 509 (1983).
21. Padgett, R.A., Hardy, S.F. & Sharp, P.A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5230 (1983).
22. Hernandez, N. & Keller, W. *Cell* **35**, 89 (1983).
23. Padgett, R.A., Mount, S.M., Steitz, J.A. & Sharp, P.A. *Cell* **35**, 101 (1983).
24. Langford, C.J. & Gallwitz, D. *Cell* **33**, 519 (1983).
25. Pikielny, C.W., Teem, J.L. & Rosbash, M. *Cell* **34**, 395 (1983).
26. Hofer, E., Hofer-Warbinek, R. & Darnell J.E. Jr *Cell* **29**, 887 (1982).
27. Salditt-Georgieff, M. & Darnell J.E. Jr *Proc. natn. Acad. Sci. U.S.A.* **80**, 4694 (1983).

Developmental biology

Morphogenesis by fibroblast traction

from Julian Lewis

THE fibroblasts that make up the extracellular matrix in the adult body may seem dull, commonplace cells, cast in a supporting role while others play the star parts. But fibroblasts in the embryo appear in a more interesting light. They are the cells that carry much of the information that dictates the structure of the skeleton[1], the locations of the muscles[2], the routes of the nerves[3] and the patterning of the skin[4]. They are the architects of connective tissue as well as the makers of the raw materials. They work on the collagen that they have synthesized and secreted, compacting it into sheets, drawing it out into cables, crawling over it, tugging upon it; and with its help, controlling the disposition of other cells and organs[5].

Though fragments of evidence have been available for a long time[6], it is only recently that experiments have brought the mechanical role of embryonic fibroblasts clearly into focus. A fibroblast alone in a culture dish will crawl about: it extends its leading edge, adheres with it to the substratum and uses the new site of anchorage to pull itself forwards, probably by an actin-based contractile mechanism. In the process, the fibroblast elongates itself for its trailing edge also is stuck to the substratum and does not easily detach.

Since action and reaction are equal and opposite, a force must be exerted on the substratum, tending to put it into compression in the region between the two ends of the fibroblast and into tension beyond them. The force can be made visible by culturing the cell on a very thin sheet of silicone rubber, which is thrown into compression wrinkles beneath the cell and into tension wrinkles beyond its ends[7]. The compression wrinkles run transverse to the long axis of the cell, while the tension wrinkles radiate from its ends.

Fibroblasts show their strength in a more dramatic way when they have collagen fibres to work on, instead of a rubber sheet. The clearest demonstration has come from experiments using an artificial collagen gel prepared from a solution of dissociated collagen subunits which is poured into a Petri dish and allowed to set by reassociation, forming a mesh of randomly oriented collagen fibrils. Living fibroblasts can be added so as to create a sort of artificial loose connective tissue in which the cells are uniformly dispersed. The gel then contracts away from the walls of the Petri dish, squeezing fluid out of the meshes of the collagen, and by the end of the week is compacted into a pellet with a diameter which may be less than a fifth of the original — corresponding to a volume less than a hundredth of the original[5-8]. The contraction occurs only if fibroblasts are present, and it can be prevented by adding cytochalasin, which is known to disrupt actin filaments and hence the intracellular contractile mechanisms that depend on them[8]. If vertical pegs are fixed to the base of the dish to prevent contraction of the gel, then collagen is stretched into rope-like bands that loop around the peg and are pulled so taut that they will often snap[5].

The contraction of the artificial gel is clearly similar to the contraction of granulation tissue in wound healing, well known to pathologists, who have coined the term 'myofibroblast' to describe the muscle-like nature of the fibroblasts that bring it about[9]. In fact, studies of wound contraction provided the earliest clear evidence of the force-generating role of fibroblasts in a collagen matrix[6].

There is, however, a major difference between the action of fibroblasts and that of muscle cells. Muscle cells are permanently attached at both ends to other structures, and bring about contraction of the tissue simply by shortening themselves. The fibroblast does more: while exerting a contractile force, it changes its points of attachment, hauling in more and more collagen, as a sailor hauls rope hand-over-hand. Indeed, the analogy goes further. Just as sailors use their ropes to manoeuvre the masts and sails, so fibroblasts use collagen fibres to pull the parts of the developing body into their proper positions; and just as sailors can swarm up the rigging, so fibroblasts can swarm along the collagen fibres that they have themselves formed and aligned.

These points are illustrated by further experiments *in vitro*[5]. If a small chunk of tissue containing fibroblasts is excised from a chick embryo and placed in a collagen gel that is otherwise cell-free, it will begin to draw in collagen from its surroundings, creating for itself a capsule of densely packed and circumferentially oriented fibres, and further out, beyond the most peripheral fibroblasts, setting up stresses that orient the collagen radially. If two such explants are placed far apart in the gel, a very striking 'two-centre effect' is seen: along the line that joins them, in the cell-free region, the collagen becomes organized into a compact band of aligned fibres[10]. Thus if embryonic cartilage rudiments, stripped of loose connective tissue but retaining a thin layer of perichondrial fibroblasts, are placed in the gel, they recruit for themselves thick new coats of perichondrial collagen and become linked by oriented collagen bands resembling

© 1984 Macmillan Journals Ltd

ligaments.

If small chunks of embryonic muscle tissue (consisting of muscle cells mingled with fibroblasts) are also placed in the dish, they become connected to the cartilage rudiments and to one another by bands of collagen equivalent to tendons; the muscle fragments, initially shapeless blobs in random positions, become stretched out into well-formed muscle bellies and are towed into place by the collagenous attachments to the skeleton[5]. Paradoxically, the muscle cells appear to be the passengers rather than the engines of such movements. In an embryonic limb bud that has been deprived of cells capable of developing into muscle, the skeleton at first forms normally, and so do the tendons, even though they have no muscles to pull on them[11].

Oriented fibrils in an extracellular matrix can orient cell movements (as a paper in this issue of *Nature*, p.453, from Norio Nakatsuji and K. Johnson demonstrates in the context of the amphibian gastrula[12]). When fragments of tissue are explanted into a collagen gel, cells — presumably fibroblasts — migrate out into the uninhabited regions of the gel; and the migrants move most rapidly, and in largest numbers, along the dense oriented bands of collagen that have been created by fibroblast traction. Thus the fibroblasts govern the disposition of collagen, and the collagen in turn affects the disposition of the fibroblasts.

Furthermore, a strong and active cluster of fibroblasts surrounded by collagenous matrix will tug towards itself not only the matrix but also any fibroblasts that may be clinging to it. Once recruited into the cluster, these cells will add to its strength. Thus inhomogeneities in the distribution of fibroblasts will tend to be self-amplifying.

Clearly, fibroblasts and collagen fibres together make a most remarkable dynamic system. A recent paper defines its properties in mathematical terms and discusses its capabilities for pattern formation[13]. For example, a homogeneous tissue of fibroblasts dispersed in a random matrix may become unstable against small departures from uniformity, and so spontaneously develop a pattern in which the cells become clustered and the collagen becomes organized into tracts.

Just such a phenomenon is seen in the skin of a bird embryo as feather tracts form. The fibroblasts of the previously almost uniform embryonic dermis gather into a regular lattice of condensations, corresponding to feather rudiments, linked by oriented bands of collagen. The whole transformation is blocked in the presence of collagenase[14]. In appropriate conditions, an artificial gel of collagen containing dispersed fibroblasts will show roughly similar behaviour[15,16]. Theory and experiment here converge in a most persuasive way.

A bolder and more dubious application of the theory of fibroblast traction aims to explain the origins of the pattern of long bones in a developing limb as a result of mechanical instabilities in the mesenchyme of the undifferentiated limb bud, creating non-uniformities where none existed before[13]. Limb-bud specialists are likely to be sceptical: there is too much evidence that cells of the apparently undifferentiated mesenchyme are already intrinsically different and determined for the formation of specific skeletal structures even before any cartilage 'condensations' are visible[1]; and the patterning of the skeleton seems too insensitive to artificial and mechanical disturbances. There is surely more to pattern formation in the developing limb than mechanical instabilities generated by fibroblast traction: but it is no less sure that fibroblast traction plays a major part in the process. □

Julian Lewis is in the Department of Anatomy, King's College London, Strand, London WC2R 2LS.

1. Summerbell, D., Lewis, J.H. & Wolpert, L. *Nature* **244**, 492 (1973).
2. Chevallier, A. & Kienny, M. *Wilhelm Roux's Arch. dev. Biol.* **191**, 277 (1982).
3. Lewis, J., Chevalier, A., Kieny, M. & Wolpert, L. *J. Embryol. exp. Morph.* **64**, 211 (1981).
4. Sengel, P. *Morphogenesis of Skin* (Cambridge University Press, 1976).
5. Stopak, D. & Harris, A.K. *Devl Biol.* **90**, 383 (1982).
6. Abercrombie, M., Flint, M.H. & James, D.W. *J. Embryol. exp. Morph.* **4**, 167 (1956).
7. Harris, A.K., Wild, P. & Stopak, D. *Science* **208**, 177 (1980).
8. Bell, E., Ivarsson, B. & Merrill, C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1274 (1979).
9. Majno, G., Gabbiani, G., Hirschel, B.J., Ryan, G.B. & Statkov, P.R. *Science* **173**, 548 (1971).
10. Harris, A.K., Stopak, D. & Wild, P. *Nature* **290**, 249 (1981).
11. Kieny, M. & Chevalier, A. *J. Embryol. exp. Morph.* **49**, 153 (1979).
12. Nakatsuji, N. & Johnson, K.E. *Nature* **307**, 453 (1984).
13. Oster, G.F., Murray, J.D. & Harris, A.K. *J. Embryol. exp. Morph.* **78**, 83 (1983).
14. Stuart, E. & Moscona, A. *Science* **157**, 947 (1967).
15. Bellows, C.G., Melcher, A.H. & Aublin, J.E. *J. Cell Sci.* **50**, 299 (1981).
16. Harris, A.K., Stopak, D. & Warner, P. (unpublished).

Obituary

Ahmad Bukhari 1943–1983

from Neville Symonds

AHMAD BUKHARI had exercised a formative influence on research in transposable elements for close to a decade and was at the height of his powers when he died suddenly of a heart attack on 19 November 1983.

His earliest major contribution, in 1975, concerned the excision of transposable elements, an issue he always felt was undervalued in the rush to understand how replicative transposition occurs. He was able to show that most bacteriophage Mu insertions in a host gene could be excised exactly with the restoration of gene activity, so demonstrating for the first time that Mu transposition normally occurs without any damage to target DNA sequences.

With regard to replicative transposition, he and Ljungquist published a crucial paper in 1977 showing that coupled transposition and replication of Mu proceeds without Mu leaving its original prophage site. Using an ingenious combination of restriction analysis and DNA–DNA hybridization, they examined the state of prophage Mu DNA after induction and were able to demonstrate conclusively that the original Mu–host junction fragments remained intact well into the latent period after many rounds of replication had occurred.

Bukhari's strength as a scientist rested on his ability to see problems as a whole and to appreciate the elements that were crucial to their understanding. This is exemplified in the beautiful review he wrote for *Annual Review of Genetics* (**10**, 389) in 1976, 'Bacteriophage Mu as a transposition element', in which he brought together the known experimental findings about Mu, assessed their importance from the viewpoint of transposition, indicated the areas where further ideas and investigations were needed and finally related Mu behaviour to that of other insertion elements.

Recently, research on Mu at Cold Spring Harbor has focused on the mechanism by which Mu transposes, paying particular attention to the findings that transposition sometimes leads to replicon-fusions (alternatively called co-integrates) and sometimes to simple insertions. These studies led Harshey and Bukhari to propose a variant to the Shapiro model for transposition which has stimulated a considerable amount of theoretical discussion and practical activity, and which is supported by observation of Mu transposition intermediates using electron microscopy.

Bukhari was born in the Punjab and was an undergraduate at the University of Karachi, leaving for the US in 1966 to carry out graduate studies at Brown University and the University of Colorado. From 1970 he worked continuously at the Cold Spring Harbor Laboratory, and was the centre of one of the most active research groups working with bacteriophage Mu.

As a person Bukhari was emotional and enthusiastic with a variety of interests and friends. He maintained his connections with Pakistan, at the personal as well as the scientific level, and was a scientific adviser to both UNESCO and UNIDO. He will be sorely missed both for his intuition as a scientist and for his humanity as a friend. □

Neville Symonds is Professor of Microbial Genetics at the School of Biological Sciences, The University of Sussex, Falmer, Brighton, Sussex BN1 9QG.

0028-0836/84/050414-01$01.00

Molecular biology

Multiple forms of mitochondrial DNA in higher plants

from Thomas D. Fox

THE study of the structure of the mitochondrial genomes of higher plants is a daunting task. Simply in terms of size there are problems enough — the genomes can contain from about 200 kilobases (kb) to over 2,000 kb of primarily unique sequence, according to species. In comparison, human mitochrondrial DNA, which appears to code for roughly the same number of products, is only 16.5 kb long. Worse yet, electron microscopy of plant mitochondrial DNA reveals a complex assortment of differently sized circular and linear molecules, and restriction digests contain fragments in sub-stoichiometric amounts, suggesting a heterogeneous structure (for a review see ref. 1).

Some semblance of order in this situation has now been provided by the restriction mapping studies of Palmer and Shields[2], reported in this issue of *Nature* (see p.437). Their results suggest that plant mitochondrial genomes are carried on discrete circular molecules which are derived from each other by recombination.

Chromosome walks along the mitochondrial DNA of *Brassica campestris* (Chinese cabbage, turnip) led around three differently sized circles. The largest, 218 kb long, contains the entire sequence complexity of the *Brassica* mitochondrial genome. This 'master chromosome' contains a major direct repeat of about 2 kb, whose elements are separated by 135 kb on one side and 83 kb on the other. The other two circles are 135 and 83 kb in size and each contains the corresponding sequences present between the repeats of the master chromosome and a single copy of the repeated element. (Other arrangements, such as dimers of the 218 kb circle, are consistent with the mapping data *per se* but fail to explain the stoichiometries observed for fragments containing the repeats and surrounding unique DNA.) Clearly, a simple way to account for these species is to assume that they are related to each other by homologous recombination between repeated elements.

As Palmer and Shields point out, this relatively simple tripartite picture may turn out upon further study to be complicated by the presence of other minor species. One would, for example, expect the presence of dimers of the 83 kb and 135 kb circles to be present. Furthermore, Palmer and Shields detected, but did not map, other minor repeated sequences in *Brassica* mitochondrial DNA which may also serve as sites for recombination.

Mapping studies on the much larger mitochondrial genome of maize by Lonsdale and co-workers at the Plant Breeding Institute and University of Cambridge (personal communication) have indeed revealed a similar but more complex picture. They have mapped a maize mitochondrial DNA master chromosome of 570 kb and located six pairs of repeated elements within it: five direct repeats and one inverted repeat. In addition, six other smaller circular maps, ranging in size from 47 to 503 kb, were generated and could be derived from the master chromosome by recombination between pairs of repeated elements. These smaller circular maps seem to correspond well in size to circles identified in maize mitochondrial DNA by electron microscopy[3]. Thus the presence of multiple circular species of DNA, related by recombination between repeated elements, may be a general feature of higher-plant mitochondrial genomes.

The study of recombination in plant mitochondrial DNA has been hampered both by the lack of genetic markers and because mitochondrial DNA is maternally inherited. Nevertheless, evidence for recombination has been obtained by somatic cell hybridization of strains differing in polymorphic mitochondrial DNA restriction fragments[4-6]. The hybrids typically contain new combinations of parental fragment types, as might be expected from the assortment of multiple circular DNAs during mitotic growth. In addition, many hybrids contain novel fragments not found in either parent, which most probably arise as a result of true crossing-over. Complete restriction maps of plant mitochondrial DNAs will allow the detailed analysis of recombination in somatic cell hybrids.

Whether plant mitochondria contain a general recombination system, such as that present in yeast mitochondria, is not yet clear. They may contain site-specific recombination systems which only function at some repeated elements — a possibility suggested by the finding of repeated elements in maize mitochondria DNA that are not surrounded by the expected recombinant set of restriction fragments (D.M. Lonsdale, personal communication). Site-specific recombination would tend to limit the number of different circles that could be generated from a master chromosome containing many repeats. (The large number of linear molecules in preparations of mitochondrial DNA from plants, as well as yeast, are assumed by most workers to be the result of *in vitro* degradation. Perhaps some of these linear molecules are recombination intermediates present *in vivo*.)

Progress in identifying plant mitochondrial genes through their homology with genes of other organisms will soon make it possible to see whether the arrangement of genes is conserved, as in animals, or not, as in fungi. Using the restriction maps of the maize genome, D.M. Lonsdale and C.J. Leaver of the University of Edinburgh (personal communication) have already shown that the genes coding for cytochrome oxidase subunit I, apocytochrome *b* and a protein homologous to that encoded by the human unassigned reading frame *URF1* are present in a cluster. The cluster also contains sequences derived from chloroplast DNA containing what appear to be pseudogenes for chloroplast 16S rRNA[7] and the large subunit of ribulose bisphosphate carboxylase[8]. About 200 kb away is another gene, for cytochrome oxidase subunit II[9], and midway between them, the rRNA genes.

Comparisons between species such as *Brassica* and maize will also help give some clues as to why the majority of plant mitochondrial DNA sequences appear not to code for useful products. That chloroplast DNA sequences[7,8] are found in maize mitochondrial DNA led Lonsdale to suggest that the ability to take up foreign DNA could in part account for the large and variable sizes of plant mitochondrial genomes. It will be interesting to see how true this is of other species, and whether *bona fide* nuclear DNA sequences also turn up in plant mitochondria. □

Thomas D. Fox is at the Section of Genetics and Development, Cornell University, Ithaca, New York 14853.

1. Leaver, C.J. & Gray, M.W. *A. Rev. Pl. Physiol.* **33**, 373 (1982).
2. Palmer, J.D. & Shields, C.R. *Nature* **307**, 437 (1983).
3. Levings, C.S. in *Extrachromosomal DNA, ICN-UCLA Symposium on Molecular and Cellular Biology* (eds Cummings, D., Borst, P., Dawid, I., Weissman, S. & Fox, C.F.) 63 (1979).
4. Belliard, G., Vedel, F. & Pelletier, G. *Nature* **281**, 401 (1979).
5. Nagy, F., Torok, I. & Maliga, P. *Molec. gen. Genet.* **183**, 437 (1981).
6. Boeshore, M.L., Lifshitz, I., Hanson, M.R. & Shamay, I. *Molec. gen. Genet.* **190**, 459 (1983).
7. Stern, D.B. & Lonsdale, D.M. *Nature* **229**, 698 (1982).
8. Lonsdale, D.M., Hodge, T.P., Howe, C.J. & Stern, D.B. *Cell* **34**, 1007 (1983).
9. Fox, T.D. & Ledaver, C.J. *Cell* **26**, 315 (1981).

100 years ago

BULLETIN No. 3 of the Entomological Division of the U.S. Department of Agriculture (Washington, 1883), when stripped of the "red-tape" that appears to be even more necessary on official documents in the States than it is in this country, is of more than usual interest. The notorious "army-worm" appears in a new character, viz, as destructive to cranberries, which form an important feature in the production of the States. A long chapter is devoted to the "cotton-worm" in which (in addition to interesting biological information) elaborate contrivances for distributing arsenical solutions are described.
From *Nature* **29**, 344, 7 February 1884.

© 1984 Macmillan Journals Ltd

Geophysics

Palaeobathymetry from sinking shells

from Peter J. Smith

THE variety of phenomena from which information can be wrung about the geophysical past is a constant surprise. Consider, for example, the case of the sinking cephalopods.

A few years ago, J.S. Weaver and J.A. Chamberlain, building on earlier ideas and making use of the equations derived by naval architects to describe the sinking of ships, managed to work out the physics of the sinking of coiled and chambered shells (see, for example, *Math. Geol.* **10**, 673; 1978). They were able in particular to demonstrate, both theoretically and by laboratory experiment, that the depth to which an intact coiled cephalopod will sink with its plane of symmetry remaining vertical is governed only by the geometry of the shell. What was not clear at the time was whether, given the more chaotic conditions of the natural environment, observations of *in situ* vertical cephalopod shells could be interpreted sensibly in terms of palaeobathymetry. R.E. Crick has now shown that they can be (*Bull. Geol. Soc. Am.* **94**, 1109; 1983).

This is possible because of the remarkable series of events that overtakes chambered cephalopods after death. Gases released during the decay of the visceral mass often increase the buoyancy of a dead cephalopod so that the shell rises to the water surface where, unless it is stranded on a shoreline, it becomes waterlogged and sinks. But it doesn't just drop. Initially, the shell floats with its plane of symmetry vertical; but as it gradually absorbs water and sinks (and assuming it remains undamaged) it begins to rock from side to side until it finally becomes horizontal. The depths at which these changes occur depend on the shell's geometry. If the water is shallower than the rocking depth for a particular type of cephalopod, the shell will reach the bottom and become anchored, infilled and buried in a vertical position. Therefore, any ancient cephalopod shell discovered in a vertical position must have been deposited in water whose maximum depth can be estimated from the shell's geometry.

Of course, there are potential difficulties. For example, could a sinking shell, having reached the depth at which it becomes horizontal, nevertheless end up in a vertical position through impact at the bottom? That seems unlikely, for cephalopod shells float down much too gently to undergo a disorienting impact.

Second, could shells reaching the bottom in a horizontal state subsequently be turned to the vertical by bioturbation? They could indeed, although there are ways of detecting that in particular cases.

Third, it is necessary to assume (reasonably) that gravity, seawater density and atmospheric pressure have not changed since the shells sank.

Finally, if the method is to work, it is essential that not all shells that end up in a vertical position at the bottom should subsequently topple over; but as vertical shells are actually observed, this appears not to be too much of a problem.

Crick has applied the technique to the top of the Fort Worth limestone, which extends in a narrow belt southwards from the Texas–Oklahoma border and which formed during the extistence of the East Texas Embayment (Lower Cretaceous). There are enough vertical cephalopods (ammonites and nautiloids) to provide data at points throughout the whole length of the formation; and there were several different types of cephalopod present, so that maximum water depths can be determined over the range 1.6–2.6 m. Moreover, at the few points at which there are no vertical shells and hence at which it is not possible to specify maximum water depths, it has at least been possible to estimate minimum depths, for the depths must have exceeded those at which the particular types of cephalopod begin to deviate from vertical sinking.

The conclusions that Crick reaches are that the Forth Worth cephalopods were laid down in water depths of generally less than 2.6 m and that depths increased slightly to the east and south. In the context of local geology, this is a considerable advance in that previous estimates of water depth based on less reliable (sedimentological and palaeoecological) criteria lie in the range 15–40 m. More important than the specific results, however, is that the method appears to work and is evidently of wider applicability. The only condition is that the formation to be studied should contain cephalopods laid down in water of a depth not exceeding the depths at which the various types of shell begin to depart from vertically oriented fall. □

Peter J. Smith is a Reader in the Department of Earth Sciences, The Open University, Milton Keynes MK7 6AA, UK and editor of Open Earth.

Astronomy

IRAS circular 7

The source name consists of four parts: (1) the letters 'IRAS' to indicate the origin; (2) the right ascension (RA) in hours and minutes, seconds omitted; (3) declination (Dec) in decimal degrees, multiplied by 10 and then truncated (thus + 32° 42.3 becomes + 327); (4) an appendix starting with 'P' and followed by the number of the circular; this appendix stresses that the data are preliminary. Position is given at Equinox 1950.0. The measurements were made between epochs 1983.1 and 1983.6.

Source IRAS	RA (1950) h min s	Dec (1950) deg arc min	Flux density (Jy) 12 μm	25 μm	60 μm	100 μm
0000+818P07	00 00 12	+81 45.9	<0.2	<0.2	0.6	<1.6
0007+821P07	00 07 33	+82 08.4	<0.2	<0.2	0.6	<1.6
0119+868P07	01 19 26	+86 49.5	<0.2	<0.2	0.6	<1.5
0147+891P07	01 47 23	+89 06.7	<0.2	<0.2	0.8	1.9
0354+226P07	03 54 54	+22 33.8	<0.2	<0.2	<0.5	3.5
0358+194P07	03 58 04	+19 22.5	<0.2	<0.3	0.9	1.6
0358+202P07	03 58 12	+20 13.7	<0.2	<0.3	0.6	2.1
0359+169P07	03 59 52	+16 56.9	<0.2	<0.3	0.9	2.6
0412+024P07	04 12 11	+02 23.2	<0.2	<0.2	1.0	2.1
0413+023P07	04 13 40	+02 21.0	<0.2	<0.2	<0.6	2.1
0413+011P07	04 13 58	+01 03.8	<0.2	<0.2	<1.0	2.3
0417+008P07	04 17 40	+00 45.1	<0.2	<0.2	0.6	<1.5
0712+880P07	07 12 40	+87 57.8	<0.2	<0.4	0.9	<1.6
0845+515P07	08 54 16	+51 32.2	<0.2	<0.2	0.8	<1.4
0854+210P07	08 54 30	+21 00.4	<0.2	<0.3	0.9	<1.0
0855+108P07	08 55 59	+10 53.0	<0.2	<0.3	1.3	2.2
0902+128P07	09 02 33	+12 53.7	<0.4	<0.3	0.5	<0.7
0904+210P07	09 04 09	+21 00.2	<0.4	<0.3	0.5	<1.1
0910+234P07	09 10 58	+23 29.8	<0.2	<0.3	0.8	2.5
0915+511P07	09 15 08	+51 09.6	<0.2	<0.2	0.5	<1.3
0920+023P07	09 20 05	+02 19.6	<0.3	<0.3	0.6	<1.1
1010+865P07	10 10 21	+86 28.6	<0.2	<0.2	0.6	<2.0
1100+792P07	11 00 51	+79 15.6	<0.2	<0.2	0.8	1.5
1108+772P07	11 08 36	+77 12.9	<0.2	<0.2	0.9	2.1
1150+829P07	11 50 23	+82 52.8	<0.3	<0.2	0.5	<1.6
1157+860P07	11 57 35	+85 59.9	<0.2	<0.2	0.5	<1.6
1221+844P07	12 21 11	+84 26.7	<0.2	<0.2	0.6	<1.6
2359+846P07	23 59 08	+84 35.1	<0.2	<0.2	0.8	<1.6

0028-0836/84/050416-01$01.00

The molecular biology of immunoglobulin D

Frederick R. Blattner* & Philip W. Tucker†

* Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706, USA
† Department of Microbiology, University of Texas Southwestern Medical School, Dallas, Texas 75235, USA

Immunoglobulin D (IgD) is co-expressed with immunoglobulin M (IgM) on the membranes of most B cells, yet its biological function has remained a mystery. Recent detailed information on the structure and transcription of the unusual IgD heavy chain (δ) gene in mouse suggests a complex genetic control. A model is presented for the developmental regulation of IgM and IgD and roles suggested for the membrane and secreted forms of IgD in the immune network.

VERTEBRATES respond to foreign substances by the production of antibodies, which are proteins consisting of two heavy and two light chains. In the higher vertebrates this response involves the sequential production of multiple classes of antibody with the same antigenic specificity but different effector functions. The effector or class of the antibody is determined by the constant (C) region of the heavy chain (C_H region). For the mouse, eight immunoglobulin classes have been identified: IgM, IgD, IgG3, IgG1, IgG2b, IgG2a, IgE and IgA; the H chains are designated with corresponding Greek characters and the genes encoding them are arranged in the order μ, δ, $\gamma 3$, $\gamma 1$ $\gamma 2b$, $\gamma 2a$, ε, α in a cluster spanning some 200 kilobase pairs (kbp) of DNA on chromosome 12 (reviewed in refs 1, 2). The light chains are encoded separately. The antigen-binding variable (V) region of the H chain is coded by a V_H gene that is assembled from separate *V*, *D* and *J* germ-line segments during B-cell ontogeny (for review see ref. 3). It is located to the left of the C_H cluster. A similar mechanism—but without *D* segments—applies to the light-chain variable region, which also contributes to antigen binding.

The primary response, elicited by the first exposure to an antigen, consists of antibody of the IgM class. The secondary response to the same antigen, however, involves the production of antibodies of different classes—IgG in serum and IgA in gut as well as IgM. Thus, between the primary and secondary responses, immune 'memory' for antigen is established. This is accompanied by a class switch in which the original V_H gene is expressed with a new C_H gene.

Antibodies are produced by B lymphocytes, each of which expresses immunoglobulin of unique antigenic specificity on its surface. Each immunoglobulin class can exist in both membrane-bound (m) and secreted (s) forms which differ in the carboxyl-terminal sequences of their respective H chains. One consequence of antigenic stimulation of a B cell is a shift of expression from the membrane-bound form to the secreted form which takes place without any change in either the specificity or the class of the antibody[4,5]. Thus, the surface immunoglobulin serves as an external signal of what the B cell, if stimulated, will produce. This is crucial for three reasons: first, it ensures that secreted antibody elicited by the interaction of membrane immunoglobulin with antigen will be appropriately directed; second, it permits B cells of the secondary response (memory cells) to be regulated as separate pools; and third, it allows maintenance of a repertoire of potential responses in the resting B-cell population at little metabolic cost.

IgD behaves paradoxically in relation to the above simplified view of the humoral immune response. It is a predominant surface immunoglobulin on most B lymphocytes[6,7], yet it is not secreted on antigenic stimulation of the cells that bear it[8]. What its role may be is a fascinating question, for elaborate mechanisms have evolved to make this immunoglobulin class behave oppositely to the others.

In this review we shall begin with a description of biological data about IgD and then summarize, from the molecular biologist's viewpoint, what has been learned about the δ gene. Next, the expression of the μ and δ transcription unit will be examined with an eye to understanding the mechanisms controlling RNA splicing in that system. Then, we shall make some suggestions as to how IgD may fit into the immune system. Although the predominant focus will be mouse, we shall also discuss the comparison between human and mouse structures, along with evolutionary implications.

Biology of IgD

IgD on the surface of B cells is co-expressed with IgM[9–12], but appears later[13,14] and is considered to be an indicator of the mature or 'virgin' resting B cell. On a given B cell, membrane IgD has the same antigen-binding specificity as IgM[15,16]. IgD antibody is a very minor component of serum (micrograms per ml compared with milligrams per ml for IgG)[17,18], so it seems unlikely that it has an important direct role in the neutralization of antigens. On the other hand, it can function as a receptor, for when heterologous antibodies against the μ or δ constant regions are used to mimic stimulating 'antigen', both stimulate replication[19–23]. When the cell is stimulated either in this way or with mitogens[24,25] or T-cell factors[26,27], IgM is synthesized and secreted whereas IgD production is shut off. This is consistent with the low levels of IgD but raises the question of how this regulation is achieved.

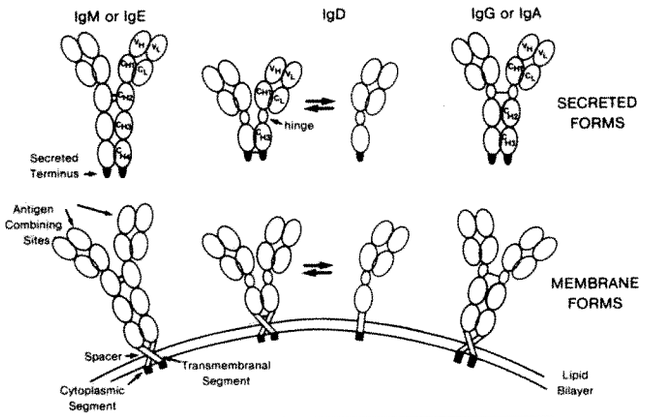
A controversial issue is the role of IgD in memory. Some hold[28,29] that the majority of cells giving rise to the secondary response have lost IgD from their surface, whereas others argue[30,31] that IgD loss is not a necessary stage in memory cell maturation.

Ablation of the population of B cells that carries IgD on their surface (IgD^+) by administration of heterologous anti-δ antibodies to the animal continuously from birth does not abolish the immune response[32]. This contrasts with the profound immunodeficiency engendered by suppression of IgM^+ cells with anti-μ antibodies[33,34]. This difference may simply be due to the fact that IgM^+ B cells are produced early in ontogeny and are precursors of most or all of the other cells involved in producing antibody, whereas IgD expression occurs after the B cell is competent to respond. IgD suppression *in vivo*[35] and anti-δ antibodies administered *in vitro*[36] do give rise to subtle shifts in the quantitative responsiveness of B cells to T-dependent antigens. This may indicate that IgD is involved in interactions

© 1984 Macmillan Journals Ltd

between T and B cells and in the regulation of B-cell populations.

Although the function of IgD remains obscure, some insight may emerge from the genetic and structural information that has recently begun to accumulate. These results reveal an unusual structure for the IgD protein, considerable complexity in its genetic regulation, and surprising species differences between man and mouse.



Class	IgM	IgD	IgG3	IgG1	IgG2b	IgG2a	IgE	IgA
H chain	μ	δ	$\gamma 3$	$\gamma 1$	$\gamma 2b$	$\gamma 2a$	ε	α
Number of Domains	4+V	2+V	3+V	3+V	3+V	3+V	4+V	3+V
Hinge	0	35	17	13	22	16	0	14
Spacer	12	26	17	17	17	17	18	25
Transmembranal Segment	26	26	26	26	26	26	26	26
Cytoplasmic Segment	3	3	28	28	28	28	28	14
Secreted Terminus	20	21	2	2	2	2	8	20

Fig. 1 Structure of membrane (m) and secreted (s) mouse immunoglobulins. The constant regions have from two to four domains depending on class, and in some cases a hinge segment is present between the first and second constant region domains. This is thought to introduce flexibility into the structure to allow the antigen binding site to achieve better contact with antigen[96]. Shown at the top are the monomer s forms (H_2L_2) for the three structural types as well as the IgD half-monomer forms (shown in equilibrium with the monomer by arrows). Large and small ellipsoids represent domains and hinge regions, and dark ellipsoids represent the secreted carboxyl termini of the H chains. Non-covalent domain contacts are shown by the overlaps, and covalent H–H disulphide bonds by straight lines. Below, the three immunoglobulin types are illustrated attached to a B-cell membrane. Most of the molecule (including the antigen binding site constant regions and m-form specific spacer region) is on the outside of the lipid bilayer. The transmembranal segment spans the membrane and the cytoplasmic segment (box at the C-terminal end) protrudes inside. At the bottom of the figure is a structural summary for s and m forms of the eight mouse H chains. All numbers, except for domain numbers, refer to amino acids. Specific references used are: μ[42,43], δ[37,38], $\gamma 3$ (J. Wells *et al.*, in preparation), $\gamma 1$[102–105], $\gamma 2b$[103,104,106], $\gamma 2a$[105,107], ε[108,109] and α[109–110]. [A discrepancy between the genomic[37,38] and cDNA[37] sequences in the secreted terminus and an uncertainty in the position of the beginning of the $C_\delta 1$ domain have been resolved through analysis of peptide sequences[95] of murine δ protein. In other respects, the DNA sequence was confirmed by the peptide analysis. Thus the sequence of the myeloma protein can be considered to be quite solid. There is no proof that the secreted terminus of the myeloma protein is the same as the terminus of serum δ found in minute quantities in the normal animal. The structure of the membrane form[38] was established entirely from genomic DNA sequence using realistic, but unproven, assignments for the membrane exon boundaries. This structure has been confirmed in part by the demonstration that an anti-peptide antibody against the membrane spacer will precipitate δ-chain obtained from the surface of normal spleen cells (T. Shinnick, F.R.B. and P.W.T., unpublished observations). However, further protein analyses will be desirable to ensure that all splice boundaries have been correctly established.

The unusual IgD protein

Through DNA sequence analysis we now know the structure of the δ gene and hence the amino acid sequence of the δ protein of the BALB/c mouse (summarized and compared with other H chains in Fig. 1). The outstanding feature of the molecule is that whereas the constant regions of other H chains have three or four domains, δ has only two[37]. These domains, termed $C_\delta 1$ and $C_\delta 3$ (C_{H1} and C_{H3} in Fig. 1) by homology with other classes, are separated by a unique hinge segment which is quite long and lacks the cysteine residues that normally provide the links between the two H chains in the standard H_2L_2 subunit structure of immunoglobulin. However, both the membrane spacer region of the membrane-bound form of δ protein and the secreted terminus of the secretory form of δ protein have a cysteine which could cross-link the respective chains[38]. The presence of both IgD monomers (H_2L_2) and IgD 'half-monomers' (one H chain and one L chain) on B-cell membranes, has been well documented[39,40], and there seems to be an equilibrium between the two forms on the cell surface. It is not known whether the secreted form exists in the same sort of equilibrium, although the H_2L_2 form has been demonstrated electrophoretically[41]. The transmembrane segment of δ is very similar to that of μ, and the carboxyl-terminal residues that extend into the cell are identical[38,42]. On the other hand, the portions encoded by membrane exons that might lie just external to the cell-surface (spacers) differ radically in length and amino acid composition. This structural dichotomy may bear on whether antigenic stimulation through μ or δ gives the same cellular response.

The μ–δ gene locus

The detailed genetic map of the μ–δ region is shown in Fig. 2. The four constant region exons of the C_μ gene, including the secreted terminus (coded as part of the $C_\mu 4$ exon and membrane terminal exons $\mu_m 1$ and $\mu_m 2$) are located within a span of 4 kbp of DNA[42–45]. The δ gene begins 2 kbp beyond and spans a much larger area (10 kbp)[38,46–49]. The δ gene is unique in that both the membrane and secreted termini are separated from the body of the gene by a large (5 kbp) intervening sequence[37,38,46,48]. In between δ_s and $\delta_m 1$ is a potential third carboxyl-terminal exon, termed δ_x (ref. 38), which has not been demonstrated to be translated into protein but which would encode a peptide with the hydrophilic characteristics of a secreted terminus.

In the course of B-cell differentiation these exons are differentially expressed. The first clue to how this is achieved came from analysis of B-cell tumours or hybridomas, which have served as models for resting B cells. The two lines studied in greatest detail are the BCL_1 lymphoma[50], which arose spontaneously in a BALB/c mouse, and the GLC28 hybridoma[51], which was made by fusion of a hamster lymphoma to a mouse B cell. The BCL_1 tumour maintains many of the characteristics of resting B cells including the ability to secrete IgM after stimulation with lipopolysaccharide[52]. The GLC28 hybridoma retains characteristics of the hamster parent, including expression of hamster immunoglobulin[48], but both tumours clearly express murine IgM and murine IgD on the cell surface[48–53].

In each of these cell lines, both μ and δ constant regions are expressed with the same V_H gene[48,49], and that V_H gene remains immediately 5′ to the μ gene in cells producing IgD (see Fig. 2*b*). Thus, δ must be expressed from an RNA precursor containing both μ and δ and from which the μ gene is spliced out.

Myelomas

A well studied cellular model for the immunoglobulin secreting, terminal stage of B-cell differentiation is the myeloma. Analyses of many examples led to the discovery that the expression of the secreted forms of all immunoglobulin classes except for μ is invariably associated with the deletion of DNA (including μ), juxtaposing the expressed class with the V region. These deletions are mediated by recombination between 'switch sites'[1,2] located immediately 5′ of each of the C_H genes except for δ[54,55] (Fig. 2) and the process is termed class switch recombination.

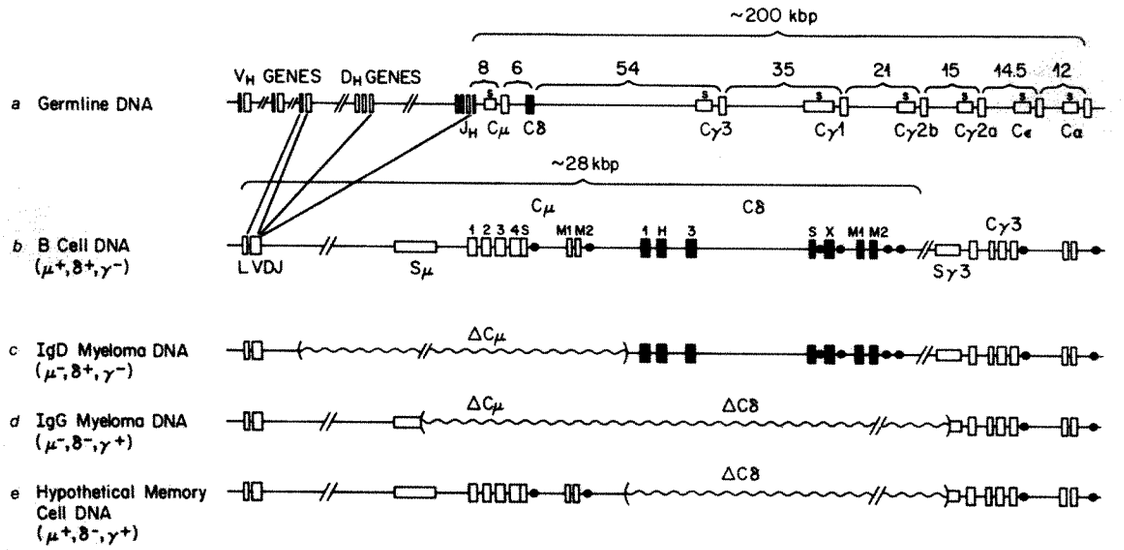


Fig. 2 Immunoglobulin gene arrangement and rearrangement during development. Tall boxes are protein encoding regions (δ regions are always in black), thin boxes are switch (S) recombination sequences, and dark dots are transcription (*end*) sites (see text). Unless indicated (//), distances are drawn to the scale. *a*, Map of the germ-line (unrearranged) H-chain locus. Distances, where indicated in kbp, are from centre to centre of a gene (for simplicity shown as a single box) or gene cluster. Switch sites are located 5′ to all C_H genes except C_δ. *b*, Expanded map of the μ–δ transcriptional unit in B-cell DNA. As indicated by the lines from *a*, only the variable region segments (V_H, *D* HHand *J*) are rearranged in cells expressing IgM (μ-chains) or IgM and IgD (δ-chains) simultaneously. Here and in *c*–*e*, exon fine structure is shown (see text for details). *c*, IgD-secreting tumours delete (Δ) C_μ. The approximate length of the deletion (determined in this case from the myeloma TEPC 1017) is indicated by a wavy line, and the left and right recombination points are represented by parentheses. Note that this deletion does not utilize conventional switch sites (see text). *d*, IgG-secreting cells delete all genes between *V*–*D*–*J* and the expressed (in this example, γ3) gene. Conventional switch sequences are utilized. *e*, Hypothetical DNA rearrangement for putative double-producing ($\mu^+\gamma3^+$) memory cells. The proposed deletion occurs at a recombination site 3′ to C_μ and 5′ to at least part of $S_\gamma3$, thereby deleting C_δ and providing a reasonably short transcriptional unit for μ–γ membrane expression.

The switch site regions are composed of tandemly repeated pentamers: GAGCT mixed with GGGGT[1,2,56] and similar variants. The boundaries of the deleted DNA are near, but not necessarily within, the switch site repeat[1,2,57,58]. Despite the absence of a δ switch site, examples of IgD-secreting myelomas have been found[41,46,59,60]. In these cases, deletion of C_μ has also occurred. In the one case so far examined, TEPC 1017 (ref. 55 and A. C. Gilliam, A. Shen, J. F. Mushinski, F.R.B. and P.W.T., in preparation), the deletion endpoints were not anywhere near sequences that resemble switch sites. Thus, in rare cases rearrangements can occur that activate IgD secretion but which are not mediated by switch sites. It is not clear, however, whether such deletion is related in any way to the biological mechanism of secretory expression of IgD. Since membrane IgD expression in B cells does not involve DNA deletion[48,49], we suspect the secretory expression is also controlled at the transcriptional level. Therefore, non-switch rearrangement may be an artefact limited to the transformed cells.

μ–δ RNA analysis

Additional information has been obtained by direct analysis of lymphocyte RNA. Table 1 lists the most important mRNAs and the cells in which they have been detected. The putative structures of precursors of some of them are shown in Fig. 3 (for details see figure legends).

Initiation of H-chain transcription begins at a promoter located just upstream of the active *V* gene[61]. So in a given B cell, as far as is known, all the H-chain RNAs have the same 5′ end. The principal H-chain RNAs of the B cell are the membrane forms of μ and δ. In both the μ and δ myelomas, the secreted forms predominate, although membrane RNA is also synthesized. Several minor components not illustrated in Fig. 3 are described in the legends to Table 1 and Fig. 3. Thus, depending on its state of differentiation, the B cell can produce multiple forms of mRNA from the same DNA, each with a different 3′ end and spliced in a unique pattern so that in some cases what is an intron in one RNA is an exon in another.

The donor and acceptor RNA splice sites at the ends of the immunoglobulin intervening sequences are typical of those of other gene systems[62] and immunoglobulin splicing appears to be no less accurate. Specifically, there is no noticeable tendency for the splicing apparatus to skip correct splice sites, thereby deleting exons from the message. This despite the fact that the μ–δ genetic region contains many sequences that are similar to the donor and acceptor splice sites of the exons which are not used as splice sites as far as is known. Control of splicing is thus as much a matter of avoiding incorrect splicing choices as of choosing the correct sites; and the pattern must be altered according to the state of cell differentiation.

Control of splicing

The study of adenovirus (reviewed in refs 63, 64) has shown that a critical step in determining differential expression of the late genes is the selection of the 3′ end of the mRNA precursor. This is thought to occur by endonucleolytic cleavage of the precursor, with possible exonucleolytic trimming[65,66], followed by addition of polyadenylate [poly(A)] residues to the 3′end. RNA splicing to remove intervening sequences generally follows both clipping and polyadenylation[63,65,67].

It is not known what structural feature of the precursor RNA determines the position of 3′-end clipping, so we have adopted the neutral term '*end* site' for this hypothetical complex recognition element. The sequence AATAAA[68] or AATTAAA[69] that occurs near the 3′ end of most eukaryotic mRNAs may be a component of the recognition site. However, these sequences occur at 16 places in the μ–δ region and only 6 are (apparently) used (J. E. Richards, unpublished data). The locations of the six functional *end* sites of the μ and δ transcripts are indicated in Figs 2–4.

It has been proposed that control of expression of the membrane as opposed to the secreted forms of IgM[42], as in adenoviruses, is controlled at the level of 3′ end selection (end_1 versus end_2 in Fig. 3). In support of this model, a recent analysis[70] of nuclear RNA in early B-cell tumours has directly demonstrated the first two predicted μ precursors of Fig. 3.

Choice of splice sites may follow automatically from choice of *end* sites. In the case of μ, the splicing choices are simple. The last three splice sites are missing from the shorter transcript

(Fig. 3), so they cannot be used and the S terminus is coded as an extension of the $C_\mu 4$ exon. The choice in the IgD myeloma TEPC 1017 is also simple. end_1 and end_2 are deleted from the DNA (Fig. 2*c*), thereby allowing straightforward expression of the δS mRNA from end_3. Membrane or secreted IgD expression in B cells is more difficult to understand for it requires removal of μ sequences from a long δ mRNA precursor containing both μ and δ exons. Thus the splice sites of μ, which must not be skipped in the μ transcript, most definitely must be skipped in order to produce δ mRNA.

In these more complex cases, how might the selection of splice sites be made? One possibility would be a developmentally regulated change in the specificity of the splicing apparatus, but this assumes sequence differences in the splice sites that might be differentially recognized, and a computer search (F.R.B., unpublished) has not suggested such differences. We would therefore like to propose that RNA precursors of different lengths might adopt different secondary structures, so exposing alternative splice sites to the splicing machinery. The developmental regulation of heavy-chain gene expression could in this case be simply a matter of regulation of 3′ *end* site selection. According to the model of Fig. 4, 3′ *end* site selection could be regulated simply by alterations in the level of the enzyme ('endase') that cuts the RNA precursor at the *end* site. Since an RNA molecule must survive a cross fire of endonuclease cutting to reach full length, we dub this the 'gauntlet' model. We assume that endase is present in the resting B cell in limiting amounts. The level of expression of various 3′ ends in B cells, then, would depend on the relative affinities of different *end* sites for endase. To account for the mRNA abundances discussed above and in the legend to Table 1, we have postulated the relative strengths of *end* sites—as shown in Figs 3 and 4.

In the differentiation of a B cell to a secreting cell, we propose that the quantity (or activity) of endase is increased. The effect would be to force the usage of more 5′ (promoter-proximal) *end* sites, regardless of affinity, because the first cut defines the end of the RNA regardless of any downstream cuts (gauntlet effect). In the limit this would drive the system to produce only the shortest possible precursor RNA in the fully differentiated plasma cell. It would also permit a continuous progression from resting B cell to plasma cell rather than an abrupt jump, which could help explain how IgM antibodies can be secreted by B cells (blasts) that have not become fully differentiated into plasma cells. The model also explains why, in all myelomas, the most strongly expressed mRNA is the one that is defined by the first *end* site in the DNA after the promoter, and why the switch site recombination is needed to obtain efficient expression of downstream classes in myelomas.

Other observations which this model helps to explain are: (1) The wide and continuous variations of μ to δ ratios observed in B-cell populations[71,72]. (2) The transitory nature of IgD expression on the resting B cell and its disappearance on activation[24–27]. (3) The observation (ref. 73 and D. J. Kemp, personal communication) of an RNA fragment containing only μ_m sequences whose size corresponds roughly to the end_2 to end_3 interval. Such '*end–end*' fragments would be expected if the RNA polymerase continues down its DNA template beyond the first *end* site, as suggested in adenovirus[63,64], and the endase nicks the downstream *end* sites as well. (4) The observation[74] that neonatal B cells seem to produce some secreted IgM. This could be due to an intermediate level of endase in these very early B cells.

An aspect of immunoglobulin expression that is not easily explained by the gauntlet model is the expression of downstream classes (IgG, IgE or IgA) in memory B cells, if these are coded from an RNA precursor up to 200 kbp in length as has been reported[75].

Selection of 3′ ends by cutting of RNA precursors at *end* sites clearly is only one of several levels at which immunoglobulin expression is regulated. Others include control of transcription initiation, possibly involving enhancer-like elements[76–78], transcription termination and chromatin structure alterations[74].

Table 1 Classification of μ and δ mRNAs

3′ Terminus	Size (kb)	Name	Occurrence
end_1	2.4	μ_s	Spleen and B-cell tumour (weak), μ myelomas (stro
end_2	2.7	μ_m	Spleen and B-cell tumour (strong), μ myelomas (we
end_3	1.75	δ_s	δ myelomas (strong)
end_3	3.2	δ_s	Spleen, B-cell tumours an myelomas (very weak)
end_4	2.65	δ_x	Spleen and B-cell tumour (weak) δ myelomas, (very weak)
end_5	2.1	$\delta_{m\text{-}minor}$	Spleen and B-cell tumour (weak), δ myeloma (very weak)
end_6	2.9	$\delta_{m\text{-}major}$	Spleen and B-cell tumour (strong), δ myelomas (we
end_6	2.9-	$\delta_{m\text{-}major}$ + 3′ heterogeneity	Spleen and B-cell tumour (very weak), δ myelomas (very weak)
end_2	2.0	μ_m 3′ fragment	μ myelomas and T cells (weak)

Sizes of the end_3, end_5 and end_6 δ mRNAs listed above[59,98] also measured as 1.6 (ref. 47) and 1.8 (ref. 48), and 2.7 (refs 47 respectively. The 2.4-kb μ_s and 2.7-kb μ_m mRNAs that were orig described in IgM-secreting myelomas[42,44,45] correspond to *end* end_2. These RNAs are also observed in splenic B cells[99]. The qu of μ_s form mRNA is considerably reduced when large spleen cells (l and plasma cells) are selectively removed (D. Yuan, unpubl observations). A number of 'sterile' μ RNAs, lacking some e component required for normal translation, have been observed i cytoplasm of both B and T cells[100,101]. In all δ^+ or $\mu^+\delta^+$ examined[48,59,98], two forms of δ mRNA are found, which we term as $\delta_{m\text{-}minor}$ and $\delta_{m\text{-}major}$ according to their relative abundance. terminate at end_5 (~2.1 kb) and end_6 (~2.9 kb), respectively. 10–50-fold difference in level of expression between these form may be due to differences in the strength of termination. These mR apparently code for the same protein[38] and differ only in the len the 3′-untranslated region. The $\delta_{m\text{-}major}$ form also shov heterogeneity caused by removal of intervening sequences with 3′ untranslated region of some molecules but not others[38,48].

The 1.75-kb transcript for δ_s, ending at end_3, is strongly exp only in the IgD-secreting tumours from which the μ gene has deleted at the DNA level[47,48,59,98]. In spleen cells and B-cell tun the only RNA that can be found ending at end_3 is a 3.2-kbp spe This species, however, is a true B-cell product since it is enrich the small B-cell population (D. Yuan and P.W.T., unpublished re No information is yet available as to its splicing pattern, nor is it whether this RNA codes for δ_s of the myeloma type. Also fou spleen and B-cell tumours is an RNA of 2.65 kb ending at *end* 38, 98). This RNA may code for δ_x, although there is no eviden this terminus being used in a protein. Either the 3.2-kbp or 2.6 RNAs might be unspliced precursors and thus might not be *bor* mature mRNAs.

There is also an indication that post-translational regu occurs in this system, since 10-fold less δ mRNA than found in the cytoplasm of splenic B cells which have amounts of the Htwo proteins on their surfaces[80].

Elucidation of the relative importance of these mecha will require detailed analysis of RNA in a variety of ty cells. We have recently observed substantial δ specific R nuclei of neonatal B cells that are expressing μ but not the surface (unpublished data of D. Yuan and P.W.T.) RNA could reflect *end–end* fragments as predicted by the let model. Such RNA is not found in myelomas commit IgM, however. This may indicate that transcription termi at a site between μ and δ comes into play in fully differen plasma cells.

The role of IgD

IgD is different from other immunoglobulins in structure arrangement, physiological localization and in its behavi the immune response. Yet we have no idea of its fun

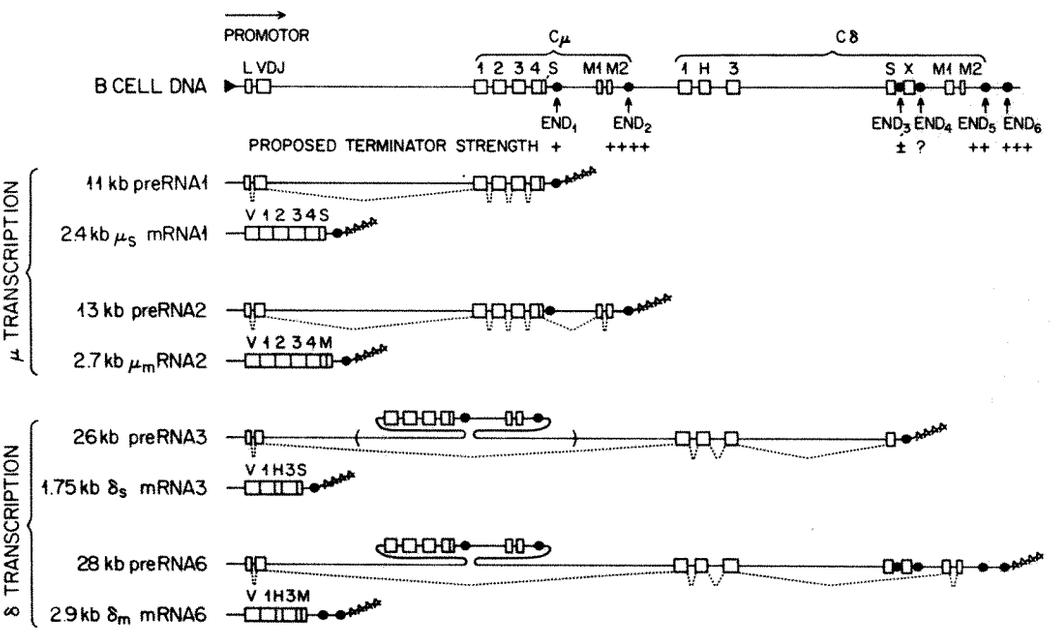


Fig. 3 Transcription and splicing patterns of major μ and δ mRNA precursors. A detailed scale map of the μ–δ transcriptional unit including the promoter (►) is given at the top (also see Fig. 4 legend). The relative strengths, based on mRNA abundances (see Table 1 legend) of transcription *end* sites are indicated below each one with +. RNAs are shown as precursor (preRNA)–product mRNA pairs. Sizes of the preRNAs are predicted from the DNA sequence, assuming that 3′-end termination results from RNA cleavage (see text for details). Sizes of the mRNAs have been determined experimentally. Splicing is indicated by dotted lines and poly(A) tails are shown as AAAA. Splicing of the μ- and δ-containing preRNAs 3 and 6 to yield exclusively δ mRNA products may depend on eliminating the μ sequences from the splicing complex through local secondary RNA structures (simplistically illustrated in the figure by a total looping out of μ). In δ-expressing myelomas, the C_μ gene and its associated *end* sites are already removed by DNA deletion (denoted by parentheses on the preRNA3). See text for references.

Perhaps it simply serves as an auxiliary antigen receptor on the B cell[22,23,81], but this seems a rather trivial explanation for the evolution of such a complex system. We prefer to suppose that it has a role in regulation of B-cell populations, because this would help explain the paradoxical aspects of IgD expression. (A third possibility, of course, is that it has no function.) Since IgD appears on the mature virgin B cell and decreases when the B cell is stimulated by antigen, the virgin cell would be the one in whose regulation IgD would be likely to participate.

These cells arise by differentiation from stem cells of the bone marrow or fetal liver in a process that involves random generation of variable regions from the germ-line V gene segments, thus producing cells with the antigenic specificities that represent a sampling of the entire repertoire of V_H regions that can be constructed from the germ line. B cells have an average lifetime of a few weeks, so their population is constantly turning over. The pool is depleted by cell death and fed by the influx of virgin cells plus replication of cells that may be stimulated by antigens or other regulatory influences which may be responsible for maintenance of immunological memory at the B-cell level. One way of accounting for the persistence of memory cells is through the network theory[82], in which antibodies themselves function to regulate the distribution of V regions represented in the B-cell population by binding to idiotypic determinants of surface immunoglobulin on B cells so as to affect differentially the replication of B cells expressing various determinants. Class-specific and/or idiotype specific factors produced by T cells play the part of regulators in modern formulations of the network theory[83–86].

IgD and especially its spacer segment (Fig. 1 and ref. 38) would be well suited to serve as a receptor on the B cell for the class-specific component of the network influences regulating the primary response. Since IgD expression is shut down on antigenic stimulation, this marker could serve to focus these influences specifically onto the virgin B-cell population. Presumably, the spacers of other membrane immunoglobulins could serve the same role for regulation of the other classes. The reason the spacer in particular is proposed for this function is that it is encoded on the same exon as the transmembrane segment (also true of other immunoglobulins). Thus, this determinant cannot appear on the surface of a cell by cytophilic adsorption of serum antibody nor could serum antibody compete for sites with a factor directed against the spacer portion.

It is more difficult to account for the secreted form of IgD (either δ_s, δ_x or unknown terminus). However, secreted IgD could complete a feedback loop by supplying afferent signals representative of all the idiotypes present in the B-cell repertoire to the T-cell population. This role would fit with the prediction of the gauntlet model that the s form of IgD would be secreted from resting B cells, not IgM-secreting ones. The low stability of serum IgD[22] would also serve a function, since an intercellular messenger molecule should not have an excessive half life if it is to function properly in that role. There is, in fact, some evidence that a factor emitted by B cells is needed for the development of a class of isotype-specific T_H cells[87]. This factor might be the s form of IgD.

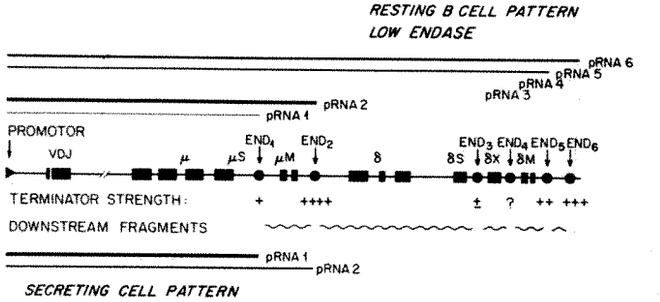


Fig. 4 The gauntlet model for modulation of μ–δ transcription. The μ–δ B-cell transcriptional unit is shown in the centre with exons as boxes and 3′ *end* sites as dots (see Fig. 3 legend). All primary transcripts (preRNA 1–6) initiate at the promoter (►) left of V–D–J and terminate at *ends*[1–6] as indicated by the relative length of the lines. The relative abundances of the preRNAs, reflected by experimentally observed abundances of their mRNA products (Table 1 and its legend) are indicated by the thickness of the lines. The model predicts that in the resting cell (top of figure), the enzyme (endase) that cleaves the preRNAs at *end* sites is in low concentration. Since fewer molecules are cleaved, longer transcripts (membrane forms) would predominate with their relative abundances weighted by *end* sequence affinities (+) for endase. On activation to secretion (patterns at the bottom of the figure), the endase activity and/or concentration is increased dramatically, resulting in almost complete dominance of promoter-proximal *end* sites regardless of affinity. The wavy lines represent hypothetical *end–end* fragments.

Human and mouse IgD

The human δ protein chain, sequenced from myeloma material[88–91], does not show all of the oddities of the mouse molecule. The human chain is conventional in having three rather than two constant region domains. Its hinge is even longer than the murine one and contains a bizarre cluster of charged amino acids in its C-terminal portion, as well as a cysteine residue that could be used for normal inter-H-chain linkage.

The sequence match between murine and human IgD molecules is poor in the first domain (~33% amino acid match). Only the third domain, exclusive of the secreted terminus, has the high degree of homology (52%) expected for corresponding structures.

The human δ gene has been cloned and a fragment sequenced to show that its position in human DNA is analogous to that in the mouse[92]. However, the disappearance of an entire domain in the mouse clearly represents a dramatic evolutionary difference that raises a serious question about whether the two molecules could serve the same function (or any function) in the two species. Could the apparent loss of a domain result from a technical artefact? We think that this is unlikely because data for rat[93,94] and another strain of mouse[95] indicate that both have the same two domain δ-chain structure that we deduced for BALB/c[37,38]. Moreover two independent human δ-chains have been sequenced with identical results. It seems that there must have been a dramatic divergence in the δ gene after the separation of human from rodent evolutionary lines.

It is unclear how these differences should be interpreted. Logic would indicate that the second domain is not important to IgD function, and would focus attention on the third domain or possibly the spacer segment, whose sequence is unknown in man. On the basis of recent studies of domain deletion mutants (V. Oi, personal communication), there is reason to believe that the absence of a $C_\delta 2$ domain may not cause extensive disruption of general immunoglobulin folding patterns[96]. In any event, the existence of IgD in seven species (ref. 97 and refs therein) probably indicates that the molecule must have conferred a significant advantage over evolutionary time.

We thank J. Richards, A. Shen, F. Mushinski, C. Word, A. Gilliam, J. Wells, D. Yuan and T. Shinnick for providing data before publication, J. Uhr, D. Capra, C. Janeway, R. Schwartz, V. Oi, W. Paul and M. Wabl for suggestions on the manuscript, D. Odle for the art work and N. Peterson and M. Allan for secretarial assistance.

1. Shimizu, A., Takahashi, N., Yoshita, Y. & Honjo, T. *Cell* **28,** 499–506 (1982).
2. Marcu, K. B. *Cell* **29,** 719–721 (1982).
3. Tonegawa, S. *Nature* **302,** 575–581 (1983).
4. Cosenza, H. & Köhler, H. *Proc. natn. Acad. Sci. U.S.A.* **69,** 2701–2705 (1972).
5. Eichman, K. & Rajewsky, K. *Eur. J. Immun.* **5,** 661–666 (1975).
6. Van Boxel, J. A., Paul, W. E., Terry, W. D. & Green, I. *J. Immun.* **109,** 648–651 (1972).
7. Vitetta, E. S. *et al. J. exp. Med.* **141,** 206–215.
8. Rowe, D. S. & Fahey, J. L. *J. exp. Med.* **121,** 171–178 (1965).
9. Pernis, B., Forni, L. & Luzzati, A. L. *Cold Spring Harb. Symp. quant. Biol.* **41,** 139–145 (1976).
10. Cooper, M. D., Kearney, J. F., Lydyard, P. M., Grossi, C. E. & Lawton, A. R. *Cold Spring Harb. Symp. quant. Biol.* **41,** 139–147 (1976).
11. Goding, J. W., Scott, D. W. & Layton, J. E. *Immun. Rev.* **37,** 152–186 (1977).
12. Vitetta, E. S. & Uhr, J. E. *Eur. J. Immun.* **6,** 140–143 (1976).
13. Kearney, J. F. *et al. J. exp. Med.* **146,** 297–301 (1977).
14. Layton, J. E., Johnson, G. R., Scott, D. W. & Nossal, G. J. V. *Eur. J. Immun.* **8,** 325–330 (1978).
15. Stern, C. & McConnell, I. *Eur. J. Immun.* **6,** 225–227 (1979).
16. Goding, J. W. & Layton, J. E. *J. exp. Med.* **144,** 852–857 (1976).
17. Bargellesi, A., Corte, G., Cosulich, E. E. & Ferrarini, M. *Eur. J. Immun.* **9,** 490–492 (1979).
18. Finkelman, F. D., Woods, V. L., Berning, A. & Scher, I. *J. Immun.* **123,** 1253–1259 (1979).
19. Parker, D. C. *Immun. Rev.* **52,** 115–139 (1980).
20. Vitetta, E. S., Pure, E., Isakson, P. C., Buck, L. & Uhr, J. W. *Immun. Rev.* **52,** 211–231 (1980).
21. Sieckmann, D. G., Scher, I., Asofsky, R., Mosier, D. E. & Paul, W. E. *J. exp. Med.* **148,** 1628–1643 (1978).
22. Vitetta, E. S. & Uhr, J. W. *Science* **189,** 964–969 (1975).
23. Scott, D. W., Layton, J. E. & Nossal, G. J. V. *J. exp. Med.* **146,** 1473–1483 (1977).
24. Bourgois, A., Kitajima, K., Hunger, I. R. & Askonas, B. A. *Eur. J. Immun.* **7,** 151–153 (1977).
25. Sitia, R., Abbott, J. & Hammerling, U. *Eur. J. Immun.* **9,** 859–864 (1979).
26. Parker, D. C., Fothergill, J. J. & Wadsworth, D. C. *J. Immun.* **123,** 931–941 (1979).
27. Isakson, P. C., Pure, E., Uhr, J. W. & Vitetta, E. S. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2507–2511 (1981).
28. Black, S. J., Van du Loo, W., Loken, M. R. & Herzenberg, L. A. *J. exp. Med.* **147,** 984–996 (1980).
29. Herzenberg, L. A., Black, S. J., Tokuhisa, T. & Herzenberg, L. A. *J. exp. Med.* **151,** 1071–1087 (1980).
30. Zan-Bar, I., Strober, S. & Vitetta, E. S. *J. Immun.* **123,** 925–930 (1979).
31. Coffman, R. L. & Cohn, M. *J. Immun.* **118,** 1806–1815 (1977).
32. Jacobsen, E. B. *et al. J. exp. Med.* **154,** 318–332 (1981).
33. Manning, D. D. *J. reticuloendothel. Soc.* **18,** 63–86 (1975).
34. Cooper, M. D., Kearney, J. F., Gathings, W. E. & Lawton, A. R. *Immun. Rev.* **52,** 29–53 (1980).
35. Zitron, M., Mosier, D. E. & Paul, W. E. *J. exp. Med.* **146,** 1707–1718 (1977).
36. Cambier, J. C. & Vitetta, E. S. *Proc. natn. Acad. Sci. U.S.A.* **78,** 432–435 (1978).
37. Tucker, P. W., Liu, C.-P., Mushinski, J. F. & Blattner, F. R. *Science* **209,** 1353–1360 (1980).
38. Cheng, H.-L., Blattner, F. R., Fitzmaurice, L., Mushinski, J. F. & Tucker, P. W. *Nature* **296,** 410–415 (1982).
39. Eidels, L. *J. Immun.* **123,** 891–902 (1979).
40. Mescher, M. & Pollack, R. R. *J. Immun.* **123,** 1155–1161 (1979).
41. Finkelman, F. D., Kessler, S. W., Mushinski, J. F. & Potter, M. *J. Immun.* **126,** 680–687 (1981).
42. Early, P. *et al. Cell* **20,** 313–319 (1980).
43. Kawakami, T., Takahashi, N. & Honjo, T. *Nucleic Acids Res.* **8,** 3933–3945 (1981).
44. Alt, F. W. *et al. Cell* **20,** 293–302 (1980).
45. Rogers, J. *et al. Cell* **20,** 303–312 (1980).
46. Liu, C.-P., Tucker, P. W., Mushinski, J. F. & Blattner, F. R. *Science* **209,** 1348–1352 (1980).
47. Moore, K. W. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 1800–1804 (1981).
48. Maki, R. *et al. Cell* **24,** 353–365 (1981).
49. Knapp, M. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 2996–3000 (1982).
50. Knapp, M. R. *et al. J. Immun.* **123,** 992–999 (1979).
51. Rasche, W. C. *Curr. Topics Microbiol. Immun.* **81,** 70–76 (1978).
52. Knapp, M. R., Severinson-Gronowicz, E., Schroeder, J. & Strober, S. *J. Immun.* **123,** 1000–1006 (1979).
53. Wabl, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **77,** 6793–6796 (1980).
54. Nikaido, T., Yamawaki-Kataoka, Y. & Honjo, T. *J. biol. Chem.* **257,** 7322–7329 (1982).
55. Richards, J. E., Gilliam, A. C., Shen, A., Tucker, P. W. & Blattner, F. R. *Nature* **306,** 483–487 (1983).
56. Nikaido, T., Nakai, S. & Honjo, T. *Nature* **292,** 845–848 (1981).
57. Davis, M. M., Kim, S. K. & Hood, L. *Cell* **22,** 1–2 (1980).
58. Marcu, K. B., Lang, R. B., Stanton, L. W. & Harris, L. J. *Nature* **298,** 87–89 (1982).
59. Mushinski, J. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **77,** 7405–7409 (1981).
60. Sablitzky, F., Radbruch, A. & Rajewsky, K. R. *Immun. Rev.* **67,** 59–72 (1982).
61. Mather, E. L. & Perry, R. P. *Nucleic Acids Res.* **9,** 6855–6866 (1981).
62. Mount, S. M. *Nucleic Acids Res.* **10,** 459–472 (1982).
63. Darnell, J. E. *Nature* **297,** 365–371 (1982).
64. Ziff, E. B. *Nature* **278,** 491–499 (1980).
65. Nevins, J. R. *Cell* **28,** 1–2 (1982).
66. Hofer, E., Hofer-Warbinek, R. & Darnell, J. E. *Cell* **29,** 887–893 (1982).
67. Proudfoot, N. *Nature* **298,** 516–517 (1982).
68. Proudfoot, N. J. & Brownlee, G. G. *Nature* **252,** 359–362 (1964).
69. Hagenbuchle, O., Bovey, R. & Young, R. A. *Cell* **21,** 179–187 (1980).
70. Nelson, K. J., Haimovich, J. & Perry, R. P. *Molec. cell. Biol.* **3,** 1317–1332 (1983).
71. Defranco, A. L., Raveche, E. S., Asofsky, R. & Paul, W. E. *J. exp. Med.* **155,** 1523–1536 (1982).
72. Cohen, P., Ziff, M. & Vitetta, E. S. *J. Immun.* **121,** 973–980 (1978).
73. Kemp, D. J., Morahan, G., Cowman, A. F. & Harris, A. W. *J. Cell Biochem.* **6,** 310 (1982).
74. Melchers, F. & Cone, R. *Eur. J. Immun.* **5,** 382–388 (1975).
75. Yaoita, Y., Kumagai, Y., Okumura, K. & Honjo, T. *Nature* **297,** 697–699 (1982).
76. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33,** 717–728 (1983).
77. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33,** 729–740 (1983).
78. Queen, C. & Baltimore, D. *Cell* **33,** 741–748 (1983).
79. Rogers, J. & Wall, R. *Proc. natn. Acad. Sci. U.S.A.* **78,** 7497–7501 (1982).
80. Yuan, D. & Tucker, P. W. *J. Immun.* (in the press).
81. Pure, E. & Vitetta, E. S. *J. Immun.* **125,** 420–427 (1980).
82. Jerne, N. K. *Ann. Immun. Inst. Pasteur* **125C,** 373–389 (1974).
83. Kelsoe, G., Reth, M. & Rawesky, K. *Immun. Rev.* **52,** 75–88 (1980).
84. Hiernaux, J. *Fedn Proc.* **40,** 1484–1488 (1981).
85. Tokuhisa, T. & Taniguchi, M. *J. exp. Med.* **155,** 126–139 (1982).
86. Janeway, C. A. Jr, Sakato, N. & Eisen, H. N. *Proc. natn. Acad. Sci. U.S.A.* **72,** 2357–23[illegible] (1975).
87. Janeway, C. A. Jr & Frost, R. in *Strategies of Immune Regulation* (eds Sercarz, E. & Cunningham, A.) 179 (Academic, New York, 1980).
88. Lin, L.-C. & Putnam, F. W. *Proc. natn. Acad. Sci. U.S.A.* **78,** 504–508 (1981).
89. Putnam, F. W., Takahashi, N., Tetaert, D., Debuire, B. & Lin, L.-C. *Proc. natn. Acad. Sci. U.S.A.* **78,** 6168–6172 (1981).
90. Takahashi, N., Tetaert, D., Debuire, B., Lin, L.-C. & Putnam, F. W. *Proc. natn. Acad. Sci. U.S.A.* **79,** 2850–2854 (1982).
91. Shinoda, T. N., Takahashi, T., Takayasu, T., Okuyama, T. & Shimizu, A. *Proc. natn. Acad. Sci. U.S.A.* **78,** 785–789 (1981).
92. Rabbitts, T. H., Forster, A. & Milstein, C. P. *Nucleic Acids Res.* **9,** 4509–4524 (1981).
93. Alcaraz, G., Bourgois, A., Moulin, A., Bozin, H. & Fougeraeu, M. *Annls Immun. Inst. Pasteur, Paris* **131C,** 363–388 (1980).
94. Sire, J., Auffray, C. & Jordan, B. R. *Gene* **20,** 377–386 (1982).
95. Dildrop, R. & Beyruther, K. *Nature* **292,** 61–63 (1981).
96. Poljak, R. J. *CRC crit. Rev. Biochem.* **5,** 45–68 (1978).
97. Leslie, G. A. & Cuchens, M. A. *Ann. N. Y. Acad. Sci.* **399,** 131–141 (1982).
98. Fitzmaurice, L. *et al. Nature* **296,** 459–462 (1982).
99. Siden, E., Alt, F. W., Shinefeld, L., Sato, V. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1823–1827 (1981).
100. Alt, F. W., Rosenberg, N. E., Enea, V., Siden, E. & Baltimore, D. *Molec. cell. Biol.* **2,** 386–400 (1982).
101. Kemp, D. J., Harris, A. W., Cory, S. & Adams, J. *Proc. natn. Acad. Sci. U.S.A.* **77,** 2876–2880 (1980).
102. Honjo, T. *et al. Cell* **18,** 559–568 (1979).
103. Rogers, J. *et al. Cell* **26,** 19–27 (1981).
104. Yamawaki-Kataoka, Y., Nakai, S., Miyata, T. & Honjo, T. *Proc. natn. Acad. Sci. U.S.A.* **79,** 2623–2627 (1982).
105. Tyler, B. M., Cowman, A. F., Gerondakis, S. D., Adams, J. M. & Bernard, O. *Proc. natn. Acad. Sci. U.S.A.* **79,** 2008–2012 (1982).
106. Tucker, P. W., Marcu, K. B., Newell, N. N., Richards, J. E. & Blattner, F. R. *Science* **206,** 1303–1306 (1979).
107. Yamawaki-Kataoka, Y., Miyata, T. & Honjo, T. *Nucleic Acids Res.* **9,** 1365–1381 (1981).
108. Nishida, Y. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 1581–1585 (1981).
109. Ishida, N., Ueda, S., Hayashida, H., Miyata, T. & Honjo, T. *EMBO J.* **1,** 1117–1123 (1982).
110. Tucker, P. W., Slightom, J. S. & Blattner, F. R. *Proc. natn. Acad. Sci. U.S.A.* **78,** 7684–7688 (1981).
111. Word, C. J., Mushinski, J. F. & Tucker, P. W. *EMBO J.* **2,** 887–898 (1983).

Palaeoanthropological discoveries in the Middle Awash Valley, Ethiopia

J. Desmond Clark*, Berhane Asfaw*, Getaneh Assefa†, J. W. K. Harris‡, H. Kurashina§, R. C. Walter‖, T. D. White* & M. A. J. Williams¶

* Department of Anthropology, University of California, Berkeley, California 94720, USA
† Department of Geology, Addis Ababa University, Addis Ababa, Ethiopia
‡ Department of Anthropology, University of Wisconsin, Milwaukee, Wisconsin 53201, USA
§ Department of Anthropology, University of Guam, Mangilao, Guam
‖ Department of Geology, University of Toronto, Toronto, Canada M5S 1A1
¶ School of Earth Sciences, Macquarie University, North Ryde, New South Wales 2113, Australia

A survey of Ethiopia's Middle Awash Valley in 1981 yielded new and significant archaeological, palaeontological, geological and geochronological results. Included in the new discoveries are hominid fossils from the Pliocene which show primitive cranial anatomy and a femur adapted to bipedality. Archaeological records document human technological change spanning the past 1.5 Myr.

RESEARCH into Africa's rift system has elucidated critical stages of human technological and physical development, providing data on environments, behaviour and phylogenetic relationships of extinct hominid species. The 1981 survey of Ethiopia's Middle Awash Valley resulted in new discoveries and confirmed the importance of the region for human origins research.

The study area is situated along the Awash River Valley between the site of Hadar in the north and the town of Gewane in the south. Miocene, Pliocene and Pleistocene deposits crop out along both eastern and western valley margins as well as in the basin centre. Geologist Taieb discovered the palaeoanthropological significance of the region during geological reconnaissance in the 1960s[1]. In 1971 and 1972, Taieb, Johanson, Coppens and Kalb conducted a preliminary survey of the central Afar. They formed the International Afar Research Expedition (IARE) and began to investigate Hadar. Discoveries there yielded new insights into hominid evolution[2,3].

The Middle Awash was explored between 1975 and 1978 by Kalb's Rift Valley Research Mission in Ethiopia (RVRME). Small excavations were done and a partial hominid cranium was found in Middle Pleistocene deposits at Bodo[4]. Taieb's initial work had identified sediments along the Awash ranging between Pliocene and Quaternary[1,5]. The RVRME conducted the initial mapping and palaeontological work here. Biostratigraphical data indicated prolonged deposition[6–10].

Wendorf, an RVRME member, invited one of us (J.D.C.) to join the archaeology team in 1977 and in 1981 the Ethiopian Ministry of Culture invited J.D.C. to lead a multidisciplinary team to the Middle Awash. Two months were spent in the field examining localities in the western, central and eastern sectors of the study area. Abundant archaeologocal remains ranging from later stone age to Oldowan were identified. Biostratigraphical analysis confirmed that approximately 6 Myr of vertebrate evolution is represented within the study area. A programme of tephrostratigraphy was initiated and the first radiometric dates for the Middle Awash strata were obtained.

Stratigraphy

Exposure of sediments in the area is often good. However, lateral correlation is hindered by common NNW–SSE and NNE–SSW normal faults that dissect the deposits and bring rocks of different age into fault contact. Lithological correlation is further impeded by erosional gaps within the sequences and between the areas. Lateral facies changes and the repetitive nature of the late Neogene sedimentation complicate the geology. For these reasons the numerous volcanic horizons provide essential stratigraphical and chronological control. Kalb *et al.*[7,9] recently formalized sedimentological groups, formations and members for the entire Middle Awash area. We refer to these units below but prefer to work with the previously introduced, informal stratigraphical nomenclature[6] until tephrostratigraphy clarifies formational boundaries in the region. Our 1981 survey showed that an east–west geological transect across the Bodo and Dawaitoli areas provides a useful guide to the general stratigraphical and tectonic relationships of Plio-Pleistocene deposits of the eastern sector of the Middle Awash (Figs 1 and 2). The 1981 archaeological and palaeontological discoveries reported here are either within or firmly correlated to the stratigraphical sequence at Bodo.

Deposits that crop out closest to the Awash in the eastern sector consist mostly of sterile brown clays and silts but include additional interbedded strata in the Bodo, Dawaitoli, Hargufia and Meadura catchments. These strata, the Upper Bodo Beds of Kalb *et al.*[6] are particularly rich in Middle Pleistocene artefact assemblages and fauna. Work on volcanic horizons in these beds should help to clarify relationships between the Bodo, Meadura and Dakanihyalo members of the Wehaietu Formation formalized by Kalb *et al.*[9].

Lower Pleistocene sediments found in the Bodo area are structurally separated from the Middle Pleistocene outcrops by normal faults, downthrown to the west. Similar structural relationships are observed between Pliocene and late Pliocene/Lower Pleistocene rocks at Dawaitoli, Wilti Dora and Matabaietu. The Lower Pleistocene beds at Bodo, the Middle Bodo Beds of Kalb *et al.*[6] (Matabaietu Formation[9]) consist of brown clays interbedded with both unconsolidated and calcite-cemented sands and gravels. The cemented sands are richly fossiliferous and at least three volcanic tuffs are interbedded in the sequence.

Pliocene deposits exposed in the Bodo area are >70 m thick and constitute the Lower Bodo Beds of Kalb *et al.*[6] (Sagantole Formation, Beearyada and Kalaloo Beds[9]). Seven tuffaceous units were identified in the composite section and one of them, informally termed the Cindery Tuff (CT), was used as a primary chronostratigraphical marker horizon (Fig. 2). This tuff is a 15–30-cm thick, grey lapilli tuff. It is a primary plinian ash deposit that, in hand sample, shows grey pumice clasts, clear grey glass shards and scoracious black glass fragments ranging from sand-size particles (1.0 mm) to fine, granule-size particles

© 1984 Macmillan Journals Ltd

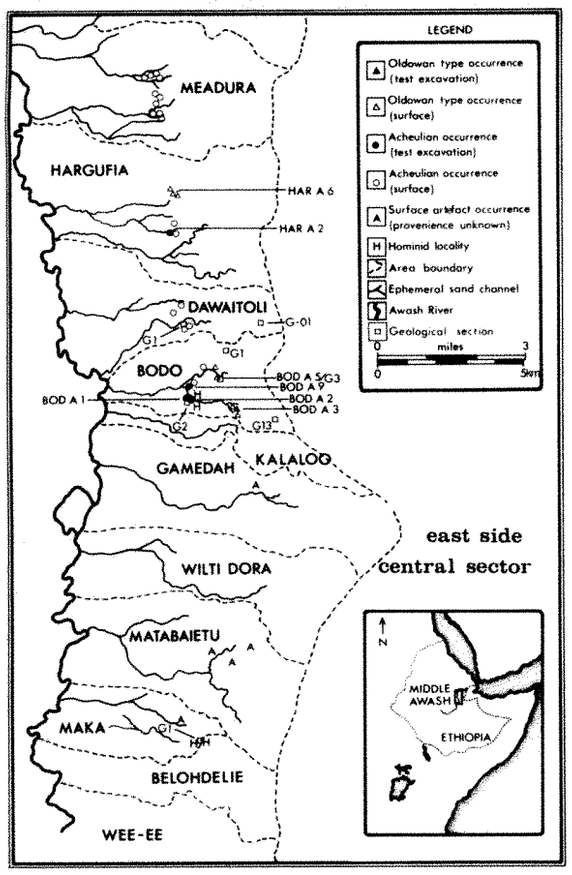


Fig. 1 The central eastern sector of the Middle Awash study area.

(up to 5.0 mm). The CT crops out extensively at Bodo and appears to have erupted into a very shallow, laterally extensive, freshwater lake, probably not highly alkaline. The description of "... a widely traceable coarse or lapilli tuff (10–35 cm)" at the base of the Kalaloo Beds[7,9] is here interpreted as the only previous description of the Cindery Tuff. Mammalian footprints found impressed into the upper CT surface at Gamedah suggest shallow lake conditions, possibly the lake margin. We have already traced the CT over a distance of ~30 km from Wee-ee to Meadura (Fig. 1).

Petrographic studies and microprobe geochemistry show that the CT is a primary ash deposit, not reworked, and containing no obvious detrital phases. The $^{40}Ar/^{39}Ar$ analyses performed on CT feldspars and glasses are concordant, yielding ages of 3.9–4.0 Myr. These results are corroborated by preliminary zircon fission track age estimates conservatively set at 4.0 ± 0.2 Myr. An isotopic age concordance of 4.0 Myr for the CT is thus a reasonable assessment of its eruptive age, and age consistent with biostratigraphic findings of the 1981 fieldwork and previous analyses[12,13].

Outcrops exposing the CT are among the most laterally continuous of this age known in Africa. At least 50 m of horizontally bedded silts, clays, diatomites, lenses of fossiliferous sand and gravel and occasional bands of volcanic ash, limestone and lignite lie below the CT. The sections beneath the CT are lithologically uniform from Wee-ee to Bodo. Diatomite is common in the upper 20 m of sediments beneath the tuff where 1–6 m thick diatomite beds alternate with grey-brown clays of comparable thickness. Facies changes are more common above the CT. Approximately 20 m of sediment overlie the CT in the Maka area. These include a vitric tuff and fossiliferous, yellow-orange stained gravel-sands. The cross-bedded sands and gravels alternate with subhorizontal silts and clays above the CT and indicate a major contrast to deposition below the tuff—a contrast which persisted during Lower and Middle Pleistocene times. Tectonic events were probably responsible for the demise of Miocene and early Pliocene lakes in the area[11].

Archaeology

Taieb[1] was the first to record artefacts coming from Plio-Pleistocene sediments in the central Afar. Kalb *et al.*[8,14–16] have also reported the existence of numbers of archaeological sites in the Middle Awash yielding stone artefacts of Oldowan, Acheulian, Sangoan and later age and have published accounts of two of these[15,17]. Corvinus and Roche[18,19] recorded Oldowan-type artefacts from conglomerates of the upper beds of the Hadar Formation in the Gona River Area. Harris[20] has subsequently test excavated and described an occurrence of Oldowan artefacts and bone *in situ* in fine-grained sediments in the Gona catchment; these, it is suggested, might be nearly 2.4 Myr old.

Our own survey concentrated on sediments of Upper Pliocene (Lower Bodo Beds[6]; Matabaietu Formation[7]) and Lower and Middle Pleistocene (Middle and Upper Bodo Beds[6]; Wehaietu Formation[7]) ages, situated on the east side of the Awash between the Maka and Meadura catchment areas and also at Dakanihyalo on the west side of the study area. Oldowan assemblages were found eroding from overbank silts in the Middle Bodo Beds[6] exposed in the Ounda Bodo catchment. Typologically Lower Acheulian occurred in a lag gravel capping Pliocene sediments at Maka and in fossiliferous fluvio-lacustrine sediments at Dakanihyalo. Numerous occurrences as previously reported by Kalb *et al.*[8] were found eroding from Middle Pleistocene (Upper Bodo beds[6]; Wehaietu Formation[7]) channel sands and silts. Two of the Oldowan and four of the Acheulian sites were test excavated.

Oldowan

Kalb *et al.*[8] have reported Oldowan artefacts eroding from Lower Pleistocene sediments at Wilti Dora but, in spite of close search, we were unable to locate any *in situ* artefacts although heavily patinated choppers and flakes traced to an overlying lag gravel lie on the slopes in some places. At Matabaietu, where a surface Oldowan occurrence had previously been reported[15], the 1981 survey found small numbers of heavily patinated artefacts on the surface and close to the bones of elephant and hippopotamus. This association could be fortuitous resulting from deflation so that, until stratified archaeological occurrences are discovered and excavated, the designation of Matabaietu as an Oldowan locality must remain uncertain.

We were, however, fortunate in locating in the Bodo drainage five Oldowan occurrences. These are the earliest stratified occurrences of stone artefacts found in the Middle Awash so far, although older occurrences can be expected. The assemblages, characterized by small cores and flakes with occasional retouched pieces, show morphological and technological similarities to Plio-Pleistocene stone artefact occurrences assigned to the Oldowan Industrial Complex found just to the north at Hadar and elsewhere in the rift system (Fig. 5, nos 7–13)[18–22].

The Oldowan sites are clustered in two fault-bounded blocks of sediment constituting the Middle Bodo Beds[6]. The northern block contains fauna which indicate an age of 1.3–1.5 Myr. The stratigraphical relationships of the archaeological occurrences in the northern and southern blocks at Bodo remain to be worked out in detail. The most informative site, BOD-A3, is situated in the south-east sector, in the Ounda Bodo catchment. A total of 389 stone artefacts and numerous fragmentary faunal remains were found in relatively fresh condition scattered over a steep-sided outcrop of fine-grained fluvial deposits. Surface artefacts include several flake scraper forms ($n = 10$) as well as modified and utilized flakes and cobbles including hammerstones ($n = 8$). The major proportion of the assemblage ($n = 371$) is cores, unmodified flakes and flaking debitage. Another 45 stone specimens were recovered *in situ* from a 10 m^2 excavation. Fine-grained basalt was the preferred raw material but artefacts were also made on glassy or aphyric rhyolite, welded ignimbrite and chert. Most faunal specimens are highly fragmentary and

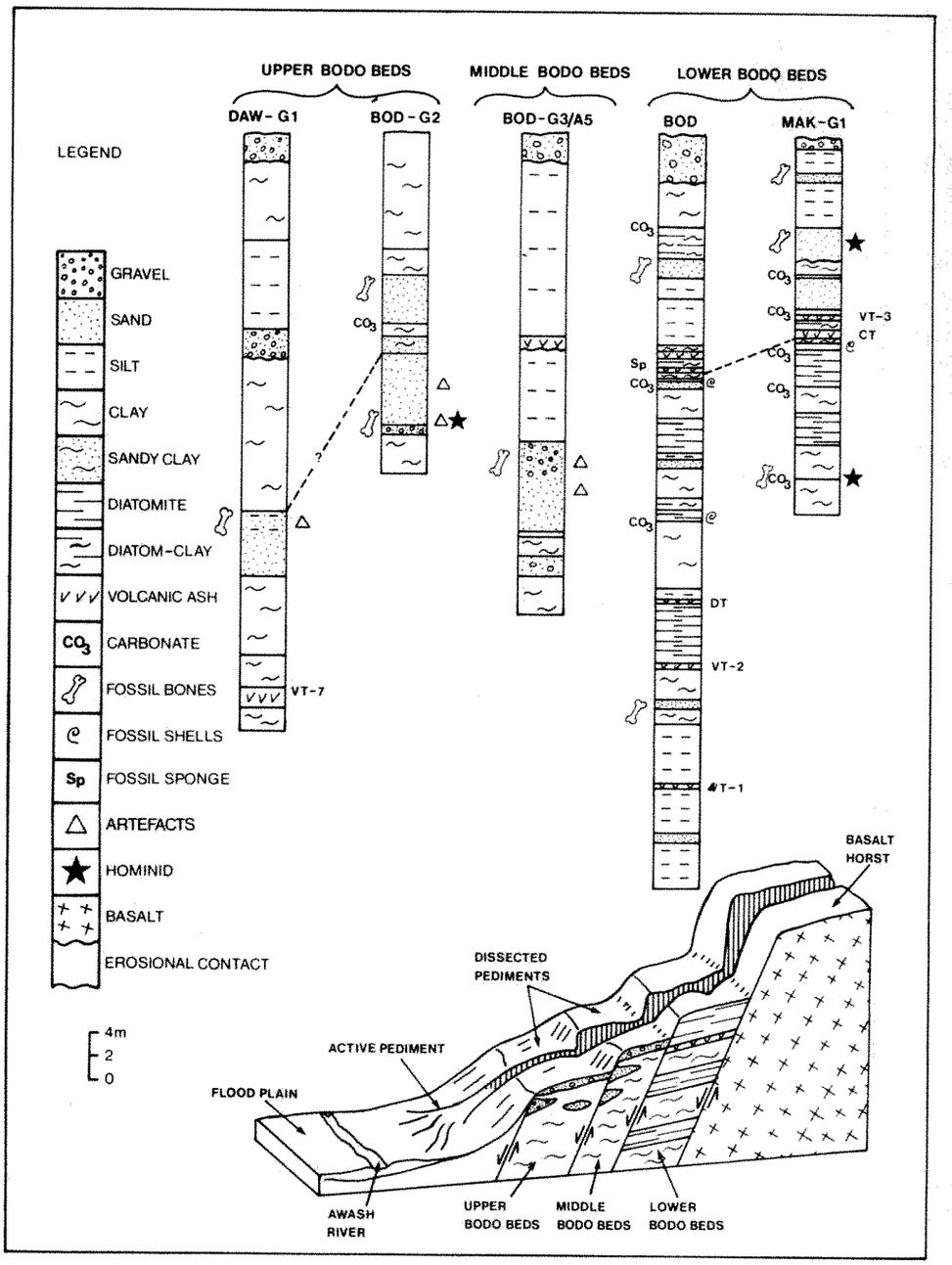


Fig. 2 Representative geological columns showing the three main stratigraphical elements present at Bodo. Also shown are the stratigraphical positions of artefacts and the three fossil hominids found in 1981. The Pliocene section at Bodo is a composite of three separate measured sections (DAW-GO1, BOD-G1 and BOD-G13, see Fig. 1) that were correlated in the field using the Cindery Tuff (CT) or Doublet Tuff (DT) as stratigraphical markers. An idealized cross-section is presented which illustrates the general geomorphic, stratigraphical and structural relationships between the main stratigraphical elements in this area.

the long bone fragments display longitudinal and spiral fracture. No cutmarks were observed. The fresh condition, lack of size sorting, lack of preferred orientation and sedimentary matrix of the specimens indicate a low-energy environment of silty overbank deposition suggesting an occurrence in minimally disturbed context.

Several similar archaeological occurrences were found nearby in overbank silty deposits. Close to one of these, designated BOD-A4, clay samples were collected from a cone-shaped, reddish area. Magneto-thermal analyses by M. Barbetti at the University of Sydney Radiocarbon Laboratory showed that the BOD-A4 clay samples had been baked. Such baked clay concentrations occur in sediments ranging in age from Pliocene to Middle Pleistocene in the Middle Awash. Stone artefacts and fractured bone occurred adjacent to some of these concentrations in the Pleistocene sediments while others again had nothing associated. Two Middle Pleistocene occurrences were sectioned and found to consist of a cone-shaped mass of burnt clay with a shallow concave base. Channels in some of these burnt concentrations are evidence of insect activity, probably termites. These features have the appearance of the burnt earth that is built up round the bases of old trees and tree stumps that have burned and are commonly to be seen in the tropics today. While it seems likely that many of these occurrences resulted from 'natural' fires, the observation made by one of us (J.D.C.) in central India early in 1982 of the way in which tree stumps were set alight intentionally by farmers, in order to have fire always available when they were working in areas distant from the homesteads, makes it possible, so great is the attraction and significance of fire for the human race, that early hominids might similarly have used and preserved fire in such a manner.

In view of the controversy surrounding the controlled use of fire by early hominids[23,24] and due to the fact that these localized, discoloured areas are a widespread phenomenon in the Middle Awash, a systematic study of these features to determine more precisely the nature of each occurrence, temperature ranges, palaeomagnetic field strengths, the agencies that caused the baking and the extent to which artefacts and bone may be significantly associated should be given high priority in the future.

Acheulian and developed Oldowan

The two occurrences that can be assigned to an earlier stage of the Acheulian Industrial Complex—from Maka and

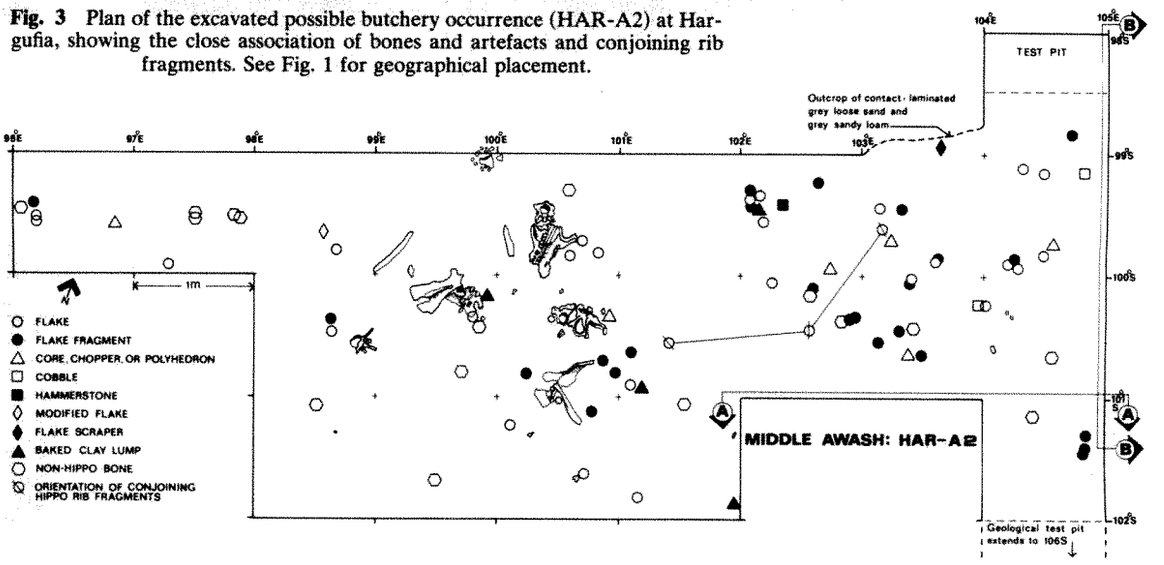


Fig. 3 Plan of the excavated possible butchery occurrence (HAR-A2) at Hargufia, showing the close association of bones and artefacts and conjoining rib fragments. See Fig. 1 for geographical placement.

Dakanihyalo—are made from lavas and comprise thick-butted and sometimes triagular-sectioned bifaces, core-choppers and flakes technologically and typologically analogous to Lower Acheulian assemblages from East Africa[22,25]. The fauna associated with the Dakanihyalo assemblage as well as the technological and typological attributes of the artefacts suggest, therefore, that the Dakanihyalo Member of the Wehaietu Formation may antedate, rather than postdate, the Bodo and Meadura Members[7,8].

On the east side of the Awash, deposits (Upper Bodo Beds[6]; Wehaietu Formation[7]) that outcrop closest to the river consist mostly of sterile brown clays and silts but interbedded strata in the Bodo, Dawaitoli, Hargufia and Meadura catchments are particularly rich in artefact assemblages that we ascribe to the Upper Acheulian and developed Oldowan industrial complexes. Those occurrences least disturbed by fluvio-lacustrine resorting or other agencies are found within massive silts and clayey fine sands which often merge laterally into coarse channel sands and gravels. Where stone artefacts are found within channel sands they may be imbricated but have not necessarily been moved very far.

Four test excavations were carried out in 1981. Assemblages are mostly of two kinds—either (1) large concentrations of Acheulian handaxes and cleavers with flaking waste but a significant paucity of light-duty artefacts and fauna (for example, BOD-A2) or (2) developed Oldowan-type assemblages of flakes (some retouched), core/choppers, and so on; the latter are associated with much broken bone material. In the Acheulian assemblages cleavers predominate over handaxes and, though these were retouched at the sites where they were used, the primary flakes from which they were made were obtained elsewhere and carried into the activity areas. These flakes appear to have been struck from 'giant polyhedron' cores of basalt that occur as erratics in the Upper Bodo Beds[6]; Wehaietu Formation[7]. Twelve of these cores were studied; each weighed more than 100 kg, averaged ~30 cm on a side and showed the scars

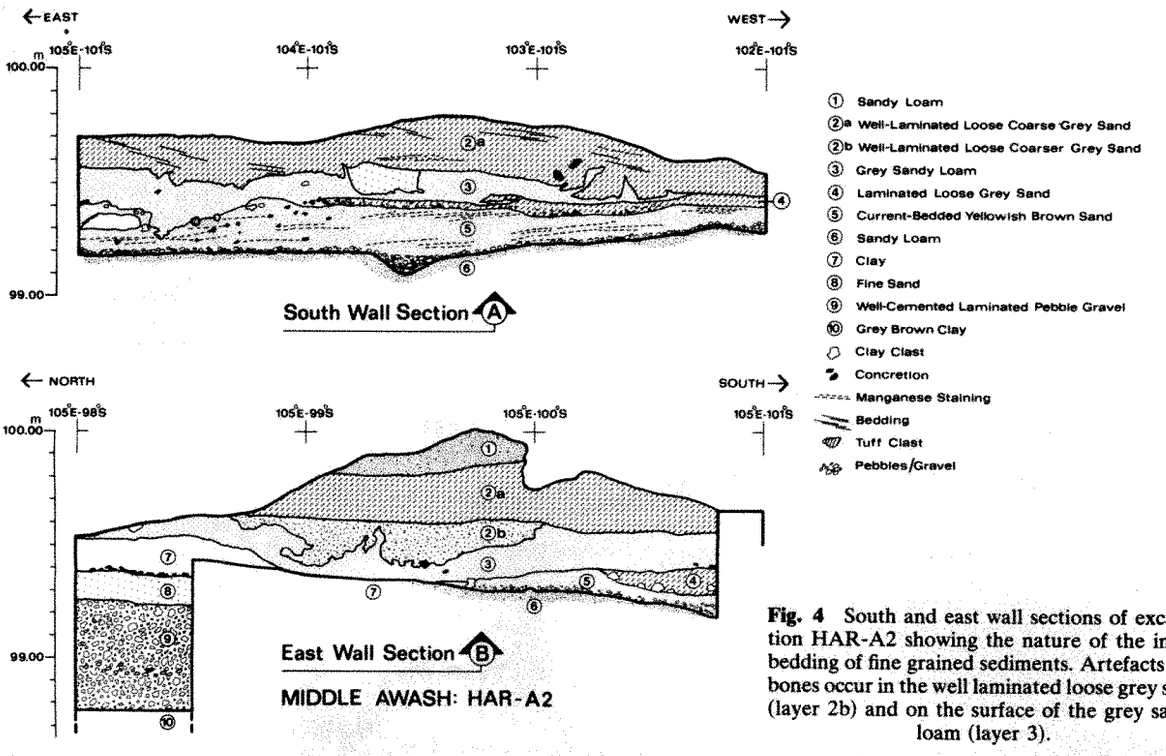


Fig. 4 South and east wall sections of excavation HAR-A2 showing the nature of the interbedding of fine grained sediments. Artefacts and bones occur in the well laminated loose grey sand (layer 2b) and on the surface of the grey sandy loam (layer 3).

of on average from four to six large flakes that had been struck from them.

Assemblages of Developed Oldowan light duty artefacts associated with bone are of two kinds. First, occurrences similar to those from the excavated site BOD-A1 which contains a small concentration (4 × 3 m) of broken bone representing possible food waste and fresh flakes, all in a fine silty sand. The bone represents fragmentary remains of large, medium and small bovids, a canid, crocodile and catfish. Associated artefacts (n = 136) consist of small primary flakes having an irregular planform and mostly plain striking platforms. Also present was a single, radially-flaked biconical core which is likely to have been the source of most of the flakes. Second, occurrences consisting of the remains of a single large animal, usually a hippopotamus, with associated artefacts. These have been considered to be butchery sites[8]. Most of those studied in 1981 were found to be in channel sands and gravels and to represent fortuitous secondary context accumulations of artefacts and bone brought together by fluvial agencies. However, one potential hippo butchery site was partially excavated at Hargufia (HAR-A2, Fig. 3). While the artefacts (n = 76) and bone (n = 38) are in fresh and unweathered condition and occur within an area of 25 m^2, they are also in secondary context. The crossbedded and poorly sorted, laminated, grey sand in which they occur (layer 2b; Fig. 4) indicates a relatively low energy current. Hippo bones (including a complete cranium, vertebrae, ribs, an innominate and scapula) show a narrow range of alignment. Joining fragments of a single rib have been displaced by little more than 1 m (Fig. 3) and both heavy and light elements occur together, evidence suggesting that the assemblage had been minimally disturbed before burial. The artefacts represent a low density occurrence and are made mostly from basalt but also from chert, rhyolite and obsidian (Fig. 5, nos 1–6). Shaped tools comprise convex side-scrapers (n = 2), polyhedron (n = 1) and sub-spheroid (n = 1), and there are three modified flake fragments. The remainder consist of unmodified small flakes (n = 26), cores (n = 5, including two discoid), flake fragments and chunks. Also associated are four lumps of burnt clay. The tight spatial relationship of bones and artefacts makes it likely that this is a butchery site and kit used by middle Pleistocene hominids.

While it is the light duty flake component that has come to be more generally associated with butchery, it should be noted that a single plotted but unexcavated surface scatter (HAR-A4) of 55 handaxes and five cleavers with hippopotamus bone ~1 km north of HAR-A2 may support Jones[26] contention that Acheulian bifaces were primarily butchery tools. The absolutely fresh and unabraded condition of the artefacts and bone fragments at HAR-A4 suggests that a large part of a single animal may still remain buried while the uniformity in biface symmetry, size and shape and the absence of trimming flakes suggest that these tools were made and used on a single occasion only.

Larger concentrations of bifaces do exist in the Afar but they appear to be rare and this pattern of small, concentrated occurrences of bifaces and light duty artefacts made for one occasion only is that which we have found to predominate in the area. Moreover, the Acheulian (bifaces) and developed Oldowan (flake/core-chopper) components are usually not found together in the same assemblage, the possibility is suggested that each may represent a different set of cultural activities. Unlike, therefore, the large concentrations of the Ethiopian and south-east plateaux sites where both components occur together[27,28], these sites in the Afar depression may provide a better opportunity for identifying what the activities may have been.

Fossil hominid discoveries

The 1981 survey resulted in the recovery of the posterio–inferior corner of a large left hominid parietal (BOD-VP-1/1, found by T.D.W.) ~400 m south of the original Bodo hominid discovery[4] and from the same Middle Pleistocene stratum. The new specimen preserves a part of the vault not seen on the original Bodo find. It represents a second, geologically contemporary adult

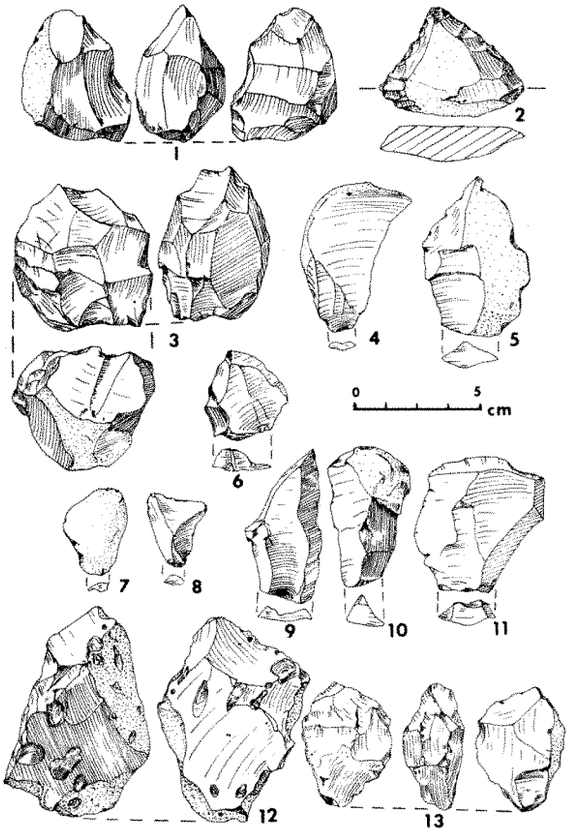


Fig. 5 Nos 1–6: artefacts from the 'Upper Bodo Beds' in the Hargufia drainage. *In situ* in HAR-A2 excavation; possible hippo butchery occurrence. All in fresh condition. 1, Angle core (side chopper): basalt (A2-19). 2, *Dejete* scraper on cortex flake: rhyolite (A2-59). 3, Multi-platformed core (three platforms): basalt (A2-4). 4, Cortex-backed knife on irregular end-struck flake: basalt (A2-20). 5, Irregular end-struck flake with marginal notching: basalt (A2-2). 6, Short sub-triangular flake: basalt (A2-10). Nos 7–13: artefacts from the 'Middle Bodo Beds' in the Bodo drainage. Oldowan Industry artefacts *in situ* from BOD-A3 excavation. All in fresh condition. 7, Irregular end-struck cortex flake: rhyolite. (A3-905). 8, Irregular end-struck flake: basalt (A3-904). 9, Irregular end-struck flake: basalt (A3-907). 10, Irregular end-struck flake (core trimming flake): basalt (A3-880). 11, Irregular end-struck flake: basalt (A3-911). 12, Single platform core (unifacial side and end chopper): vesicular basalt (A3-899). 13, Angle core (bifacial side chopper): basalt (A3-908).

individual of the same taxon. The parietal is robust, measuring 20.8 mm across asterion, the greatest such dimension recorded for a hominid. The specimen features a strong supramastoid crest extension, a massive angular torus and a wide temporal overlap[29].

An adolescent left proximal femur of a Pliocene hominid (MAK-VP-1/1) was found at Maka (by T.D.W.) only 7 m above the projected CT horizon in a limonite-stained gravelly sand that yields a diverse and abundant fauna. An erosional disconformity separates the hominid from the radiometrically dated CT horizon. However, the presence of primitive *Notochoerus euilus*, *Nyanzachoerus kanamensis*, *Elephas recki brumpti*, primitive *Theropithecus*, *Ugandax* sp., *Predamalis deturi* and *Ceratotherium praecox* demonstrates that the Maka femoral fragment is at least as old as the Sidi Hakoma member of the Hadar Formation immediately to the north and the Laetolil Beds in Tanzania. The age of the former deposits remains unresolved[30–32] while the latter are well dated to ~3.7 Myr (ref. 33). We therefore assign an age of 3.5–4.0 Myr to the Maka femoral fragment.

Approximately 700 m north-east of the Maka femur find a second Pliocene hominid fossil was recovered, 11 m below the

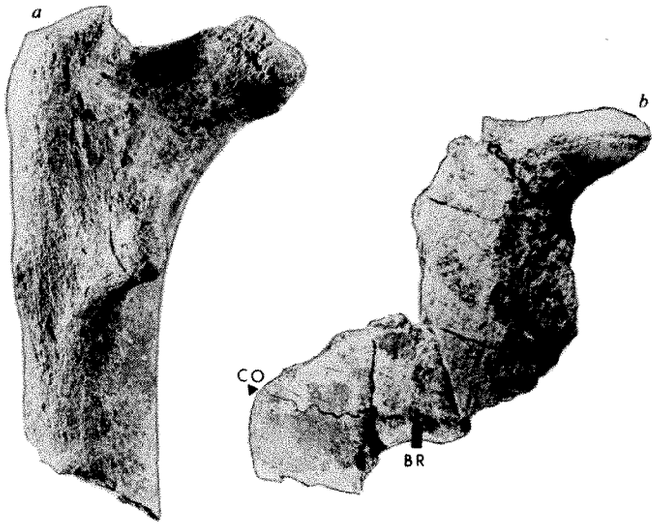


Fig. 6 Pliocene hominids from the Middle Awash, 1981. *a*, Proximal femur from Maka (MAK-VP-1/1). *b*, Frontal from Belohdelie (BEL-VP-1/1). CO indicates the coronal suture and BR indicates bregma. See text for details. See Figs 1 and 2 for geographical and stratigraphical placement. ×, 60%.

CT in the Belohdelie drainage. The specimen (BEL-VP-1/1, found by L. Krishtalka) consisted of seven vault fragments eroding from Pliocene sediments. Three of the fragments join to comprise much of an adult hominid frontal. The geological correlation for the two Pliocene hominids with respect to the CT is firm. Matrix on the fossils indicates that these surface finds eroded from the deposits on which they rested. Limited stratigraphical exposure at both localities argues against the fossils' being derived from younger sediments so that these cranial fossils are securely placed at >4.0 Myr, an age consistent with associated fauna.

The new Pliocene fossils from the Middle Awash (Fig. 6) are important because of their antiquity and because they represent functionally critical parts of the hominid skeleton. The Pliocene femur fragment represents an individual whose greater and lesser trochanters had fused. The femoral head was beginning to fuse but is now lost leaving the subepiphyseal surface partially intact. The specimen was virtually adult in size and is intermediate between the smallest (A.L. 288-1, 'Lucy') and the largest (A.L. 333-3) Hadar femora. It agrees in detail with the anatomy seen in *A. afarensis*[34]. A suite of external morphological features diagnostic of habitual bipedality is evident on the Maka specimen. These characters include the position of the lesser trochanter, the length of the neck, the morphology of the obturator insertion, the shape of the superior notch, the height of the greater trochanter and the clear obturator externus groove. The specimen is susceptible to a variety of X-ray techniques, including xeradiography and computerized tomography. The presence of thickened cortex through the ventral portion of the neck and the strong calcar also indicate habitual bipedality.

The Belohdelie specimen is stratigraphically older than the Maka femur. This fossil is of outstanding importance because it fills the last unknown area of adult cranial anatomy in the earliest Pliocene hominids. The only other hominid vault remains approaching this age belong to *A. afarensis*[35]. The Belohdelie frontal resembles that of the Laetoli child (L.H.-21)[35] and conforms well to predictions made by a composite cranial reconstruction of an adult Hadar male[36]. The new fossil is thick, measuring 8.4 at 10.0 mm anterior to bregma. Frontal length is diminutive and the overall size of the bone is equivalent to that of an adult chimpanzee. The small size and apelike morphology of the supraorbital corner combine to indicate a primitive status for the fossil. The lack of marked postorbital constriction or strong temporal line convergence is different from the robust *Australopithecus* condition. The small vault size and long, low, flat frontal squama set the specimen apart from *Homo*. Vault thickness and lack of a supratoral sulcus are traits not seen in African pongids. The morphology of BEL-VP-1/1 suggests that the fossil is best considered to belong to *Australopithecus*, a hominid genus. The specimen constitutes a significant addition to our knowledge of Pliocene hominid form and emphasizes the primitive nature of early hominid cranial architecture.

We anticipate that further fieldwork in this area will yield additional significant results.

We thank the following for assistance: The Government of Socialist Ethiopia, including the Ministry of Culture and Sports Affairs, the Ministry of Mines and Energy, the Game Commission; Addis Ababa University; the Centre for Research and Conservation of the Cultural Heritage and the National Museum of Ethiopia. Funding was provided by the NSF (BNS 80-19868) and the L. S. B. Leakey Foundation. Photographic assistance was granted by the National Geographic Society. D. C. Johanson provided critical field equipment and support. The following individuals provided valuable comments and data: D. A. Adamson, J. Aronson, M. Barbetti, M. Beden, E. Delson, F. Gasse, C. Hall, W. H. Kimbel, C. O. Lovejoy, E. Meikle, E. Vrba, J. Westgate and D. York. Very special thanks go to all the 1981 expedition members for their participation and for making this fieldwork such a success: especially to Dr L. Krishtalka, R. J. Blumenschine, B. C. Clark, T. Kaleu, C. E. Sussman, M. Tesfaye, Tafesse G. Egziabher and C. Yirgu. Their work is gratefully acknowledged. The assistance of the Afar people of Gewane and Aramis is gratefully appreciated.

Received 11 July; accepted 14 September 1983.

1. Taieb, M. thesis, Univ. Paris VI (1974).
2. Johanson, D. C. & White, T. D. *Science* **202,** 321–330 (1979).
3. White, T. D., Johanson, D. C. & Kimbel, W. H. *S. Afr. J. Sci.* **77,** 445–470 (1981).
4. Conroy, G. C., Jolly, C. J., Cramer, D. & Kalb, J. E. *Nature* **276,** 67–70 (1976).
5. Taieb, M. *C.r. Somm. Soc. Geol. Fr.* **13,** 63–65 (1971).
6. Kalb, J. E. *et al. Palaeogeogr. Palaeoclimatol. Palaeoecol.* **30,** 107–120 (1980).
7. Kalb, J. E. *et al. Nature* **298,** 17–25 (1982).
8. Kalb, J. E. *et al. Nature* **298,** 25–29 (1982).
9. Kalb, J. E., Oswald, E. B., Mebrate, A., Tebedge, S. & Jolly, C. J. *Newslett. Stratig.* **11,** 95–127 (1982).
10. Kalb, J. E. *et al. J. Vert. Palaeont.* **2,** 237–258 (1982).
11. Aronson, J. L. & Taieb, M. in *Hominid Sites: Their Palaeoenvironmental Settings* (eds Rapp, G. & Vondra, C.) 165–195 (American Association for the Advancement of Science, 1981).
12. Walter, R. C., Westgate, J. A., Hall, C. M., Gorton, M. P. & York, D. *Nature* (in the press).
13. Hall, C. M., Walter, R. C., Westgate, J. A. & York, D. *Isotopic Age Results for the Cindery Tuff.* Preprint (Univ. Toronto).
14. Kalb, J. E. *Sinet. Eth. J. Sci.* **1,** 87–98 (1978).
15. Larson, P. A. *Nyame Akuma* **11,** 6–10 (1977).
16. Kalb, J. E. *Nyame Akuma* **21,** 10–11 (1982).
17. Kalb, J. E., Jaeger, M., Jolly, C. J. & Berhane Kana. *J. Archaeol. Sci.* **9,** 349–363 (1982).
18. Corvinus, G. *Nature* **256,** 468–471 (1975).
19. Corvinus, G. & Roche, H. *Proc. 8th Panafr. Congr. Prehist. Quat. Stud. Nairobi, 1977* 186–188 (TILLMIAP, Nairobi, 1979).
20. Harris, J. W. R. & Johanson, D. C. *Proc. XI Cong. INQUA*, Moscow **2,** 98 (1982).
21. Isaac, G. L. & Harris, J. W. K. in *Koobi Fora Research Project*, Vol. 1 (eds Leakey, M. G. & Leakey, R. E. F.) 64–85 (Clarendon, Oxford, 1978).
22. Leakey, M. D. *Olduvai Gorge* Vol. 3 (Cambridge University Press, 1972).
23. Gowlett, J. A. J., Harris, J. W. K., Walton, D. & Wood, B. A. *Nature* **294,** 125–129 (1981).
24. Barbetti, M., Clark, J. D., Williams, F. M. & Williams, M. A. J. *Anthropologie* (Brno) **18,** 299–304 (1980).
25. Isaac, G. L. in *Background to evolution in Africa* (eds Bishop, W. W. & Clark, J. D.) 229–257 (University of Chicago Press, 1967).
26. Jones, P. R. *World Archaeol.* **12,** 153–165 (1980).
27. Clark, J. D. & Kurashina, H. *Nature* **282,** 33–39 (1979).
28. Chavaillon, J., Chavaillon, N., Hours, F. & Piperno, M. *Quaternaria* **21,** 87–114 (1979).
29. Asfaw, B. *Am. J. phys. Anthropol.* **61,** 367–371 (1983).
30. Brown, F. H. *Nature* **300,** 631–633 (1982).
31. Boaz, N., Howell, F. C. & McCrossin, M. *Nature* **300,** 633–635 (1982).
32. White, T. D., Moore, R. & Suwa, G. *J. Vert. Palaeont.* (in the press).
33. Leakey, M. D. & Hay, R. L. *Nature* **278,** 317–323 (1979).
34. Lovejoy, C. O., Johanson, D. C. & Coppens, Y. *Am. J. phys. Anthropol.* **57,** 679–700 (1982).
35. White, T. D. *Am. J. phys. Anthropol.* **53,** 487–504 (1980).
36. Kimbel, W. H. & White, T. D. *Am. J. phys. Anthropol.* (submitted).

Origin and evolution of the Mediterranean vegetation and climate in Europe

J.-P. Suc

Laboratoire de Palynologie, Institut des Sciences de l'Evolution (LA 327 CNRS), Université des Sciences et Techniques du Languedoc, 34060 Montpellier Cedex, France

Palynology provides information about Pliocene and early Pleistocene vegetation and climate evolution in the north-west Mediterranean area in relation to high northern latitude climatic trends. An important change occurred approximately 3.2 Myr ago with the appearance of the Mediterranean climatic rhythm (summer drought) causing the individualization of the modern Mediterranean floral elements. Quaternary-type mediterranean climatic fluctuations started approximately 2.3 Myr ago (early Glacial) causing the Mediterranean-type of vegetational organization.

MEDITERRANEAN climatic conditions are today distinctive and consist of a temperate climate characterized by dry summers with rainfall concentrated during the other seasons and low temperatures during winter. Floral assemblages and the vegetational organization are therefore very typical. To understand present day ecosystems it is essential to establish when and how the summer drought first occurred.

A model based on fossil macrofloras was proposed for California[1] (a region now displaying the same climatic and vegetational features as the Mediterranean area[2,3]) but there is at present no equivalent for the Mediterranean area where recent advances in palaeoclimatic knowledge are based mainly on studies of marine deposits, particularly from DSDP site 132, according to foraminifera[4,5], isotopic measurements[6] and clay minerals[7]. This research has yielded information about water temperatures and about rainfall rhythm. Until recently, reliable palaeoenvironmental floristic records based on pollen grains[8–10] and macroremains[11,12] were scarce.

Our own palynological research has been concentrated in the Gulf of Lion (off-shore sequences and terrestrial sections in Languedoc, Roussillon and Catalunya). New data have been obtained from off-shore sections in the Adriatic Sea and from lacustrine deposits in southern Italy (Fig. 1). Long palynological sequences covering the whole Pliocene and early Pleistocene were yielded by off-shore sections (Autan 1[13] and Garraf 1[14] in the Gulf of Lion and the Adriatic Sea, J. Cravatte and J.-P.S., unpublished data) in which the biostratigraphic control is based on planktonic foraminifera and nannoplankton. The foraminiferal zonation adopted in this article has been determined in the Gulf of Lion[15] with reference to the DSDP site 132 chronology[16]: *Globorotalia margaritae s.l.* zone, the top of which is marked by *G. puncticulata* decreasing or absent just above the *G. margaritae* extinction (from approximately 5.4 to 3.2 Myr ago); *G. crassaformis* zone, the top of which is marked by the first appearance of *G. inflata* (3.2–2.2 Myr ago); *G. inflata* zone, the top of which is marked by the first appearance of *Hyalinea balthica* (2.2–1.8 Myr ago). Short terrestrial sections (marine coastal or lacustrine deposits) have been investigated[14,17–19] and their chronological placement obtained from mammal biochronology[20] and sometimes from K–Ar (ref. 21) and palaeomagnetic measurements (refs 22, 23 and N. A. Mörner, personal communication).

Appearance of Mediterranean climate

During the Lower Pliocene (Tabianian stage, approximately represented by the *G. margaritae s.l.* zone[15]), the north-west Mediterranean area had a dense forest vegetation composed of: (1) a coastal association in which Taxodiaceae predominated (*Taxodium*-type especially), *Myrica*, *Symplocos*, *Nyssa*; and (2) just behind it, assemblages with *Engelhardtia*, *Carya*, *Rhoiptelea*, *Hamamelis*, *Embolanthera* growing in less damp biotopes.

Moist climatic conditions (with rainy summers) prevailed during this time (P I pollen zone[24]; Fig. 2*A*) and climatic fluctuations are revealed by pollen assemblages of the subzone P Ib, denoting less humid conditions. Such assemblages can be compared with the present evergreen sclerophyllous broad-leaved forest in China[25].

A great change occurred approximately 3.2 Myr ago that modified the composition and structure of the forests. The coastal forest swiftly grew thinner and disappeared. Components of the sclerophyllous forest also changed and *Quercus* species and *Alnus* (in damp biotopes) became predominant. Furthermore, xerophytic taxa (those of the present-day Mediterranean) rose by degrees to higher frequencies (*Phillyrea*, *Olea*, *Cistus*, *Quercus ilex*-type, *Pistacia*). During the beginning of this phase all species of Taxodiaceae disappeared from Languedoc-Roussillon[26].

The P I–P II boundary (Fig. 2*A*) is a climatic event, rather than related to the local Piacenzian transgressive phase, not only because of the disappearance of the coastal forest which does not occur everywhere (in Catalunya, it diminished but did not disappear at that time), but also because of the change in the structure and the composition of the sclerophyllous forest. Moreover, this boundary precedes (Rhône Valley) or follows (Catalunya) the transgressive movement. During the same time, some subtropical planktonic foraminifera disappeared from the Mediterranean Sea (*G. margaritae*, *G. puncticulata* and *Sphaeroidinellopsis*[4]).

Vegetational assemblages were certainly complex. Although a latitudinal and altitudinal zonation existed, a mosaic distribution most likely existed in relation to the relief, and different types of soils: for instance, *Cedrus* pollen grains are only abundant in western Languedoc and less so in Catalunya deposits which are located near south-facing metamorphic and granitic mountains; *Abies*, *Picea* and *Fagus* pollen grains are numerous at the opening of the Rhône Valley.

At the beginning of the P II pollen zone (Fig. 2*A*), all taxa requiring a year-long degree of moisture decreased or disappeared while xerophytic ones increased (frequencies and absolute counts[24]). *Phillyrea*, *Olea*, *Cistus*, *Quercus ilex*, *Pistacia*, which ultimately came to characterize the Mediterranean flora, were already abundant and appear to have been the most resistant to the new climatic conditions and the most adaptative but did not yet form specialized associations. Today, similar assemblages are developed in the eastern Mediterranean (Pontic) area where *Phillyrea media*, *Quercus ilex*, *Rhamnus alaternus* grow under humid conditions (maximum rainfall in summer) and are associated with deciduous forest (*Quercus*, *Pterocarya*, *Fagus orientalis*)[27]. *Pistacia*, *Phillyrea*, *Cistus*, *Olea* and the *Quercus ilex*-group (evergreen species) were present in the north-west Mediterranean area at the base of the Miocene beds (sometimes in high percentages) and mixed with tropical assemblages[28].

Also during P II, sedimentary trends evolved into coarse and red deposits chiefly in Languedoc-Roussillon. The significance

0028-0836/84/050429—04$01.00

© 1984 Macmillan Journals Ltd

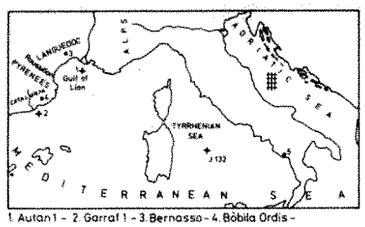


Fig. 1 Location map of the Plio-Pleistocene palynological sequences utilized in this paper. The planktonic foraminifera biostratigraphy of Autan 1 and Garraf 1 wells is referred to the DSDP site 132 chronostratigraphy.

of this has been explained as a shrinking of the forest surface caused by an increase in xericity[29]. The composition of the Ruscinian mammal faunas which mostly correlate with the P II pollen zone is in accordance with the new vegetational and climatic characteristics of that time. For instance, the Muridae, which predominated among rodents, are now living in open forest subtropical biotopes[20].

According to all these recent data, we consider that at the beginning of the Piacenzian stage (approximately 3.2 Myr ago) the climate of the north-west Mediterranean area evolved towards a seasonal rhythm of great contrast and the summer drought became stable approximately 2.8 Myr ago (Fig. 3). This opinion is supported by the changes in vegetation occurring later and hereunder described.

Earliest modern-type climatic fluctuations

In the upper part of the *G. crassaformis* zone which overlaps with the first appearance in the Mediterranean Sea of the planktonic foraminifera *G. inflata* (until then characteristic of northern latitudes)[15], 'steppic' pollen assemblages were on the increase (*Artemisia*, *Ephedra*, other Compositae, Caryophyllaceae, Amaranthaceae–Chenopodiaceae, *Erodium*)[30] compared with low frequencies of arboreal pollen grains (*Pinus* excepted). The P III pollen zone, as defined in the Autan 1 and Garraf 1 sections, has a climatic significance without any relation to the marine regression in the Gulf of Lion which occurred much earlier (Fig. 2*A*)[24]. Moreover, this climatic zone, which lasted about 0.2 Myr (approximately from 2.3 to 2.1 Myr ago[31]), was also registered in the Adriatic series (J. Cravatte and J.-P.S., unpublished data). Pollen spectra clearly demonstrate a reduction of forest cover and the enlargement of 'steppic' vegetational associations denoting drier and probably milder climatic conditions[24] (Fig. 3).

The next pollen zone (P VI–Pl. I) where the Pliocene–Pleistocene boundary occurs was recognized in southern Italy[19] (Camerota) and in the Adriatic sections. Forest associations predominated again but *Quercus*, *Carya*, *Carpinus*, *Ulmus–Zelkova* were then the most abundant taxa along with *Acer*, *Pterocarya*, *Parrotia persica*. Palaeogeographic reconstructions and comparisons between pollen- and macro-floras show an established Mediterranean vegetational zonation (P. Brenac, personal communication) as follows:

(1) The coastal *Olea–Ceratonia* association (accompanied by *Pistacia*, *Phillyrea* and *Myrtus* that is, thermo-Mediterranean).

(2) The *Phillyrea–Quercus ilex* association (accompanied by *Olea*, *Carpinus orientalis* and *Rhamnus*; that is, meso-Mediterranean).

(3) The deciduous *Quercus* forest with *Carpinus* (*C. orientalis* and *C. betulus*), *Carya*, *Ulmus* and *Zelkova* (the supra- or humid-Mediterranean zone).

(4) Mountain forest including *Cedrus*, *Pinus*, *Abies*, *Picea* and *Tsuga*.

This is an altitudinal zonation characterized by increase in moisture and decrease in temperature. The present model of such forests must be sought in Pontic and to a less degree in Caspian areas[27,32]. From a climatic point of view, the P VI–Pl. I phase

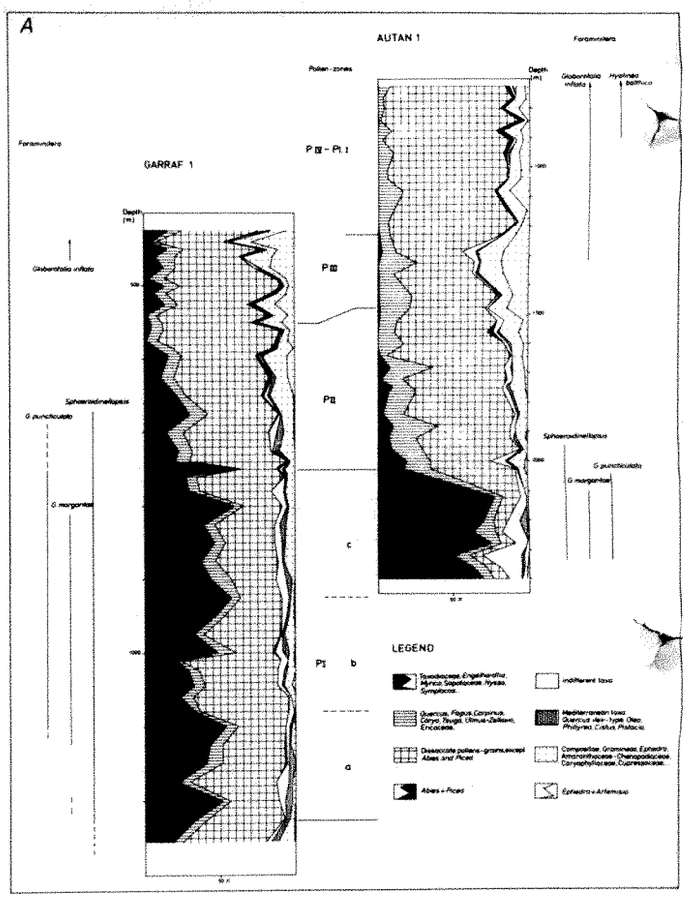


Fig. 2 Synthetic pollen diagrams based on ecological assemblages. *A*, Garraf I 1 and Autan 1 with the planktonic foraminifera first and last appearance data; the pollen zones are founded on the vegetational changes; notice the decrease of taxa requiring a year long degree of moisture (P I–P II boundary) and the increase of 'steppic' elements (P III pollen zone). *B*, Bernasso: two 'steppic' phases are bordering a deciduous forest period (interstadial). *C*, Bòbila Ordis: an interglacial deciduous forest phase.

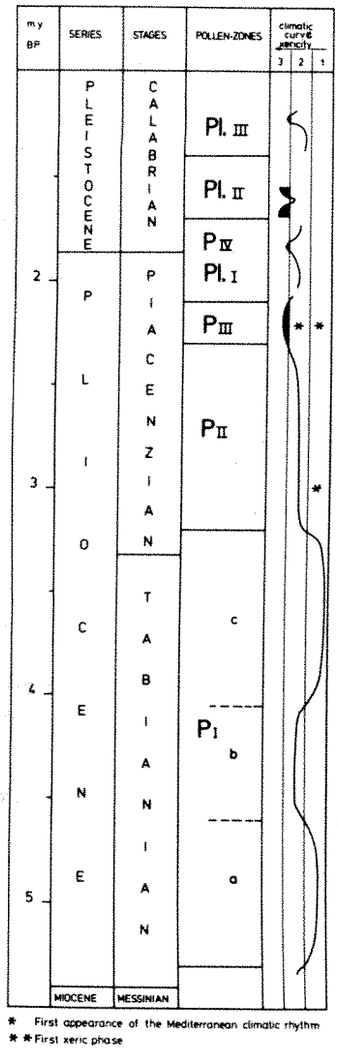


Fig. 3 Climatic evolution in north-west Mediterranean area during the Pliocene and Lower Pleistocene. The Quaternary-type climatic fluctuations began with the oldest 'steppic' phase P III.

indicates a return to moister (rainfall increasing) and warmer conditions[24] (Fig. 3).

With the P III period, the climate started fluctuating as it did in the late Pleistocene. The oldest cycle is constituted by P III and P IV–Pl. I phases, the second one[24] (Fig. 3) includes Pl. II (recognized at Bernasso[18] and in Adriatic sections—Fig. 2*B*; drier and probably cooler than P III) and Pl. III phases (recognized at Bòbila Ordis[33] and Nogaret—Fig. 2*C*; certainly warmer than P IV–Pl. I); the next cycle is incompletely known up till the lowermost oscillation in the Tenaghi Philippon pollen diagram (northern Greece) which is dated at 0.7 Myr ago[34].

These mean early climatic periods can be subdivided into several minor phases (Figs 2*B*, 2*C*, 3). The abrupt transition between two alternating minor phases appears difficult to understand but the possibility of an intermediate climate oscillating over both sides of a threshold should be considered. This is one of the reasons why we do not consider that early 'steppic' phases denote cool conditions but only drier and perhaps milder ones. Another argument is provided by some differences between the oldest and most recent 'steppic' associations as is indicated in the early Pleistocene by the presence of thermophilous elements (*Cistus*, *Phlomis* cf. *fruticosa*, *Erodium*). Thus it would be possible to discriminate between ancient Mediterranean 'steppes' and recent ones by reference to the differentiation now existing between steppes with dry and cool determinism[27]. This supports the idea of dividing the Mediterranean Pleistocene into two distinct climatic periods, the earliest warmer and the latest characterized by important temperature fluctuations[5,35]. On the other hand, the transition between two main successive vegetational (then climatic) phases appears to have been gradual as proved by the different noticeable subdivisions in the P VI–Pl. I pollen zone. In short, the modern type of Mediterranean climatic fluctuations started in the late Pliocene; the periodicity and the amplitude of these successively dry and humid periods increased up to the present.

Conclusions

Chronological relationships between the Mediterranean Plio-Pleistocene climatic fluctuations and those defined in north-west Europe[36,37] can be established. Correlations are based both on independent chronological data and on the correspondence between Mediterranean 'steppic' and tundra-like pollen spectra on the one hand and forest pollen assemblages on the other corresponding respectively to late Glacial and Holocene models[31]. Before the P III pollen zone, relationships between the Mediterranean area and north-west Europe are less obvious than in later periods; nevertheless an equal number of climatically connectable changes is observed in the two areas[31].

The following relationships[31] are thus proposed (Fig. 3):

Pl. III	Waalian	P III	Praetiglian
Pl. II	Eburonian	P II	Reuverian
P VI–Pl. 1	Tiglian	P I	Brunssumian

Therefore, climatic changes which occurred in the Mediterranean area are essentially related to temperature changes in the high northern latitudes[31].

The cooling dated at approximately 3.2 Myr ago was a global event that has been recognized in planktonic foraminifera[4,5,38] and oxygen isotopic curves[6,39,40]. In the Mediterranean area, the same trend in isotopic curve could indicate "an increased evaporation relative to precipitation"[6] which would be in accordance with information deduced from pollen records. The P I–P II boundary climatic event is probably related to this cooling caused by the beginning of the permanent glaciation in the Arctic area which in turn is related to the Gulf Stream intensification due to gradual uplift of the Panama isthmus[41]. This climatic event was preceded by the less humid P Ib phase in the Mediterranean area (that is, Brunssumian B in north-west Europe[31]) as also indicated by planktonic foraminifera data[5]. The climatic trends as revealed by palynological records in the Mediterranean area are quite similar to those indicated by data obtained from planktonic foraminifera and isotopic studies. Furthermore, palynology yields additional information about changes in moisture. The two main climatic changes occurred at around 3.2 and 2.3 Myr ago, the greater importance of the later event being generally accepted today (L. D. Keigwin, Jr. personal communication). Moreover, according to these different methods there is wide agreement that the Pliocene–Pleistocene boundary is not characterized by climatic cooling[4,5,24,31].

The pollen analysis allows distinction in the Mediterranean area between interglacial and interstadial forest phases. For instance, the forest phase in the Bòbila Ordis pollen diagram corresponds to an interglacial period shown by high frequencies of deciduous oaks (that is, the interglacial forest climax in the Mediterranean area), the presence of the two hornbeam species (*Carpinus orientalis* and *C. betulus*) and *Parrotia persica* as opposed to low percentages of Mediterranean 'steppic' elements even in the upper part of the pollen diagram (Fig. 2*C*); moreover, the relative high representation of Mediterranean taxa and the succession in vegetational features are characteristic. In contrast, very low frequencies of deciduous oaks and only one hornbeam species (*C. orientalis*) characterize the interstadial forest phase in Bernasso pollen diagram while Mediterranean 'steppic' components reach high values (Fig. 2*B*).

The beginning of the evolution of the Mediterranean-type climate has been characterized more by fluctuations in moisture (especially in rainfall rhythm) than in temperature. From the early Pliocene onwards, the climate was already fluctuating but the period and the amplitude increased during the Pleistocene.

Two very important steps are located at approximately 3.2 Myr ago (progressive establishment of the Mediterranean rhythm with dry summers; individualization of the modern Mediterranean floral elements) and 2.3 Myr ago (the oldest xeric phase; starting of the Quaternary-type oscillations; oldest signs of a Mediterranean-type vegetational organization). The previous flora was decimated and the vegetation thoroughly modified because of the intensity and rapidity of the climatic changes and, finally, human activity had a considerable effect.

We thank M. Van Campo, Ph. Guinet, J. Michaux and J.-L. Vernet for discussing the results, J. Holmes for reading the manuscript and Dr L. D. Keigwin Jr and R. C. Thunell for helpful comments. This work was supported by CNRS and the Compagnie Française des Pétroles.

Received 7 July; accepted 6 October 1983.

1. Axelrod, D. I. *Mediterranean Type Ecosystems, Origin and Structure* (eds Castri, F. Di & Mooney, H. A.) 225–227 (Springer, Berlin, 1972).
2. Nahal, I. *Ecosystems of the World* (ed. Castri, F. Di.) 63–86 (Elsevier, Amsterdam, 1981).
3. Quézel, P. & Shevok, J. *Ecol. Médit.* **8**, 389–408 (1982).
4. Ciaranfi, N. & Cita, M. B. *Init. Rep. DSDP* **13**, 1387–1399 (1973).
5. Thunnel, R. C. *Sed. Geol.* **23**, 67–79 (1979).
6. Keigwin, Jr, L. D. & Thunnel, R. C. *Nature* **282**, 294–296 (1979).
7. Chamley, H. *Bull. Gr. fr. Argiles* **27**, 97–137 (1975).
8. Pons, A. *Ann. Sci. Nat.* **5**, 499–722 (1964).
9. Bertoldi, R. *Atti Acad. Naz. Lincei* **62**, 547–555 (1977).
10. Bertolani-Marchetti, D. *L'Ateneo Parmense* **11**, 467–485 (1975).
11. Roiron, P. *Paléobiol. cont.* **12**, 27–42 (1981).
12. Vernet, J.-L. *Paléobiol. cont.* **12**, 43–51 (1981).
13. Cravatte, J. & Suc, J.-P. *Pollen et Spores* **23**, 247–258 (1981).
14. Suc, J.-P. & Cravatte, J. *Paléobiol. cont.* **13**, 1–31 (1982).
15. Cravatte, J., Dufaure, P., Prim, M. & Rouaix, S. *Notes Mém. Comp. fr. Pétroles* **11**, 209–274 (1974).
16. Cita, M. B. & Ryan, W. B. F. *Init. Rep. DSDP* **13**, 1405–1415 (1973).
17. Suc. J.-P. *Paléobiol. cont.* **12**, 7–26 (1981).
18. Suc, J.-P. *Pollen Spores* **20**, 497–512 (1978).
19. Baggioni, M., Suc, J.-P. & Vernet, J.-L. *Geobios* **14**, 229–237 (1981).
20. Michaux, J. *Bull. Ass. fr. ét. Quaternaire* **36**, 24–40 (1973).
21. Ildefonse, J.-P., Bellon, H., Pantaloni, A. & Philippet, J.-C. *Earth planet. Sci. Lett.* **14**, 249–254 (1972).
22. Lindsay, E. H., Opdyke, N. D. & Johnson, N. M. *Nature* **287**, 135–138 (1980).
23. Sémah, F. & Biquand, D. *Paléobiol. cont.* **12**, 167–173 (1981).
24. Suc, J.-P. *C. r. hebd Séanc. Acad. Sci., Paris* **294**, 1003–1008 (1982).
25. Wang, Chi-Wu. *The Forests of China* **5**, 313 (Maria Moors Cabot Foundation, Cambridge USA, 1961).
26. Michaux, J., Suc, J.-P. & Vernet, J.-L. *Rev. Palaeobot. Palynol.* **27**, 185–191 (1979).
27. Quézel, P., Barbero, M., Bonin, G. & Loisel, R. *Nat. Monsp.* 89–100 (1980).
28. Bessedik, M. *C. r. hebd. Séanc. Acad. Sci., Paris* **293**, 469–472 (1981).
29. Barrière, J. *Bull. Ass. fr. ét. Quaternaire* **8**, 142–144 (1971).
30. Zohary, M. *Geobotanical Foundations of the Middle East* (Fisher, Stuttgart, 1973).
31. Suc, J.-P. & Zagwijn, W. H. *Boreas* **12**, 153–166 (1983).
32. Emberger, L. & Sabéti, A. *Nat. Monsp. Bot.* **14**, 55–61 (1962).
33. Juliá Bruguès, R. & Suc, J.-P. *Geobios* **13**, 5–19 (1980).
34. Hammen, T. van der, Wijmstra, T. A. & Zagwijn, W. H. *The Late Cenozoic Glacial Ages*, (ed. Turekian, K. K.) 391–424 (Yale University Press, New Haven, 1971).
35. Cita, M. B., Chierici, M. A., Ciampo, G., Zei, M., D'Onofrio, S., Ryan, W. B. F. & Scoriziello, R. *Init. Rep. DSDP* **12**, 1263–1339 (1973).
36. Zagwijn, W. H. *Meded. Geol. Sticht* **C3**, 1–78 (1960).
37. Zagwijn, W. H. *Boreas* **3**, 75–97 (1974).
38. Berggren, W. A. *Init. Rep. DSDP* **12**, 953–963 (1972).
39. Shakleton, N. J. & Opdyke, N. D. *Nature* **270**, 216–219 (1977).
40. Shackleton, N. J. & Cita, M. B. *Init. Rep. DSDP* **47**, 433–445 (1979).
41. Keigwin, Jr, L. D. *Science* **217**, 350–353 (1982).

Construction, expression and recognition of an H–2 molecule lacking its carboxyl terminus

Cornelis Murre*, Carol S. Reiss†, Carmelo Bernabeu§, Lan Bo Chen‡, Steven J. Burakoff† & J. G. Seidman*

* Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA
† Divisions of Pediatric Oncology and ‡ Cell Growth and Regulation, § Laboratory of Molecular Immunology, Dana Farber Cancer Institute, Boston, Massachusetts 02115, USA

A mouse major histocompatibility antigen (H–2) gene, encoding a novel H–2L^d molecule lacking its intracytoplasmic domain, has been constructed and introduced into mouse L-cells. The novel H–2 molecule is found on the surface of the transfected cells at the same level as L-cells transfected with the native H–2L^d *gene. Allo- and influenza-specific cytotoxic T lymphocytes can recognize the truncated* H–2 *gene product nearly as efficiently as the normal* H–2L^d *gene product. However, vesicular stomatitis virus-specific cytotoxic T lymphocytes recognize the truncated H–2L^d molecule less efficiently than the complete H–2L^d product. The rate of capping of the truncated H–2L^d molecule was investigated and found to be the same as that of the complete* H–2L^d *gene product.*

THE major histocompatibility complex (MHC) of the mouse encodes a variety of cell-surface glycoproteins involved in the immune response[1,2]. H-2K, H-2D and H-2L are the three murine major histocompatibility antigens that have been defined by classical genetic and serologic critera. These transplantation antigens are members of a family of proteins referred to as class I antigens. The class I molecules are glycoproteins that consist of two subunits, β_2-microglobulin and H-2. The 12,000 molecular weight β_2-microglobulin chain is found only on the extracellular part of the protein. The H-2 subunit is about 345 amino acids long and is encoded by a gene comprised of 8 exons[3–5] (Fig. 1). The first 280 residues are on the extracellular side of the cell membrane, 40 residues are thought to be imbedded in the cell membrane and the final 25 residues are inside the cell. The role of these intracellular residues of the H-2 molecule is the subject of this article.

Class I molecules serve as targets for T-cell mediated cytolysis in allogeneic immune responses, such as *in vivo* graft rejection and destruction of allogeneic cells by cytotoxic T lymphocytes (CTLs) *in vitro*. However, the physiological role of transplantation antigens might be to serve as a restriction element in T-cell lysis of virus-infected cells. That is, virus infection of mice generates CTLs whose receptor(s) must interact with the foreign viral antigens and a class I molecule in order to lyse the virus-infected cell[6,7].

Genomic clones encoding H–2L^d and H–2D^d can be introduced into thymidine-kinase deficient mouse L-cells by co-transformation with the herpes thymidine kinase gene[8,9]. The gene products are functionally expressed and can mediate immunologically specific cellular interactions[10,11]. Recently, we have constructed *H–2D^d/H–2L^d* hybrid genes expressing the N and C_1 domains of one molecule and the C_2, M and cytoplasmic domains of the other[12]. The restricting elements for allo- and antiviral CTLs were mapped to the N and/or C_1 domains[13,14]. This approach allows us to begin to define the portions of these molecules important for CTL recognition.

In the present studies, we sought to determine whether the cytoplasmic portion of the H–2 molecule has a role in the process by which T cells lyse their targets. A variety of functions can be postulated for the intracytoplasmic portion of the H–2 molecule. To test these models, we constructed an *H–2L^d* gene in which the cytoplasmic domains have been deleted. H–2 anti-

0028-0836/84/050432—05$01.00

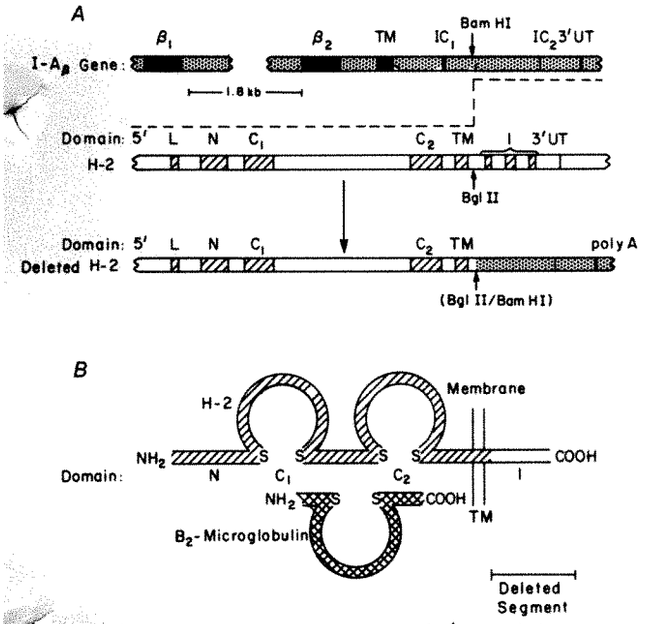


Fig. 1 Construction of the truncated *H-2L*^{d} gene (*A*) and the predicted structure of the deleted H-2 antigen (*B*). The truncated *H-2L*^{d} gene was made from restriction enzyme fragments using standard recombinant DNA technology[32]. A fragment encoding a deleted gene was isolated after a partial digestion of pL^d-4 plasmid DNA with *Bgl*II. A 2.5-kb fragment encoding the last cytoplasmic domain of the murine *I-Aβ* gene cloned into the *Bam*HI site of pBR322 was provided by Dr E. Choi. Partial digestion of this plasmid DNA with *Bam*HI yielded a single fragment containing the 2.5 kb *Bam*HI fragment and the pBR322 sequence. The *Bgl*II fragment encoding the deleted *H-2L*^{d} gene was ligated to the pBR322-I-Aβ fragment, and used to transform *Escherichia coli* strain LE322[33]. The *Bgl*II and *Bam*HI sites destroyed by this procedure are indicated as (*Bgl*II/*Bam*HI) in the deleted gene (*A*). Colonies were screened for the insert having the correct orientation by a 'mini-DNA' preparation followed by restriction enzyme digestion and agarose gel electrophoresis[34]. The resulting plasmid was extensively mapped by restriction-endonuclease cleavage to confirm the reconstruction and that no other rearrangements of the coding regions had occurred. Black areas represent the region coding for the *I-Aβ* gene[16]. Hatched areas represent the regions coding for the *H-2L*^{d} gene[4,5]. Stippled areas represent the intervening sequences and 3' untranslated region of the *I-Aβ* gene. β_1, β_2, TM, IC_1, IC_2, L, N, C_1 and C_2 represent the coding domains of the *I-Aβ* and *H-2L*^{d} genes. The portion of the H-2 molecule that should be deleted by this procedure is indicated in the lower panel.

gens, like other cell-surface molecules, 'cap'. Several models to explain the formation of these caps have been proposed[15]. We have compared the capping of the native and deleted H-2 molecules in order to understand the role of the intracytoplasmic region of the molecule in the capping process.

H-2 molecules with cytoplasmic part deleted

A variety of approaches are available for removing the segment of a gene encoding the COOH end of the protein. For example, Rose and his collaborators have deleted the 3' end of a cloned cDNA of the vesicular stomatitis virus (VSV) G protein by exonuclease digestion (J. Rose, personal communication). The one constraint imposed on restructuring the gene at the 3' end appears to be that a poly (A) addition site must be encoded at the 3' end of the gene. The approach we have taken involves replacement of the exons that encode the intracytoplasmic portion of the H-2 molecule with the portion of the *I-Aβ*^{d} gene that encodes three amino acids and a poly (A) addition site. One advantage of this approach is that the mRNA from the mutant gene is significantly different from the original H-2 gene in that it hybridizes to *I-Aβ* probe.

Detailed structural analysis of the *H-2L*^{d} gene including the determination of the complete DNA sequence, revealed a restriction nuclease site in the intron between the membrane and first cytoplasmic domain[4,5]. Specifically, four *Bgl*II sites are present in the plasmid pL^4-4 (not shown in Fig. 1). Two *Bgl*II sites are located 5' to the promoter region. One *Bgl*II site is located in the C_2 domain and one in the intervening sequence between the membrane and first cytoplasmic domain. Plasmid pL^d-4 DNA was partially digested with *Bgl*II and a fragment was isolated encoding the H-2L^d molecule without the cytoplasmic portion. Recently, the nucleotide sequence of the coding portions of the mouse *I-Aβ* gene has been determined[16]. A 2.5-kilobase (kb) *Bam*HI fragment from the 3' end of the *I-Aβ*^{k} gene encodes a 3' acceptor site, three amino acids, a terminator, a 3'-untranslated region and a poly(A) addition site (Fig. 1*A*). The *Bgl*II fragment encoding the H-2L^d molecule, without the cytoplasmic portion, was ligated to the 2.5 kb *Bam*HI fragment. This construction resulted in an *H-2L*^{d} gene in which the H-2 cytoplasmic domains were deleted (Fig. 1).

Figure 2 shows the amino acid sequence of the transmembrane region and the 25 amino acids comprising the cytoplasmic domain of the native H-2L^d protein. We expected the constructed *H-2L*^{d} gene to encode an H-2L^d molecule with the same transmembrane region as the native H-2L^d molecule, but lacking its cytoplasmic portion. The IC_1, IC_2 and IC_3 encoded portions of the native H-2L^d molecule should be substituted by three amino acids encoded by the cytoplasmic domain from the *I-Aβ*^{k} gene (Fig. 2).

Table 1 H-2L^d antigens on the surface of L-cells containing native and deleted *H-2L*^{d} genes

Cell	*H-2L*^{d} gene	^{125}I-Protein A bound
L cell	—	700
T1.1.1	Native	25,620
T123.8	Native	14,620
D3.8	Deleted	24,642
D3.4	Deleted	19,337

Cloned *H-2* genes encoding either the native or deleted *H-2L*^{d} gene were introduced into thymidine kinase deficient mouse L-cells (DAP-3) by DNA-mediated gene transfer using the herpes simplex thymidine kinase gene as a selectable marker as described previously[4,8]. The amount of H-2L^d expressed on the cell surface was measured by radioimmunoassay[8,12]. 2×10^5 cells were incubated in 50 μl of a 1:1 dilution of a monoclonal H-2L^d antibody (28.8.14) for 2 h at 4 °C. The cells were extensively washed, ^{125}I-labelled Protein A was added, and the cells were incubated for an additional 2 h at 4 °C. After extensive washing the amount of ^{125}I bound to the cells was measured. Each value represents the mean of triplicate samples with a standard deviation of less than 3% of the mean. Although the relative amounts of H-2L^d on the surface varied somewhat from experiment to experiment, the amount of H-2L^d on the surface of T1.1.1 was always very similar to the amount on the surface of the D3.8 cells, while the T123.8 and D3.4 cells had about 50% and 80% respectively of the H-2L^d found on the surface of T1.1.1.

Deleted *H-2* gene products in transformants

To establish whether the truncated gene is expressed when reintroduced into mouse cells, DNA from either the truncated or the complete *H-2L*^{d} gene together with the herpes viral thymidine kinase gene, was used to co-transform thymidine kinase negative mouse L-cells as previously described[17,18]. Transformants were selected in hypoxanthine–aminopterin–thymidine medium and screened with monoclonal antibodies specific for H-2L^d transformants[19,20]. Cell-surface antigen expression was measured quantitatively by radioimmunoassay. Two cloned lines transformed with the deleted *H-2L*^{d} gene, D3.4 and D3.8, were chosen for further analysis. Table 1 shows that clone D3.8 had as much H-2L^d on its surface as was found on the surface of clone T1.1.1 (an L-cell transformed with the complete *H-2L*^{d} gene, as described previously[3]). Clone D3.4, used in the biochemical studies described below, had slightly less H-2L^d molecules on its surface. Apparently, the deleted H-2L^d expressed after introduction into L-cells can be recognized by monoclonal antibodies.

To demonstrate that the deleted gene product was associated with β_2-microglobulin and shorter than the native protein, the deleted protein was isolated from the cell surface. Membrane

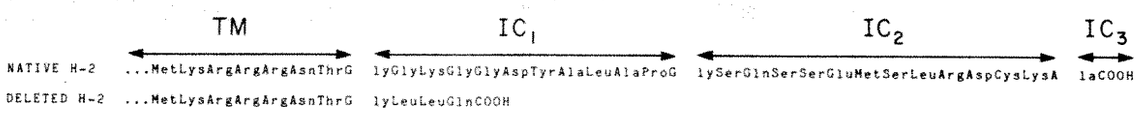


Fig. 2 Amino acid sequence of the transmembrane and cytoplasmic domains of the native and deleted H-2L^d molecules. The amino acid sequence of the transmembrane and cytoplasmic domains of the complete *H-2L^d* gene were predicted from DNA sequence analysis[4,5]. The four amino acids encoding the cytoplasmic domain in the truncated *H-2L^d* gene were derived from the second cytoplasmic domain of the murine *I-Aβ* gene[16].

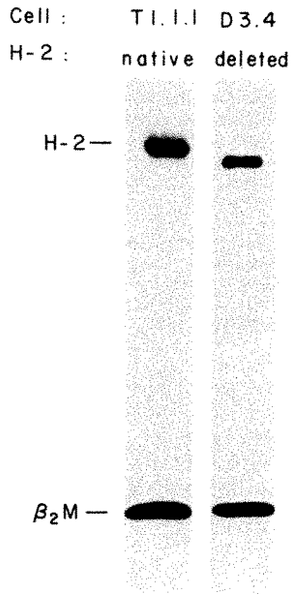


Fig. 3 SDS-polyacrylamide gel electrophoresis analysis of ^{125}I-labelled H-2L^d antigens immunoprecipitated from clone T1.1.1, expressing the complete *H-2L^d* gene, and clone D3.4 expressing the truncated *H-2L^d* gene. Cell-surface iodination with the ^{125}I was catalysed by lactoperoxidase[35]. The radiolabelled cells were lysed with 1% Nonidet P40 in 0.01 M Tris-HCl, *p*H 7.0, 0.15 M NaCl, 1 mM phenylmethylsulphonyl fluoride, 0.02 mg ml^{-1} ovomucoid trypsin inhibitor. Nuclear debris was removed from the lysates by centrifugation at 13,000*g* for 15 min at 4 °C. Further pretreatment of the lysates was done by centrifugation at 100,000*g* for 30 min and by pre-clearing with formalin-fixed *Staphylococcus aureus* bacteria (strain Cowan I) and with a preformed complex of mouse IgG and rabbit-anti-mouse IgG as described previously[36]. Precleared lysates were incubated for 3–4 h with a preformed complex with the monoclonal 30.5.7 and rabbit anti-mouse IgG at 4 °C and immunoprecipitates were removed from the lysates by centrifugation at 13,000*g*. Precipitates were resuspended in 0.2 ml Tris-HCl buffer with 0.5% deoxycholate sodium salt and spun through on a discontinuous gradient consisting of 0.4 ml of 10% sucrose, 0.5% Nonidet P-40 and 0.8 ml of 20% sucrose. SDS-polyacrylamide electrophoresis was carried out on discontinuous vertical slab gels according to a modification of the Laemmli procedure[37]. A gradient gel was made in 10–15% acrylamide. The gels were dried and exposed for 3 days. The figure shows H-2L^d antigens from clone T1.1.1 expressing the complete *H-2L^d* gene and clone D3.4 expressing the truncated *H-2L^d* gene.

Table 2 Normalized T-cell mediated lympholysis of normal and deleted *H-2L^d* genes expressed on L-cells

Target	BALB/c anti-VSV BALB/c %	*n*	BALB/c anti-PR8 BALB/c %	*n*	C-H-2^{dm2} anti-BALB/c %	*n*	BALB/c anti-C3H %	*n*
T1.1.1	100		100		100		100	
T123.8	84±10	(5)	81±22	(4)	106±19	(8)	87±7	(9)
D3.4	20±5	(7)	74±17	(5)	72±11	(15)	83±12	(9)
D3.8	19±7	(5)	90±4	(4)	92±16	(8)	74±12	(9)

BALB/c mice were immunized intraperitoneally with either wild-type VSV (10^7 plaque-forming units (PFU)) or influenza A/PR/8/34 virus (100 haemagglutinating units). One week later, the mice were killed and their spleens used as a source of virus-immune lymphocytes. Five-day secondary *in vitro* cultures were established with 5×10^6 primed lymphocytes and 1×10^6 irradiated BALB/c splenocytes which were infected with either 10 PFU tsG41 VSV or 10 EID_{50} (50% egg infectious dose) PR8 virus per cell in 17 mm Costar dishes with RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine, and 5×10^{-5} M 2-mercaptoethanol. Primary allospecific CTLs were generated in 5-day cultures of 5×10^6 naive lymphocytes derived from either BALB/c or C-H-2^{dm2} mice cultured with 1×10^6 irradiated C3H or BALB/c stimulator cells using the same medium. Virus-specific or allo-specific effectors were collected and dispensed in duplicate at 1×10^6, 2×10^5 and 5×10^4 cells per V-bottom microtitre well (Linbro) in 0.1 ml minimum essential medium supplemented with 10% FBS, nonessential amino acids, penicillin, streptomycin, and L-glutamine. L-cell targets, detached by versene treatment, were divided into three aliquots and infected with either 10 PFU wild-type VSV or 10 EID_{50} PR8 virus, or not infected. Target cells were pulsed with 0.1 m Ci Na_2 $^{51}CrO_4$ for 1 h at 37 °C, incubated an additional 1 h, and then washed three times. L-cell targets were dispensed at 1×10^4 cells in 0.05-ml volumes into wells containing effectors, normal spleen cells to measure spontaneous release or 0.1 ml 5% Triton X-100 (to measure total releasable ^{51}Cr). Plates were centrifuged at 100*g* and then incubated at 37 °C for 5 h. Supernatants (0.1 ml per well) were collected and counted for ^{51}Cr release. Per cent specific release was calculated by the formula = 100 × (experimental c.p.m. − control c.p.m.)/(total c.p.m. − control c.p.m.)[15]. In order to present the results from multiple experiments, data were normalized such that lysis of T1.1.1 cells was 100%. Lysis of the T1.1.1 cells was equal to or greater than 70% in all experiments. Standard deviation and number of experiments (in parentheses) are indicated. Data are presented for lysis on appropriate targets (for example PR8 CTLs assayed on PR8 infected targets). Lysis on uninfected targets was always less than 10%.

proteins were labelled by lactoperoxidase-mediated iodination of the transformed cells. The proteins were immunoprecipitated by monoclonal antibody 30.5.7 which recognizes a determinant in the N/C_1 domain of the H-2L^d molecule[12]. The immunoprecipitates were analysed by SDS-polyacrylamide gel electrophoresis (Fig. 3). Both the deleted and wild-type proteins are associated with β_2-microglobulin. As expected, the molecular weight of the truncated H-2L^d antigen is significantly smaller than the complete *H-2L^d* gene product.

S_1 analysis of truncated H-2 transcripts

The fact that the protein is shorter does not guarantee that the mRNA has spliced correctly and that the protein has the expected sequence. To determine if the truncated gene product is the same as the predicted structure shown in Fig. 2, S_1 nuclease mapping was used to analyse total RNA obtained from transformants expressing the truncated gene product. This analysis was done using a single-stranded uniformly labelled DNA probe of high specific activity that was prepared by primer extension of a recombinant phage M13 DNA template. A *Bam*HI-RI fragment derived from the murine *I-Aβ* gene was cloned into the M13 phage (Fig. 4). After hybridizing mRNA with the labelled DNA probe, the reaction mixture was digested with S_1 nuclease and the products were analysed on denaturing polyacrylamide gels (Fig. 4). Only probes that had been hybridized to either mRNA from the transformed L-cells or from mRNA made from B cells expressing the *I-Aβ* gene were protected from S_1 nuclease digestion. An 89-nucleotide fragment was protected by mRNA isolated from clone D3.8 (Fig. 4, lane *D*). The protected fragment is identical to the fragment protected by mRNA (poly $(A)^+$) isolated from A20, a B-cell lymphoma expressing the *I-Aβ* gene (Fig. 4, lane *D*). Furthermore, the protected fragment is about the size (85 base pairs) predicted by comparison of the *I-Aβ* cDNA sequence[21] to the genomic *I-Aβ* sequence[16].

These results demonstrate that splicing of the cytoplasmic domain in the truncated *H-2L^d* mRNA occurs similarly to that of the *I-Aβ* mRNA normally expressed in a B-cell lymphoma, A20. Thus, the amino acid sequence of the COOH-terminus of the truncated *H-2L^d* product is predicted in Fig. 2. The deleted gene product has removed 25 amino acid residues from the native *H-2L^d* gene product and replaced these with three new residues.

Cytoplasmic part of H-2 in recognition

To determine the effects of deleting the cytoplasmic portion of the *H-2L^d* gene, L-cells expressing the native and truncated *H-2L^d* genes were used as targets for effector T cells in four systems. We and others have previously described the use of L-cells transformed with *H-2L^d* genes as targets for killer T cells[9–12]. Allospecific T cells recognize L-cells expressing the truncated gene product nearly as well as they recognize the

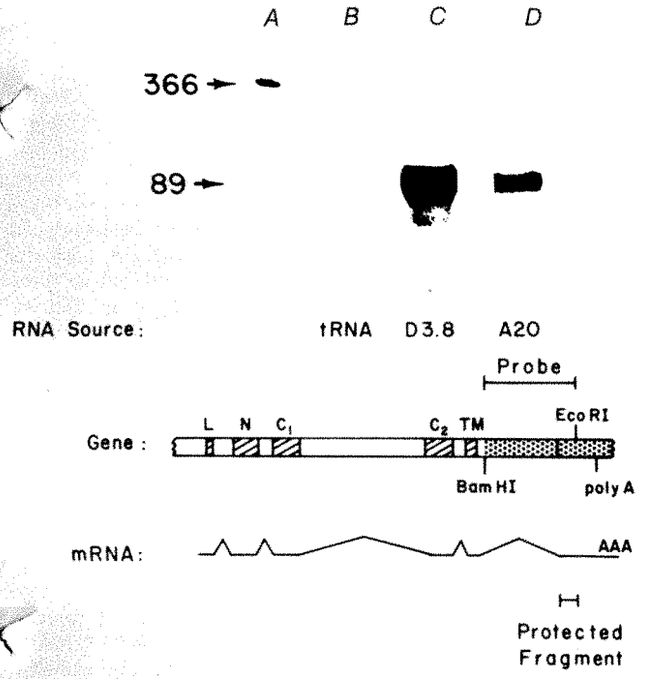


Fig. 4 Results of S_1 nuclease mapping analysis of mRNA isolated from clone D3.8 and B-cell lymphoma A20. A *Bam*HI–*Eco*RI fragment encoding the second cytoplasmic domain and 3′ untranslated region of the murine *I-Aβ* gene was cloned into M13mp9. After transfection of *E. coli* JM103 [38] appropriate recombinants, each containing the strand of the *I-Aβ* DNA that would serve as a template for the synthesis of a probe complementary to mRNA, were selected. Single-stranded DNA was recovered by using standard techniques after overnight incubation at 37 °C[38]. *A*, The probe fragment; *B*, tRNA hybridized with the probe and S_1 digested; *C*, mRNA from clone D3.8 hybridized and S_1 digested; *D*, mRNA from B-cell lymphoma A20 hybridized and S1 digested. **Methods:** A universal 24 base long primer was annealed with 0.3 μg of the single-stranded M13 recombinant; synthesis of the radiolabelled strand was then directed by the large fragment of DNA polymerase I using [α-^{32}P]dATP (400 Ci mmol^{-1}) and unlabelled dTTP, dCTP and dGTP. Synthesis of the second strand completed a unique *Hin*dIII site downstream from the insert. After synthesis of the second strand, digestion with *Hin*dIII released a ^{32}P-labelled single-stranded copy of the insert. This radiolabelled fragment was very different in size from the linear unlabelled template. The single-stranded radiolabelled probe was purified on a denaturing gel and recovered by electroelution[39]. mRNA was isolated from cells as described previously[40]. Samples containing 10 μg total mRNA from clone D3.8, 10 μg poly (A)$^+$ mRNA from A20 and 20 μg tRNA were hybridized overnight with 50,000 c.p.m. of single-stranded probe at 55 °C in 30 μl reaction mixture containing 75% (v/v) formamide[41]. Each reaction mixture was then digested with 700 U S_1 nuclease at 42 °C for 30 min[41,42] and the products were precipitated and analysed by electrophoresis on denaturing polyacrylamide sequence gels[43]. The size of the protected fragment was measured at 89 bases by co-migration with ^{32}P-labelled *Hin*f digested pBR322.

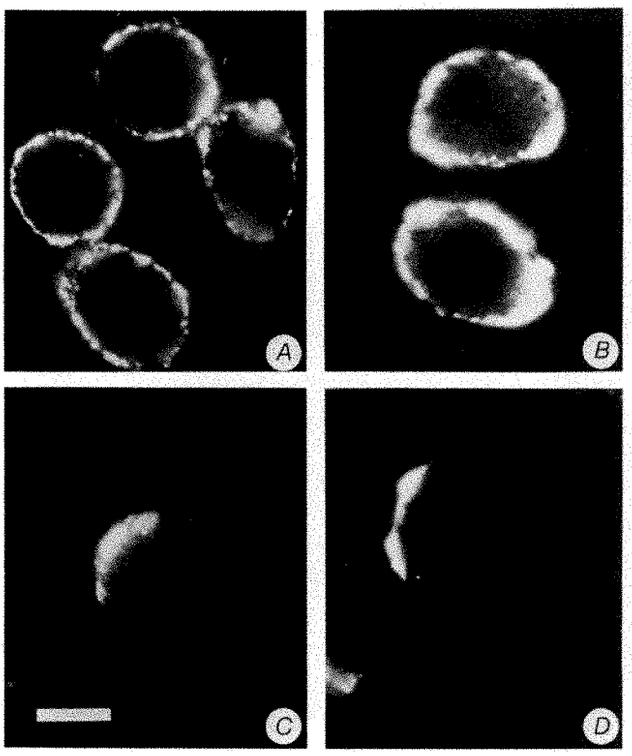


Fig. 5 T1.1.1 and D3.8 cells were detached from culture dishes with a rubber policeman and dissociated into individual cells with a Pasteur pipette in the cold room (4 °C). Cells were incubated with diluted mouse monoclonal antibodies (mixture of 30.5.7 and 28.14.8) in a sealed tube containing RPMI 1640 medium at 4 °C for 30 min. After washing in RPMI 1640 medium, cells were incubated with diluted fluorescein-conjugated goat anti-mouse immunoglobulin (Cappel) in RMPI 1640 medium at 4 °C for 30 min. After washing, an aliquot of cells was placed under a glass coverslip, sealed with nail polish and examined immediately by Zeiss Photomicroscopy III equipped with epiflorescent optics (Planapo, ×63). The rest of the cells were divided into two tubes, one kept at 4 °C, the other at 37 °C. At various times an aliquot of cells was removed and examined as above. For each time point, 300 cells were scored for redistribution of antigen. Photographs were made with Kodak Tri-X film (E1600) and developed in HC-110 dilution B for 12 min. Cells kept at 4 °C did not have a significant antigen redistribution at the end of the experiments. *A*, T1.1.1 and *B*, D3.8 immediately after staining at 4 °C. *C*, T1.1.1 and *D*, D3.8 after 2 h at 37 °C. Scale bar, 20 μm.

L-cells expressing the normal gene product (Table 2). Furthermore, influenza virus specific CTLs recognized virus-infected L-cells expressing the deleted *H-2L^d* gene product nearly as well as L-cells expressing the native *H-2L^d* gene product. By contrast, L-cells expressing the deleted *H-2L^d* product and infected with VSV were recognized less efficiently by the VSV specific killer T cells as the L-cells expressing the native H-2L^d molecule and infected by the virus (Table 2).

L-cells expressing the deleted *H-2L^d* gene product (clone D3.8, Table 1) appeared to bind the same amount of monoclonal H-2L^d antibody as the T1.1.1 cell (*H-2L^d*). Nevertheless, the possibility remained that small differences in the amount of H-2L^d on the cell surface had a significant effect on the recognition of these cells by VSV specific CTLs, even though these differences could not be measured by radioimmunoassay. To test this possibility we measured the ability of VSV specific CTLs to kill L-cells expressing significantly less native H-2L^d (Table 1, clone T123.8) than T1.1.1 cells (Table 1). These cells, expressing lower levels of H-2L^d, are killed just as effectively as cells expressing high levels of H-2L^d (Table 2). Thus, we conclude that the VSV specific CTLs are not able to recognize the truncated H-2 molecules as efficiently as they can recognize VSV associated with the normal *H-2L^d* gene product.

Cytoplasmic part of H-2L^d in capping

The H-2 molecule is normally uniformly distributed across the cell surface. Shortly after the addition of a monoclonal H-2 specific antibody and a fluorescent-labelled second cross-linking antibody, the H-2 molecules on the cell surface start to redistribute. Initially this redistribution involves the aggregation of the H-2 molecules into patches, presumably through the cross-linking of H-2 molecules by antibodies in the plane of the membrane. This event is followed by movement of these patches into a polar cap[22,23]. Other investigators have proposed that the latter is an energy-dependent process resulting from

association of transmembrane glycoproteins with cytoskeletal elements[24,25]. We investigated the nature of the interaction between the H-2 molecule and cytoplasmic proteins by studying the ability of the truncated H-2L^d molecule to make caps on the cell surface. Figure 5 shows the 'patching' and 'capping' of H-2L^d molecules expressed on the surface of T1.1.1 (complete *H-2L^d* genes) and D3.8 cells (truncated *H-2L^d* gene) after reaction with monoclonal antibody and fluorescent-labelled second antibody. Initially, the H-2L^d molecules are evenly distributed over the entire surface of the plasma membrane (Fig. 5*A, B*). After 2 h incubation the patches coalesce into a single cap on the surface of the T1.1.1 (Fig. 5*C*) and D3.8 (Fig. 5*D*) cells. To investigate if the rate of capping of the truncated H-2L^d antigen was different from the native molecule, a time course experiment was performed (see legend to Fig. 5). After 30 min incubation at 37 °C, 25% of T1.1.1 and 20% of D3.8 cells had capped. After 1 h at 37 °C, 50% of T1.1.1 and 46% of D3.8 cells had formed caps. After 1.5 h, 70% of T1.1.1 and 74% of D3.8 had capped. After 2 h at 37 °C, 80% of the T1.1.1 and 85% of the D3.8 cells had capped. Apparently the deleted *H-2L^d* gene product caps at precisely the same rate as the native H-2L^d molecule. Thus, we suggest that the interactions of the intracytoplasmic portion of the H-2 molecule with other proteins do not have a significant role in the movement of cell-surface antigens into caps.

Conclusions

We have constructed an *H-2L^d* gene whose product lacks the intracytoplasmic portion. The shortened protein is expressed on the cell surface. The product of this truncated *H-2* gene is efficiently recognized by allo- and influenza-specific cytotoxic T lymphocytes. Although VSV specific CTLs do not recognize this truncated H-2L^d molecule as efficiently as the complete *H-2L^d* product they are still able to recognize the truncated H-2 antigen. Thus, many of the cellular functions of the H-2 antigen can be performed by the truncated molecule. These findings suggest that interactions between the cytoplasmic proteins of the cell and the H-2 molecule do not have a critical role in the function of the H-2 molecule.

The nature of the interaction between viral antigens and H-2 antigens on the cell surface has puzzled immunologists for a long time. Several studies have indicated that some viral antigens and H-2 antigens are co-precipitated[26–28] or co-capped[29,30] by the appropriate antiserum. By contrast, no association has been identified between influenza virus antigens and H-2 antigens[31]. Presumably, these different results reflect differences in the interactions between particular viral antigens and the H-2 antigens. These differences might also account for the differences in the ability of the VSV specific CTLs and influenza specific CTLs to recognize cells expressing the truncated H-2 antigen. If interactions between viral and H-2 antigens do occur, these interactions must be quite subtle. Thus, small differences in experimental conditions may have a significant effect on the apparent function of an H-2 molecule. The weakness of these interactions also raises the question of which of these interactions are important to the animal rather than those detected in tissue-culture conditions. The introduction of the truncated *H-2* gene described here directly into the mouse genome may allow a direct test of this question.

The truncated *H-2L^d* gene product and the wild-type H-2L^d molecule function identically in most of the cellular assays, suggesting that the structure of the protein is not grossly altered by the deletion of the cytoplasmic portion. To test whether a physical parameter, the ability of these molecules to form caps, is affected by changes in the cytoplasmic portion we compared the rates of the cap formation on cells expressing the two proteins. We observed no differences in the rate of capping between the truncated and complete H-2L^d antigen. We conclude that if interactions between other cellular proteins and the intracytoplasmic portion of the H-2 molecule have an important role in cap formation, then these interactions are non-specific. Co-capping experiments of viral antigens and truncated H-2L^d molecules will be examined to determine if the truncated H-2L^d is able to co-cap viral antigens as well as the wild-type antigen co-caps viral antigens.

We thank Ms J. Borst for help with the immunoprecipitation, Ms Lisa Liu for technical assistance and Ms T. Broderick for preparation of the manuscript. C.S.R. is supported by a New Investigator Award (AI18083). This work was supported by grants from the NIH to L.B.C. (CA22659), S.J.B. (AI17258) and J.G.S. (AI18436 and AI19148). S.J.B. and L.B.C. are recipients of an American Cancer Society Research Award and J.G.S. is the recipient of an ACS award (JFRA-66).

Note added in proof: Zuniga *et al.*[44] recently reported the synthesis and characterization of an H-2 gene encoding an H-2 molecule similar to the one described here.

Received 21 July; accepted 6 October 1983.

1. Klein, J. *Biology of the Mouse Histocompatibility Complex* (Springer, Berlin, 1975).
2. Snell, G. D., Dausset, J. & Nathenson, S. G. *Histocompatibility* (Academic, New York, 1976).
3. Steinmetz, M. S. *et al. Cell* **25,** 683–692 (1981).
4. Evans, G. A., Margulies, D. H., Ozato, K., Camerini-Otero, R. D. & Seidman, J. G. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1994–1998 (1982).
5. Moore, K. W., Sher, B. T., San, Y. H., Earle, K. A. & Hood, L. *Science* **215,** 679–682 (1982).
6. Doherty, P. C. & Zinkernagel, R. M. *J. exp. Med.* **191,** 502–507 (1975).
7. Zinkernagel, R. M. & Doherty, P. C. *Adv. Immun.* **27,** 51–177 (1979).
8. Goodenow, P. S. *et al. Science* **215,** 677–679 (1982).
9. Margulies, D. H. *et al. J. Immun.* **130,** 463–470 (1983).
10. Woodward, J. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 3613–3617 (1982).
11. Mellor, A. L. *et al. Nature* **228,** 529–534 (1982).
12. Evans, G. A., Margulies, D. H., Shykind, B., Seidman, J. G. & Ozato, K. *Nature* **300,** 755–757 (1982).
13. Ozato, K., Evans, G. A., Shykind, B., Margulies, D. H. & Seidman, J. G. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2040–2043 (1983).
14. Reiss, C. S., Evans, G. A., Margulies, D. H., Seidman, J. G. & Burakoff, S. J. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2709–2712 (1983).
15. Braun, J. & Unanue, E. R. *Fedn Proc.* **42,** 2996–2951 (1983).
16. Choi, E., McIntyre, K., Germain, R. N. & Seidman, J. G. *Science* **221,** 283–286 (1983).
17. Graham, F. L. & van der Eb, A. J. *Virology* **52,** 546–553 (1973).
18. Wigler, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **76,** 1373–1377 (1979).
19. Ozato, K., Hansen, T. H. & Sachs, D. H. *J. Immun.* **125,** 2973–2977 (1980).
20. Ozato, K. & Sachs, D. H. *J. Immun.* **126,** 317–321 (1981).
21. Robinson, R. R., Germain, R. N., McKean, D. J., Mescher, M. & Seidman, J. G. *J. Immun.* **131,** 2025–2031 (1983).
22. Taylor, R. B., Duffus, R. W., Ruff, M. & de Petris, S. *Nature new Biol.* **233,** 225–229 (1971).
23. Loor, F. L. *Eur. J. Immun.* **4,** 210–215 (1974).
24. Nicholson, A. L. *Biochim. biophys. Acta* **457,** 57–108 (1977).
25. Flanagan, J. & Koch, G. L. E. *Nature* **273,** 278–281 (1978).
26. Bubbers, J. E., Chew, S. & Lilly, F. *J. exp. Med.* **147,** 340 (1979).
27. Kirst, S., Ostberg, L., Persson, H., Philipson, L. & Peterson, P. A. *Proc. natn. Acad. Sci. U.S.A.* **75,** 5674–5678 (1978).
28. Signas, L., Katze, M. G., Persson, H. & Philipson, L. *Nature* **299,** 175–178 (1982).
29. Ciaverra, R. & Forman, J. F. *Immun. Rev.* **50,** 73–78 (1981).
30. Geiger, B., Rosenthal, K. L., Klein, J., Zinkernagel, R. M. & Singer, J. *Proc. natn. Acad. Sci. U.S.A.* **76,** 4603–4607 (1979).
31. Hackett, L. J. & Askonas, B. A. *Immunology* **45,** 431–436 (1982).
32. Maniatis, T., Fritisch, E. F. & Sambrook, J. *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
33. Dagert, M. & Ehrlich, S. D. *Gene* **6,** 23–28 (1979).
34. Holmes, D. S. & Grigley, M. *Analyt. Biochem.* **114,** 193–197 (1981).
35. Haustein, D., Marchalonis, J. J. & Harris, A. W. *Biochemistry* **14,** 1826–1829 (1975).
36. Borst, J., Prendiville, M. A. & Terhorst, C. *J. Immun.* **128,** 1560–1565 (1982).
37. Laemmli, U. K. *Nature* **227,** 680–685 (1970).
38. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. *J. molec. Biol.* **143,** 161–171 (1980).
39. Ley, T. J., Anagnon, N. P., Pepe, G. & Nienhuis, A. W. *Proc. natn. Acad. Sci. U.S.A.* **79,** 9775–9779 (1982).
40. Chirgwin, J. M., Prsybyla, G., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18,** 5294–5299 (1979).
41. Nienhuis, A. W., Immer, P. & Bens, E. J. *Proc. natn. Acad. Sci. U.S.A.* **79,** 3960–3964 (1977).
42. Berk, A. J. & Sharp, P. A. *Cell* **12,** 721–732 (1977).
43. Weaver, R. F. & Weissman, C. *Nucleic Acids Res.* **7,** 1175–1193 (1979).
44. Zuniga *et al.* Cell **34,** 535–534 (1983).

Tripartite structure of the *Brassica campestris* mitochondrial genome

Jeffrey D. Palmer*‡ & Clark R. Shields†

* Department of Plant Biology, Carnegie Institution of Washington, Stanford, California 94305, USA
† Department of Agronomy and Range Science, University of California, Davis, California 95616, USA

Physical mapping studies indicate a novel sequence arrangement, consisting of three circular chromosomes, for the mitochondrial genome of Brassica campestris *(Chinese cabbage, turnip). A master chromosome, 218 kilobases (kb) long, contains the entire sequence complexity of the mitochondrial genome, including two copies of an ~2-kb element present as direct repeats separated by 135 and 83 kb. The two smaller circles are 135 and 83 kb in size, contain one copy each of the 2-kb repeat element, and are postulated to interconvert with the master chromosome via a co-integration–resolution pathway mediated by reciprocal recombination within the 2-kb repeat.*

HIGHER plant mitochondrial genomes are unusually large and variable in size, ranging from ~200 kilobases (kb) in *Oenothera*[1] and *Brassica*[2,3] to almost 2,500 kb in muskmelon[4]. The way in which this DNA is organized into discrete molecules is unclear[5–7]. Electron microscopic analysis of mitochondrial DNA isolated from native plant tissue reveals a broad size range of molecules, primarily linear with a low percentage of circles[8–10]. A much larger percentage of the mitochondrial DNA from cultured cells can be isolated as intact circles, with predominant size classes in the range 2–30 kb[11–13]. Several models have been proposed which attempt to reconcile the large kinetic and restriction complexities of plant mitochondrial DNA with the failure to find a discrete size class of molecules large enough to accommodate this information (see refs 5 and 6 for a review). At one extreme, it has been proposed that the plant mitochondrial genome may exist as a collection of different size classes of circular molecules, with each size class containing a distinct, non-overlapping subset of the total sequence complexity of the genome[14]. Alternatively, it has been suggested that the genome is organized primarily as a single large linkage group, with frequent recombination and rearrangement leading to the array of smaller molecules more easily isolated and observed via electron microscopy[15]. Data in support of these and other models are hardly overwhelming, although the last model is consistent with restriction mapping of corn mitochondrial DNA[16–18], where several linear linkage groups have been determined that are larger than any of the observed circular molecules[9].

We now report the first complete restriction map for a higher plant mitochondrial genome, that of *Brassica campestris* (Chinese cabbage, turnip). The model most consistent with our mapping data is that this genome is organized into three physically distinct circular molecules. The largest circle of 218 kb bears the entire sequence complexity of the genome and thus constitutes a 'master' chromosome, and the two smaller circles contain distinct, 135-kb and 83-kb subsets of the master molecule. These three chromosomes seem to interconvert via reciprocal recombination across the major repeat element present on all three molecules.

Physical mapping studies

A clone bank containing 96% of the *B. campestris* mitochondrial genome was constructed by inserting *Pst*I and *Sal*I fragments into the plasmid vector pUC8 (ref. 19). Each of these cloned fragments was labelled with ^{32}P and hybridized to nitrocellulose filters containing single digests of total mitochondrial DNA with each of five enzymes (*Pst*I, *Sal*I, *Bgl*I, *Kpn*I, *Nru*I) and also selected double digests with various combinations of these enzymes. The mapping strategy is illustrated in Fig. 1. A cloned *Sal*I fragment of 28 kb (S28) hybridized to eight *Pst*I fragments, ranging in size from 10.2 to 0.9 kb (Fig. 1). Hybridization to a *Sal*I–*Pst*I double-digest lane indicated that six of these fragments (9.7, 7.5, 2.9, 2.0, 1.3 and 0.9 kb in size) are uncut by *Sal*I, and thus lie completely internal to S28, while P10.2 and P5.7 are cut by *Sal*I and thus overlap the junctions between S28 and two flanking *Sal*I fragments (Fig. 1). P10.2 hybridized to a double-digest fragment of the same size (2.4 kb) as one of the two terminal S28 double-digest fragments. We conclude, therefore, that this fragment represents the overlap between S28 and P10.2, and that the other terminal S28 double-digest fragment, of 2.2 kb, must represent its overlap with P5.7. P10.2 also hybridized to internal *Sal*I fragments of 4.4 and 2.8 kb and to a second *Sal*I fragment, S10.1, cut by *Pst*I (Fig. 1). The length of the overlap between P10.2 and S10.1 is given by the 0.6-kb double-digest fragment to which they both hybridize (Fig. 1). Thus, using only three hybridizations, a region of 60 kb of DNA, from P19.4 to P5.7, was mapped for these two enzymes (Fig. 2).

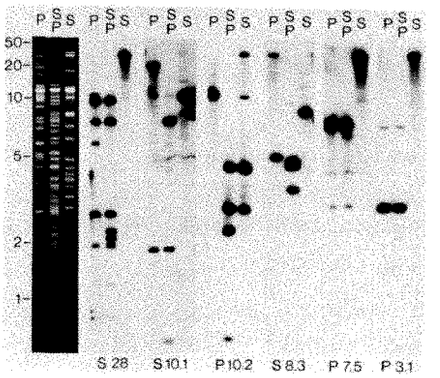


Fig. 1 Representative mitochondrial DNA mapping hybridizations. Left-hand panel: DNase I-purified[25] mitochondrial DNA from *B. campestris* was digested with *Pst*I (P), *Sal*I–*Pst*I (SP) and *Sal*I (S), and fragments were separated by electrophoresis in a 0.7% agarose gel[38]. The size scale on the left is in kb and was determined using λ phage DNA restriction fragment markers generated with *Sal*I, *Hin*dIII and *Eco*RI. Right-hand panels: six cloned mitochondrial DNA restriction fragments were each labelled with ^{32}P by nick-translation[39] and hybridized[38] to a replica nitrocellulose filter imprint[38] of the gel shown in the left panel. In this and the other figures, and also in the text, a given mitochondrial DNA restriction fragment, or a plasmid containing the fragment, is designated both by a letter, indicating the enzyme used to generate the fragment (P = *Pst*I, S = *Sal*I, B = *Bgl*I, K = *Kpn*I, N = *Nru*I) and a number, indicating the size of the fragment in kb.

‡ To whom correspondence should be addressed at Department of Zoology, Duke University, Durham, North Carolina 27706, USA.

© 1984 Macmillan Journals Ltd

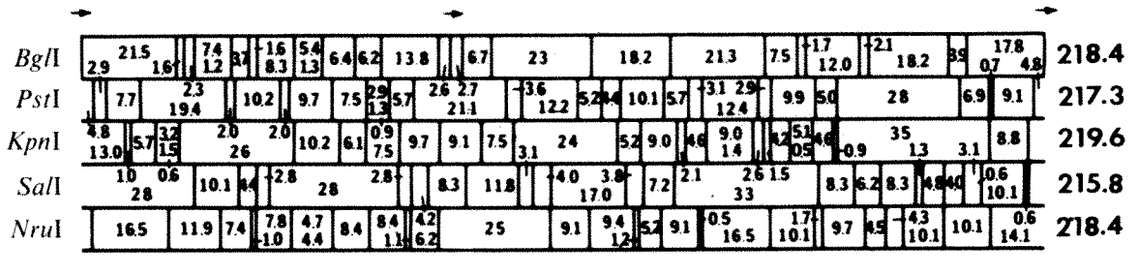


Fig. 2 Restriction map of the master chromosome of *B. campestris* mitochondrial DNA. The circular map for the largest mitochondrial linkage group, containing the entire sequence complexity of the genome, is shown linearized at the *Bgl*I site internal to the 2-kb repeat sequence. Arrows indicate the position and relative orientation of the two copies of the 2-kb repeat present on this linkage group. The numbers at the right-hand end of the map indicate the summation of the restriction fragments for each of the enzymes marked at the left-hand end.

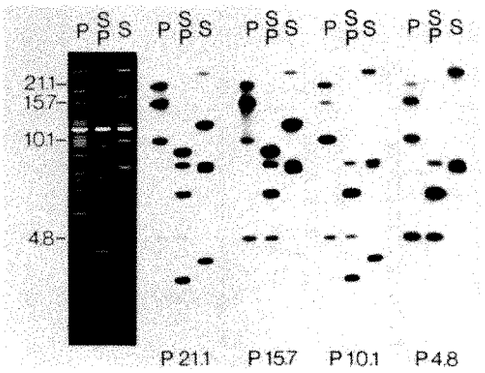


Fig. 3 Hybridization analysis of the major repeat sequence of *B. campestris* mitochondrial DNA. The four *Pst*I clones indicated were each hybridized to replica nitrocellulose blots of mitochondrial DNA digested with *Pst*I (P), *Sal*I–*Pst*I (SP) and *Sal*I (S).

In control *Pst*I–*Pst*I or *Sal*I–*Sal*I hybridizations, several cloned fragments hybridized to one or more fragments in addition to the expected fragment of the same size of the cloned insert. Except for one special case, described in detail below, these extra hybridizations were all very weak and did not confuse interpretation of the hybridization results in terms of constructing a map. In several cases, reciprocal cross-hybridizations clearly indicated that these weak hybridization signals result from small repeated sequences present in two or more copies in the mitochondrial genome (compare P7.5 and P3.1 hybridizations in Fig. 1). In other cases, the weak, additional hybridizations were to fragments larger than the probe fragment and sometimes could not easily be assigned to major fragments visible in the stained gel (see S10.1 and S8.3 hybridizations in Fig. 1). These hybridization signals could result either from slight partial digestion products, from the presence of minor repeat elements within the major bands, or from microheterogeneity in the DNA, resulting in very faint bands not clearly visible in the stained gel.

2-kb Repeat element

The major cross-hybridization between cloned fragments was observed with four *Pst*I fragments, each of which hybridized to itself and to the other three *Pst*I fragments (Fig. 3). The sequence common to these four fragments has a minimum size of 0.6 kb, as defined by common *Bam*HI and *Bgl*I sites, and a maximum possible size of 3.2 kb, as defined by *Eco*RI digestion (Fig. 4). Hybridization of each of the four *Pst*I fragments to *Bam*HI and *Bgl*I digests of all four clones (normalizing insert cross-reactions to vector hybridizations) allows a very rough quantitative estimate of the size of this repeat as 2 ± 1 kb. For convenience, this element will henceforth be referred to as the '2-kb repeat'.

Three of the 2-kb repeat-containing *Pst*I fragments, P21.1, P15.7 and P4.8, are clearly substoichiometric relative to the rest of the *Pst*I fragments as judged both by direct visualization of stained gels (Figs 1, 3) and densitometric scanning of a *Pst*I digest (Fig. 5). The fourth 2-kb repeat-containing fragment, P10.1, co-migrates with a second, non-identical fragment and its stoichiometry could not be evaluated directly. It should be emphasized that, taking into account degradation effects on the larger fragments, all the remaining *Pst*I fragments fall into a unimolar stoichiometric series except for one doublet (P5.7), one triplet (P2.9), and a bright band at 11.3 kb which corresponds to an extrachromosomal, linear mitochondrial DNA plasmid described previously[3].

The gels and gel scan suggest that P21.1 and P4.8 are present in submolar amounts relative to P15.7 (Figs 1, 3, 5). Direct support for this conclusion can also be gained from quantitative consideration of the hybridizations shown in Fig. 3. If P21.1 and P15.7 were present in equal molarity within the mitochondrial genome, then, assuming that single-stranded probe lengths are short relative to insert sizes, a P21.1 probe should hybridize to itself approximately 1.5 times (21.1 divided by 14—the approximate size of the region of homology between P21.1 and P15.7; see Fig. 4) more intensely than to P15.7, while a P15.7 probe should hybridize to itself only 1.1 times (15.7/14) more intensely than to P21.1. In fact, visual inspection reveals that P21.1 hybridizes to itself somewhat less intensely than to P15.7, while P15.7 hybridizes to itself significantly more intensely than to P21.1 (Fig. 3). Quantitation of these hybridization signals by densitometry and measurement of the area under each curve indicates that P15.7 is actually present in about twice the molarity of P21.1. These calculations can only be approximate for two reasons. First, the size of the repeat is not accurately known, and second, significant degradation of the DNA on the filter results in diminished hybridization signals for the large fragments relative to small ones. Similar pairwise comparisons of the hybridizations in Fig. 3 lead us to conclude that P10.1 is also present in approximately twice the concentration of P21.1, and that P15.7 and P10.1 are approximately equimolar and are approximately twice as abundant as P4.8. Thus, we conclude that the four *Pst*I fragments which contain the 2-kb repeat are all submolar and that they can be further subdivided into two groups, with P15.7 and P10.1 being present in approximately equal molarity and at roughly twice the concentrations of P21.1 and P4.8.

Multicircular mitochondrial DNA

The 2-kb repeat is present on all four substoichiometric *Pst*I fragments. In addition, each *Pst*I fragment is identical, on one side of the repeat, to one of the other three fragments and on the other side of the repeat to a second one of the other three (Fig. 4). In other words, the 2-kb repeat is flanked by four paired combinations of four unique sequences. The two lower molarity substoichiometric fragments, P4.8 and P21.1, are equivalent in sequence complexity to the higher molarity pair, P15.7 and P10.1, and the two pairs are interconvertible by a single reciprocal cross-over within the 2-kb repeat.

The overall arrangement in the genome of these four substoichiometric, repeat-containing *Pst*I fragments was determined by completing the chromosomal 'walk' described above. These hybridizations generate a single, circular linkage group

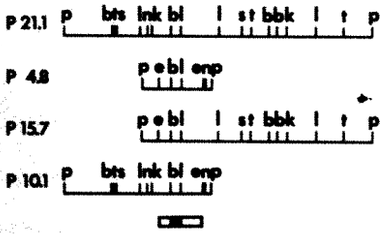


Fig. 4 Restriction maps of the four, 2-kb repeat-containing, substoichiometric *Pst*I fragments of *B. campestris* mitochondrial DNA. Cleavage sites for *Bam*HI (b), *Bgl*I (l) *Bst*EII (t), *Eco*RI (e), *Kpn*I (k), *Nru*I (n), *Pst*I (p) and *Sal*I (s) were mapped by appropriate double and triple digestions of plasmids containing each of the four *Pst*I fragments indicated. All cleavage sites are shown for all enzymes except for *Eco*RI, which produced many small fragments with the three largest clones. However, as *Eco*RI cleaves P4.8 only twice, defining the maximum boundaries of the repeat sequence as presently mapped, we show these two sites as they occur on P4.8, P10.1 and P15.7. The repeat sequence (bl) is flanked by four paired combinations of four unique sequences (pbtslnk, lstbbkltp, pe, enp). The solid bar indicates the minimum extent of the repeat sequence and open extensions of the bar its maximum extent.

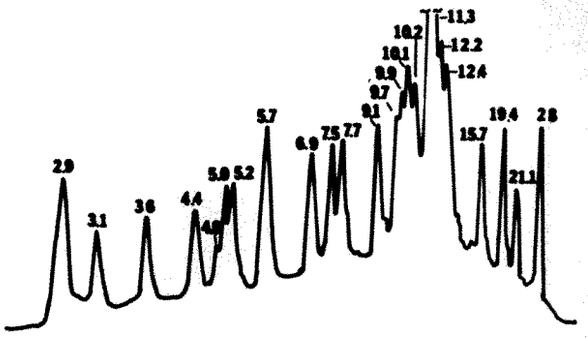


Fig. 5 Stoichiometry of *Pst*I fragments of *B. campestris* mitochondrial DNA. A negative of a gel similar to those shown in Figs 1 and 3 was densitometrically scanned in a Beckman DU spectrophotometer at an absorbance wavelength of 600 nm.

(Fig. 2) that accounts for every fragment for all five enzymes except for two fragments with each of *Pst*I (P15.7 and P10.1), *Kpn*I (K11.0, a doublet), *Nru*I (N35 and N3.9) and *Sal*I (S28 and S8.1). This circle contains the entire 218-kb sequence complexity of the *B. campestris* mitochondrial genome, including two copies of the 2-kb repeat present in direct orientation within P21.1 and P4.8 (Fig. 2). When the chromosomal walk is started at P15.7 or P10.1 and continued outwards in both directions, smaller circular linkage groups are generated, of 135 and 83 kb, respectively, each containing just one copy of the 2-kb repeat (Fig. 6).

The *Kpn*I, *Nru*I and *Sal*I fragments not accounted for by the large circle (Fig. 2) are those that span the 2-kb repeat on the two small circles (Fig. 6). It was not possible to evaluate the stoichiometry of the repeat-containing *Sal*I fragments (Figs 1 and 3) and *Kpn*I fragments due to their co-migration with other fragments. However, all four of the repeat-containing *Nru*I fragments are well separated and appear substoichiometric with approximately the same molarity relationships as described for the corresponding *Pst*I fragments (data not shown). *Bgl*I cuts within the repeat (Figs 2, 4, 6), thus no substoichiometric *Bgl*I fragments were observed.

Together, the two small circles contain the entire sequence complexity of the master chromosome. Figure 6 presents a simple model for the interconversion of these three circles via reciprocal recombination between directly oriented 2-kb repeat elements. Two recombination reactions are shown: intramolecular recombination between repeat elements present on the master chromosome will lead to its resolution into the two small circles, and the converse, intermolecular recombination between 2-kb repeats on the small circles will lead to their cointegration to form the master circle. This model portrays only two of essentially an infinite number of possible recombination reactions. Each of the two small circles could recombine with either repeat element on the large circle, generating circles of 353 or 301 kb. Recombination could occur between identical circles, leading to circular dimers. Higher order reactions could involve any of these multimeric molecules and also the simpler molecules shown in Fig. 6, leading to larger and larger circles, *ad infinitum*.

The simplest alternative to the three-circle model shown in Fig. 6 is a circular dimer model[20,21]. Head-to-head 436-kb circular dimers formed by intermolecular recombination between the P4.8 repeat present on one 218-kb circle and the 21.1-kb repeat present on a second circle would contain all four of the repeat orientations shown in Fig. 6. However, a collection of 218-kb monomers and head-to-head dimers would have higher levels of the P4.8 and P21.1 repeats (present on both monomers and dimers) than of the P15.7 and P10.1 repeats (present on dimers only). Even in the limit case, where all of the mitochondrial DNA existed as head-to-head 436-kb dimers, the four repeats would be equimolar. As P15.7 and P10.1 are approximately twice as abundant as P4.8 and P21.1, we conclude that the simplest model consistent with both mapping and stoichiometry relationships is the three-circle model (Fig. 6). While we believe it likely that most of the mitochondrial DNA exists in one of these three circular forms, this does not preclude a small percentage of the DNA existing as circular dimers or

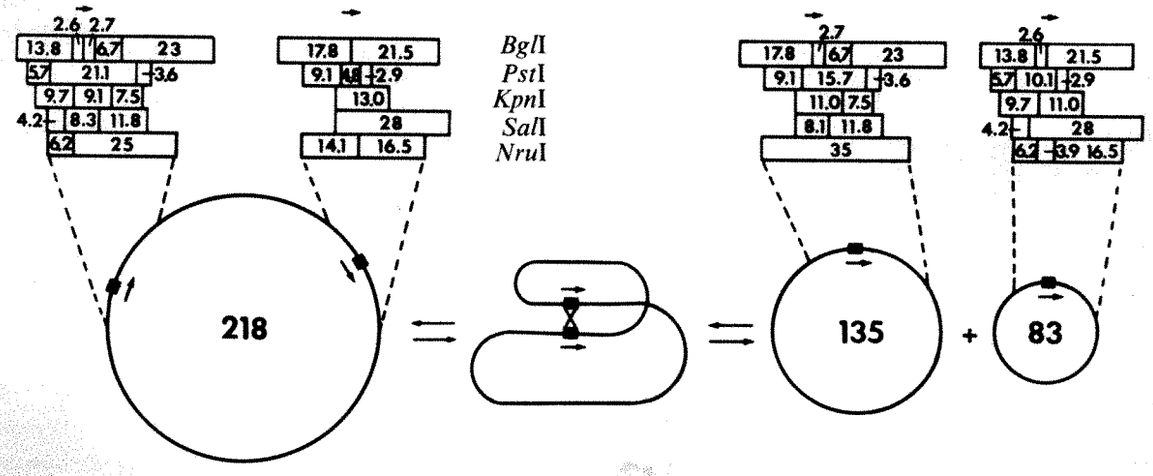


Fig. 6 Top, restriction maps of the four regions of the mitochondrial genome which contain the 2-kb repeat sequence. Bottom, predicted organization of the *B. campestris* mitochondrial genome into three circular molecules and their interconversion via recombination within the 2-kb repeat. Solid boxes indicate the position of the repeat and arrows its orientation.

any of the other multimers described in the preceding paragraph.

Regardless of the ultimate size of the molecules on which they are present, P4.8 and P21.1 seem to be inextricably physically linked and thus must be present in equal molarity. As P15.7 and P10.1 are present at roughly twice the concentration of P21.1 and P4.8, it can be concluded that there are approximately two molecules of the two small circles for each molecule of the master chromosome. That P15.7 and P10.1, and thus the circles they represent, are approximately equimolar suggests that the master chromosome may be the replicating molecule within the mitochondrion. According to this hypothesis, most recombination events would occur intramolecularly, generating the two small circles from the master chromosome, and thereby maintaining equal levels of these two circles. Clearly, however, fragment stoichiometries, approximate as they are, can provide only suggestive evidence concerning the direction of recombination and replication, and more direct experimental tests are necessary to provide conclusive answers.

Discussion

The organization of the *B. campestris* mitochondrial genome into three physically distinct circular chromosomes maintained via recombination across a common repeat element is a novel pattern of mitochondrial DNA sequence organization. While a great diversity of small extrachromosomal molecules have been found in the mitochondria of plants and fungi, the main mitochondrial genome in all eukaryotic organisms characterized to date consists of a single molecular species, usually circular, although occasionally linear[6,7,22].

Is the tripartite structure of mitochondrial DNA from *B. campestris* typical of other plant mitochondrial genomes, most of which are considerably larger? Sets of four repeat-containing restriction fragments having paired combinations of unique flanking sequences, completely analogous to those characterized here, have been found in corn[21], wheat[23] and spinach (D. B. Stern and J.D.P., unpublished data). In corn, extensive restriction mapping of the 569-kb mitochondrial genome indicates that the bulk of the genome may be organized in a tripartite fashion similar to that of *Brassica*, although secondary recombination events outside the major repeat sequence appear to generate an extra diversity of circular size forms not seen in *Brassica* (ref. 21 and D. M. Lonsdale, personal communication). Recent work also indicates that similar processes of intramolecular recombination and excision may lead to the dissolution of the 65-kb *Neurospora* mitochondrial genome into 22- and 43-kb circles in certain *stopper* mutants[24].

The process of recombination between repeated sequences to give alternate states of *Brassica* mitochondrial genome organization—either two small circles or one large co-integrate circle—is analogous to the process of λ phage integration–excision into the *Escherichia coli* chromosome[26] and that of bacterial transposable element cointegrate formation–resolution[27]. By analogy to these and mechanistically related specialized site-specific recombination systems[28–31], we speculate that there may be a specialized recombination system, possibly encoded within the *Brassica* mitochondrial genome and perhaps even physically linked to the 2-kb repeat sequence, which mediates recombination between specific recombination sites embedded within the 2-kb repeat. We are now examining these hypotheses through sequence studies of the 2-kb repeat and by testing its ability to act as a substrate in a heterologous site-specific recombination system[32].

Further analogy, to processes of low-frequency, secondary site recombination between λ DNA and the *E. coli* chromosome[26], leads to the prediction that recombination may also occur between the putative strong recombination site located in the 2-kb *Brassica* repeat and weaker recombination sites located elsewhere in the genome. This possibility will be studied in *Brassica*, and may be related to the high levels of small circular mitochondrial DNA molecules observed in suspension cell cultures of other plant species[11–13]. We hypothesize that these abundant small circles may result from secondary recombination processes which normally occur at very low frequency in native plant tissues but which are intensified when plant cells are cultured *in vitro*.

The *B. campestris* mitochondrial genome is relatively small compared with the mitochondrial genomes of most other higher plants. Therefore, we expect that it will serve as an excellent model system in which to examine many fundamental questions concerning the structure, expression and function of plant mitochondrial genes and also the precise involvement of the mitochondrial genome in cytoplasmic male sterility[3,5,6]. In addition, important evolutionary questions relating to the mode and tempo of plant mitochondrial DNA divergence can now be directly addressed. It is well known, for example, that animal mitochondrial DNA evolves extremely rapidly at the primary sequence level but is highly conserved in gene arrangement, whereas fungal mitochondrial DNA is characterized by a high degree of genomic rearrangement[7,33]. In contrast, little is known about how plant mitochondrial DNA evolves, although limited observations suggest that it may be relatively conserved among mitochondrial DNAs at the sequence level, with a moderate tendency to rearrange[7,34]. We are now addressing these questions by comparing restriction maps of mitochondrial DNA from *B. campestris* and from five other species in the genus *Brassica*. Evolutionary divergence values are well established for the chloroplast DNAs of these species[35], thus these experiments should also give valuable information on relative rates of sequence change in the two cytoplasmic organelles. Finally, we point out the utility of a complete physical map for furthering our understanding of the nature and pattern of integration of chloroplast DNA sequences within plant mitochondrial DNAs[17,36,37].

We thank W. F. Thompson for providing laboratory facilities for this research, the Department of Vegetable Crops at the University of California at Davis for financial assistance, D. M. Lonsdale and M. W. Gray for communication of results before publication, and R. B. Flavell and M. E. Zolan for helpful discussion. This is CIW-DPB publication 838.

Received 20 September; accepted 27 October 1983.

1. Brennicke, A. *Pl. Physiol.* **65,** 1207–1210 (1980).
2. Lebacq, P. & Vedel, F. *Pl. Sci. Lett.* **20,** 1–9 (1981).
3. Palmer, J. D., Shields, C. R., Cohen, D. B. & Orton, T. J. *Nature* **301,** 725–728 (1983).
4. Ward, B. L., Anderson, R. S. & Bendich, A. J. *Cell* **25,** 793–803 (1981).
5. Levings, C. S. III *Cell* **32,** 659–661 (1983).
6. Leaver, C. J. & Gray, M. W. *A. Rev. Pl. Physiol.* **33,** 373–402 (1982).
7. Gray, M. W. *Can. J. Biochem.* **60,** 157–171 (1982).
8. Synenki, R. M., Levings, C. S. III & Shah, D. M. *Pl. Physiol.* **61,** 460–464 (1978).
9. Levings, C. S. III, Shah, D. M., Hu, W. W. L., Pring, D. R. & Timothy, D. H. *ICN–UCLA Symp. molec. cell. Biol.* Vol. XV 63–73 (1979).
10. Fontarnau, A. & Hernandez-Yago, J. *Pl. Physiol.* **70,** 1678–1682 (1982).
11. Dale, R. M. K., Duessing, J. H. & Keene, D. *Nucleic Acids Res.* **9,** 4583–4593 (1981).
12. Dale, R. M. K. *Proc. natn. Acad. Sci. U.S.A.* **78,** 4453–4457 (1981).
13. Brennicke, A. & Blanz, P. *Molec. gen. Genet.* **187,** 461–466 (1982).
14. Spruill, W. M., Levings, C. S. III & Sederoff, R. R. *Devl Genet.* **1,** 363–378 (1980).
15. Bendich, A. J. in *Mitochondrial Genes* (eds Slonimski, P., Borst, P. & Attardi, G.) 477–481 (Cold Spring Harbor Laboratory, New York, 1982).
16. Lonsdale, D. M., Thompson, R. D. & Hodge, T. P. *Nucleic Acids Res.* **9,** 3657–3669 (1981).
17. Stern, D. B. & Lonsdale, D. M. *Nature* **299,** 698–702 (1982).
18. Stern, D. B., Dyer, T. A. & Lonsdale, D. M. *Nucleic Acids Res.* **10,** 3333–3340 (1982).
19. Vieira, J. & Messing, J. *Gene* **19,** 257–268 (1982).
20. Kolodner, R. & Tewari, K. K. *Proc. natn. Acad. Sci. U.S.A.* **76,** 41–45 (1979).
21. Lonsdale, D. M., Hodge, T. P., Fauron, C. M. R. & Flavell, R. B. *ICN–UCLA Symp. molec. cell. Biol.* new ser. **12,** 445–456 (1983).
22. Wallace, D. C. *Microbiol. Rev.* **46,** 208–240 (1982).
23. Falconet, D., Lejeune, B., Quetier, F. & Gray, M. W. *EMBO J.* (in the press).
24. Gross, S. R. & Levine, P. H. *Genetics* **103,** S31 (1983).
25. Kolodner, R. & Tewari, K. K. *Proc. natn. Acad. Sci. U.S.A.* **69,** 1830–1834 (1972).
26. Nash, H. A. *A. Rev. Genet.* **15,** 143–167 (1981).
27. Kostriken, R., Morita, C. & Heffron, F. *Proc. natn. Acad. Sci. U.S.A.* **78,** 4041–4045 (1981).
28. Simon, M., Zieg, J., Silverman, M., Mandel, G. & Doolittle, R. *Science* **209,** 1370–1374 (1980).
29. Broach, J. R. *Cell* **28,** 203–204 (1982).
30. van der Putte, P., Cramer, S. & Giphart-Gassler, M. *Nature* **286,** 218–221 (1980).
31. Roizman, B. *Cell* **16,** 481–494 (1979).
32. Cox, M. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 4223 (1983).
33. Brown, W. M. in *Evolution of Genes and Proteins* (eds Nei, M. & Koehn, R. K.) 62–88 (Sinauer Associates Inc., Sunderland, Massachusetts, 1983).
34. Sederoff, R. R., Levings, C. S. III, Timothy, D. H. & Hu, W. W. L. *Proc. natn. Acad. Sci. U.S.A.* **78,** 5953–5957 (1981).
35. Palmer, J. D., Shields, C. R., Cohen, D. B. & Orton, T. J. *Theor. appl. Genet.* **65,** 181–189 (1983).
36. Lonsdale, D. M., Hodge, T. P., Howe, C. J. & Stern, D. B. *Cell* **34,** 1007–1014 (1983).
37. Stern, D. B. & Palmer, J. D. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
38. Palmer, J. D. *Nucleic Acids Res.* **10,** 1593–1605 (1982).
39. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72,** 1184–1188 (1975).

On the far-infrared excess of Vega

Paul M. Harvey, Bruce A. Wilking & Marshall Joy

Astronomy Department, University of Texas at Austin, College of Natural Sciences, 15.308 R. L. Moore Hall, Austin, Texas 78712, USA

The Infrared Astronomy Satellite (IRAS) has recently detected a compact far-infrared emission source associated with Vega (α Lyrae)[1]. This flux appears to be thermal emission from dust heated by the visual/UV luminosity of Vega. Although circumstellar dust is commonly associated with young, early-type stars[2], the characteristics of the emission from Vega are quite atypical. The essential difference is the relatively low temperature of the dust for the observed source size. This low temperature implies that the dust grains producing the far-infrared emission must be very efficient 60/100-μm emitters and must, therefore, be much larger than typical interstellar and circumstellar dust grains. To place more limits on the properties of this far-infrared emission region, we observed Vega at 47 and 95 μm with the Kuiper Airborne Observatory (KAO) on the night of 12–13 August 1983. We report here confirmation of the IRAS results and that the source size may be as large as 46 arc s along some axis.

We used a 3 spatial × 2 spectral element array radiometer described in detail by Wilking *et al.*[3]. Briefly, this system measures simultaneously the flux at two wavelengths from three circular regions of sky which lie along a line in cross-elevation. The centre-to-centre separation between the beams is 1 arc min. The cross-elevation direction was rotated 15° to the north-west and south-east from a line parallel to declination. We observed at 47 μm ($\Delta\lambda = 20$ μm) and at 95 μm ($\Delta\lambda = 80$ μm). In addition to the normal short-wavelength blocking provided by a diamond scatter filter and various crystalline materials[4,5], we added a layer of 0.003-inch black polyethylene on the outside of the radiometer to eliminate any possible short-wavelength leaks in the standard filtering. The beams are roughly gaussian in shape with half-power widths of 30 and 43 arc s respectively at 47 and 95 μm. The oscillating secondary chopper of the KAO was operated with a throw of 3 arc min in cross-elevation.

The central of the three beams was placed on the visible image of Vega so that the outer beams observed points roughly 1 arc min to the north and south. The measured flux densities in all six channels after about 4,000 s integration time are listed in Table 1. The observations were calibrated relative to IRC+10420. We have measured the flux density of IRC+10420 relative to Mars, S140 (ref. 6), and NGC7027 (ref. 7), on several flights and find values of 1,270 ± 135 and 360 ± 70 Jy, respectively at 47 and 95 μm. (These measurements have also refined our estimates for the flux density of S140 to 6,600 and 6,900 Jy respectively.)

It is clear from Table 1 that far-infrared flux is definitely seen from Vega at a level similar to that reported by Aumann[1]. It is also apparent that no flux was seen in the detectors which viewed positions 1 arc min away from the star to a 2σ limit of roughly one-third that seen on Vega itself. A more stringent limit on the far-infrared surface brightness at a radius of 1 arc min from Vega may be obtained by averaging the four measurements taken off the peak. This leads to an average 50–100 μm flux density of 0.45 ± 0.35 Jy, a factor of 10 or more fainter than the central value. Therefore, the source radius is certainly less than 1 arc min, consistent with the results of Aumann *et al.*[1].

Table 1 Observed flux densities (Jy)

	1 min South	Central	1 min North
47 μm	0.55 ± 0.6	4.6 ± 0.85 (±1.25)	0.45 ± 0.65
95 μm	0.9 ± 0.65	4.5 ± 0.8 (±1.40)	−1.1 ± 1.45

Absolute calibration uncertainty ±20% at 50 μm; ±25% at 100 μm. The total uncertainty, absolute plus statistical, is shown in parentheses.

If we combine our subarc min resolution data with those of Aumann *et al.*[1], we can place better limits on the source size. For comparison, we have calculated the flux densities at our effective wavelengths of 47 and 95 μm using the IRAS flux densities and assuming a black-body spectral shape. These are then 8.2 Jy at 47 μm and 7.3 Jy at 95 μm. Our observed flux densities (Table 1) are 0.56 ± 0.15 and 0.62 ± 0.21 of the IRAS values at 47 and 95 μm, where the errors include all our uncertainties and a nominal 10% error for the IRAS data. Within 3σ error limits these are consistent with unity. But we may derive a rough estimate of source size by assuming that the lower flux densities seen by our smaller beams result because the source size is comparable to our beam size. In this case, the diameter of a uniform disk large enough to produce the above flux ratios is 42^{+10}_{-7} arc s and 53^{+18}_{-10} arc s, respectively, at 47 and 95 μm. Although the formal error limits on the source size are quite large, the fact that our observed colour temperature of 79 K is less than the IRAS value of 85 K, is also consistent with the idea that the source size is comparable to our beam size. In such a case, the larger 95 μm beam would be expected to measure a greater fraction of the total flux than the 47 μm beam, leading to a lower colour temperature than found by IRAS. In the following discussion we will assume a source diameter of 46 ± 10 arc s based on our flux measurements.

This small source size implies dust grains which have an absorption efficiency at visual–UV wavelengths nearly equal to their emission efficiency in the far-infrared as noted already by Aumann *et al.*[1]. If we equate the energy absorbed by grains at a radius of 23 ± 5 arc s with the energy re-emitted, we find that the ratio of Planck-averaged absorption efficiency to emission efficiency is 2.8 ± 1.3. This is in extreme contrast to typical values for interstellar dust of 100–1,000 (refs 8, 9 and P. Makinen, N. J. Evans, P. M. H. and M. A. Kutner, in preparation). This near equality of visible and far-infrared efficiencies implies that the 47 μm and 95 μm emission efficiencies are almost certainly equal except for any small effects dependent on dust composition. Therefore, the IRAS colour temperature of 85 K is probably quite close to the physical dust temperature.

This high emission efficiency places strong lower limits on the size of the grains seen in emission. No calculations of $\lambda \sim 100$ μm emission efficiency for 'large' grains have been found in the literature, but a rough estimate may be obtained from calculations of small grain properties at shorter wavelengths. For instance, the data of Aannestad[10] show for typical interstellar grains that as soon as the wavelength becomes larger than 1–1.5 times the grain diameter, the emission efficiency drops rapidly from unity. Scaling to $\lambda \sim 100$ μm, we expect the grains around Vega to have a diameter $d \geq 20$ μm in order to have a far-infrared emission efficiency as large as one-third the short-wavelength absorption efficiency.

We may estimate the minimum mass necessary to produce the observed emission assuming the particles have unit emissivity, a radius $r \geq 10$ μm, and density of 2 g cm^{-3}. This mass is $M = 10^{24}$ g, comparable to the mass of a small satellite in the solar system. The mass required to produce the observed level of emission scales linearly with assumed particle size. Therefore, although it is possible to produce the observed flux density with a single large 'particle', the required mass is of the order of $10^6\ M_\odot$.

The above limit on grain size, $d \geq 20$ μm, is a very strong limit based only on the assumption that the emission is thermal and that the grain emissivity is a reasonable function of wavelength. An additional size limitation is imposed by the Poynting–Robertson effect[11] which will cause grains of diameter 20 μm and density 2 g cm^{-3} to spiral in to the central star in a time of 8×10^6 yr, assuming $L_* = 58\ L_\odot$ (ref. 12), a source radius

© 1984 Macmillan Journals Ltd

$r \sim 180$ AU and a distance for Vega of 8 pc (ref. 13). Aumann *et al.*[1] have summarized a number of arguments which imply that the grains must have been produced at the time of formation of Vega. Therefore, the grains must be even larger than $d = 20$ μm if the age of Vega is $>10^7$ yr.

We have confirmed the far-infrared excess of Vega found by the IRAS survey. From a comparison of flux densities in our beam relative to the IRAS data we find evidence that the source has a diameter of the order of 46 arc s. This is roughly twice that found by IRAS using deconvolution of larger beam data. Resolution of this disagreement must await further observations: it may result either from observational errors or from a source brightness distribution which is more complicated than the assumed uniform, spherical geometry.

These data also confirm the fact that the grains around Vega must be much larger than typical interstellar grains ($a \sim 0.1$ μm). Therefore, some form of grain growth must have occurred. The Poynting–Robertson effect places a limit on the grain radius a (μm) and the time since grain formation in units of 10^6 yr, t_6, such that $a \sim t_6$ for a cloud diameter of 46 arc s. Further observations of the Vega dust cloud and theoretical modelling of grain growth should provide important insight into the kind of processes which may have led to the even greater 'grain' growth in the Solar System.

We thank the entire KAO staff for their help in obtaining the observations presented here. We also thank C. Beichman and the IRAS science team for several useful discussions.

Received 26 October; accepted 24 November 1983.

1. Aumann, H. H. *et al. Astrophys. J. Lett.* (in the press).
2. Harvey, P. M., Thronson, H. A. Jr & Gatley, I. *Astrophys. J.* **231,** 115 (1979).
3. Wilking, B. A., Harvey, P. M., Lada, C. J., Joy, M. & Doering, C. R. *Astrophys. J.* (in the press).
4. Armstrong, K. A. & Low, F. J. *Appl. Opt.* **9,** 2007 (1973).
5. Armstrong, K. A. & Low, F. J. *Appl. Opt.* **13,** 425 (1974).
6. Harvey, P. M., Campbell, M. F. & Hoffmann, W. F. *Astrophys. J.* **219,** 891 (1977).
7. Moseley, S. H. *Astrophys. J.* **238,** 892 (1980).
8. Gatley, I., Becklin, E. E., Werner, M. W. & Wynn-Williams, C. G. *Astrophys. J.* **216,** 277 (1977).
9. Whitcomb, S. E. *et al. Astrophys. J.* **246,** 416 (1981).
10. Aannestad, P. A. *Astrophys. J.* **200,** 30 (1975).
11. Lang, K. R. *Astrophysical Formulae,* 544 (Springer, Berlin, 1980).
12. Dreiling, L. A. & Bell, R. A. *Astrophys. J.* **241,** 736 (1980).
13. Jenkins, L. F. *General Catalogue of Trigonometric Stellar Parallaxes* (Yale University Observatory, New Haven, 1963).

$^{15}N/^{14}N$ ratio of dissolved N_2O in the eastern tropical Pacific Ocean

Naohiro Yoshida*§, Akihiko Hattori†, Toshiro Saino†, Sadao Matsuo* & Eitaro Wada‡

* Department of Chemistry, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan
† Ocean Research Institute, University of Tokyo, Nakano-ku, Tokyo 164, Japan
‡ Laboratory of Biogeochemistry and Sociogeochemistry, Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, Japan

Nitrous oxide (N_2O) is of prime interest for understanding stratospheric chemistry[1] and the energy budget of the troposphere[2]. Atmospheric N_2O has been considered to be mainly regulated by biological processes in soils[3–6]. However, the oceans have an important role in the geochemical cycle of N_2O both as a reservoir and a reaction site[7–12]. We report here the first ^{15}N abundance data on dissolved N_2O collected from the eastern tropical Pacific Ocean where active nitrogen transformation has been proposed to occur[12–16]. Our ^{15}N data provide additional evidence for the hypothesis that the subsurface waters act as a source of N_2O and that the extremely oxygen-depleted waters act as a sink.

§ Present address: Laboratory of Biogeochemistry and Sociogeochemistry, Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, Japan.

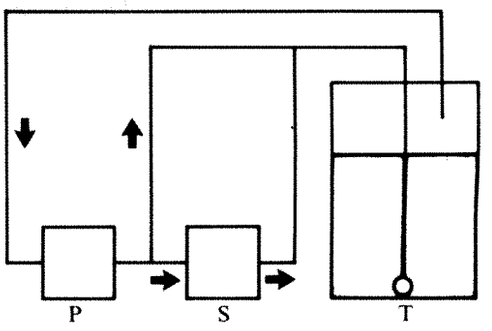


Fig. 1 The device used for extraction and collection of dissolved N_2O from seawater samples. Approximately 100 l of seawater was introduced into a stainless steel tank (T, ~150 l in volume), which had been filled with Ar. The gas above the sample water was circulated by the aid of a pump (P) through a sintered glass ball to facilitate emission of N_2O into the gas phase. A portion of the circulation gas goes through a bypass where N_2O is adsorbed on a molecular sieve 5A column in a sampling device (S) whose detail was described in ref. 17. Complete collection of N_2O was achieved within 12 h. For safety, the circulation was continued for 15 h.

Yoshida and Matsuo[17] first introduced the nitrogen isotope ratio to assess the geochemical cycle of N_2O. In their study, they maintained that basic knowledge of the nitrogen isotope ratio of dissolved N_2O in the oceans would improve our understanding of the global budget of N_2O. The existing data on dissolved N_2O concentration in oceanic waters suggest that N_2O is produced in association with bacterial nitrification in subsurface waters and consumed by denitrification occurring in extremely oxygen-depleted waters[8,9,11–13]. Low oxygen tension favours the production of N_2O associated with nitrification[18–20]. We therefore focused on the nitrogen isotope ratio of N_2O dissolved in water masses within and at the boundaries of the oxygen minimum zone.

Seawater samples were collected with 23-l Niskin bottles from various depths at six stations of the eastern tropical Pacific Ocean on legs 2 and 3 of KH-82-5 Cruise of RV *Hakuho Maru*, University of Tokyo[21]. Five seawater samples collected at 2-m depth intervals were successively and carefully introduced, without exposure to air, to a stainless steel tank which had been flushed and filled with argon. Dissolved N_2O was then extracted and collected on a molecular sieve 5A column using a device illustrated in Fig. 1. The nitrogen isotope ratio of the N_2O was determined later in the laboratory by a method described elsewhere[17]. The accuracy of the data presented here is estimated to be within ±3% for concentration and ±0.2‰ for $\delta^{15}N$ of N_2O both in the atmosphere and in the ocean.

The vertical profiles of N_2O were similar to those reported by other workers[9,11–13,16]; N_2O increased with depth, attained a maximum and decreased at greater depths. Figure 2 depicts the relationship between dissolved oxygen concentration and dissolved N_2O concentration (top) or nitrogen isotope ratio in N_2O (bottom).

According to the nitrogen isotope ratio ($\delta^{15}N$ referred to atmospheric N_2 standard), dissolved N_2O can be categorized into two groups; one is depleted in ^{15}N (average $\delta^{15}N = 5.2 \pm 1.3$‰) relative to that in equilibrium with atmospheric N_2O (8.0‰ as indicated by the dotted line in Fig. 2), and the other is enriched in ^{15}N (average $\delta^{15}N = 12.6 \pm 1.1$‰). The former was found invariably at depths where the oxygen concentration was relatively high, while the latter occurred at depths where the oxygen concentration was extremely low and the N_2O concentration almost the same as or somewhat lower than the level in equilibrium with air estimated from the solubility data of N_2O (ref. 22). The high concentration of dissolved N_2O observed above the oxygen minimum layer can be attributed to the production of N_2O associated with nitrification[18–20]. Our ^{15}N data support this inference, because we (N.Y. and S.M.,

0028–0836/84/050442—03$01.00

Table 1 Concentration and nitrogen isotope ratio of N_2O collected from maritime air over the Pacific Ocean on cruise KH-82-5 of RV *Hakuho Maru*

Sample no.	Location Latitude	Longitude	Station no.	Date (GMT)	N_2O (p.p.b.)	$\delta^{15}N$ (‰)
1	36°09′ N	156°32′ E	S4–5	25 November 1982	327	8.1
2	36°25′ N	168°19′ E	S8–9	27 November 1982	308	7.1
3	40°25′ N	175°11′ W	S14–15	29 November 1982	336	8.1
4	49°37′ N	174°07′ W	S14–15	29 November 1982	301	7.4
5	40°53′ N	172°53′ W	S14–15	29 November 1982	306	7.4
6	41°00′ N	172°04′ W	S15–16	29 November 1982	334	7.7
7	41°09′ N	170°58′ W	S15–16	29 November 1982	324	6.5
8	41°19′ N	169°56′ W	S15–16	30 November 1982	329	7.2
9	41°30′ N	168°53′ W	S16–17	30 November 1982	315	6.5
10	42°22′ N	156°00′ W	S20–21	2 December 1982	313	7.0
11	41°16′ N	135°49′ W	S26–27	5 December 1982	310	7.5
12	26°50′ N	121°47′ W	9	21 December 1982	327	3.7
14	17°15′ N	108°38′ W	13	26 December 1982	297	8.6
16	10°30′ N	105°20′ W	16	4 January 1983	287	7.9
19	2°29′ S	115°05′ W	19	8 January 1983	292	6.7
23	13°00′ N	117°00′ W	25	15 January 1983	305	6.6
Averages					313 ± 15*	7.2 ± 1.1

*As for the maritime air, Rasmussen and Pierotti[25] reported the N_2O content to be between 315 and 330 parts per 10^9 (p.p.b.). On the other hand, Weiss *et al.*[26] proposed that it was 302.8 ± 0.6 p.p.b. for this sampling time.

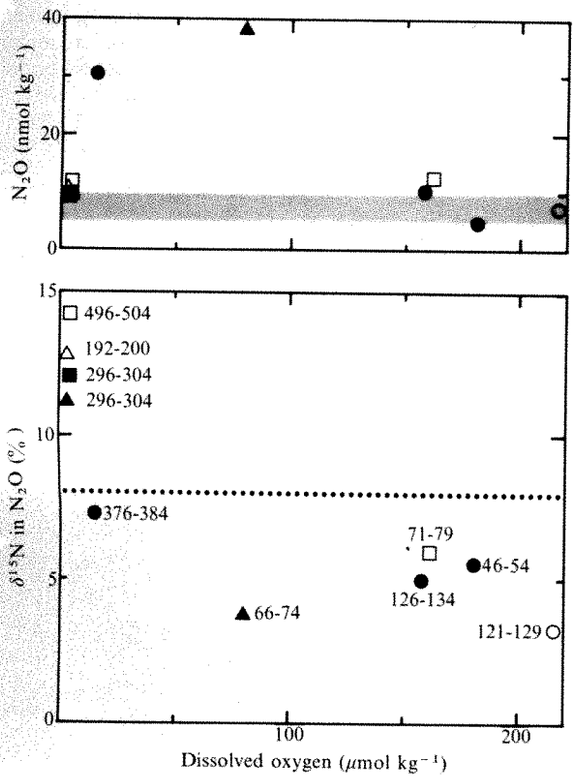


Fig. 2 Relationship between dissolved oxygen concentration and N_2O concentration (top) or nitrogen isotope ratio in N_2O (bottom) in the subsurface waters of the eastern tropical Pacific Ocean. Numerical values represent depth ranges where the water samples were collected. Symbols: ○, station 9; △, station 13; □, station 16; ●, station 19; ▲, station 25; ■, station 11′. Sampling date and station location are given in Table 1 except for station 11′ (18 January 1983, 17°27′ N and 116°58′ W). Oxygen data presented in the cruise report[21] were used. A shaded bar (top) and a dotted line (bottom) refer to N_2O concentration and $\delta^{15}N$ in N_2O in equilibrium with atmospheric N_2O (averages in Table 1), respectively. The range of equilibrium concentration corresponds to the range of temperature and salinity of the seawater samples; from 8.58 °C and 34.626‰ to 28.21 °C and 35.048‰. The nitrogen isotope fractionation factor in the process of dissolution of N_2O in seawater is substituted for that in pure water which is 0.9992 (N.Y. and S.M., unpublished) in this temperature range; this means 0.8‰ enrichment of ^{15}N in the aqueous phase.

unpublished) have found a great depletion of ^{15}N down to −66‰ in the product N_2O relative to the initial reactant ammonium during nitrification by *Nitrosomonas europaea*. The lighter isotope was preferentially used to produce N_2O. Actually, excess N_2O (N_2O concentration observed − N_2O concentration in equilibrium with air at given temperature and salinity) was linearly correlated with AOU (apparent oxygen utilization) (data not shown), as observed by Yoshinari[9], Elkins *et al.*[11] and Cohen and Gordon[13].

The ^{15}N enrichment in N_2O in the oxygen minimum zone can also be explained by the isotope ratio of the nitrate source and by the isotope fractionation associated with denitrification. Cline and Kaplan[23] demonstrated that nitrate in the oxygen-deficient waters of the eastern tropical North Pacific is highly enriched in ^{15}N. N_2O enriched in ^{15}N may result from nitrate enriched in ^{15}N. Nitrous oxide enriched in ^{15}N may also be produced by the mode of reaction kinetics associated with denitrification, as observed in the case of nitrite by Blackmer and Bremner[24]. ^{15}N enrichment in N_2O caused by isotope fractionation in reduction of N_2O is also likely. We (N.Y. and S.M., unpublished) have found nitrogen isotope fractionation factors of up to 1.027 in the reduction of N_2O by *Pseudomonas denitrificans*, where the lighter isotope was favoured in the N_2 produced. In fact, N_2O concentrations in extremely oxygen-depleted waters were far less than that expected from AOU.

The average nitrogen isotope ratio of N_2O in maritime air collected on the same cruise over the Pacific Ocean (Table 1) was 7.2 ± 1.1‰. This average was higher than that of dissolved N_2O in the subsurface layer above the oxygen minimum zone, but slightly lower than that of land surface air collected in Japan (8.1 ± 1.0‰)[17]. The observed difference suggests that the subsurface layer of the sea seems to emit ^{15}N-depleted N_2O and consequently lower ^{15}N content of N_2O in the maritime air. The results described here are still too premature to discuss this topic in further detail. However, we emphasize that nitrogen isotope studies have the potential to answer such questions.

We thank the officers and crew members of the RV *Hakuho Maru* and the scientists aboard the KH-82-5 cruise for cooperation, especially Mr H. Otobe for assistance in air and water sampling. Our special thanks also to Asahi Breweries Ltd, Japan for allowing us to use the stainless steel tanks for N_2O extraction.

Received 31 August; accepted 31 October 1983.

1. Johnston, H. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2369–2372 (1972).
2. Lacis, A., Hansen, J., Lee, P., Mitchell, T. & Lebedeff, S. *Geophys. Res. Lett.* **8**, 1035–1038 (1981).
3. Blackmer, A. M. & Bremner, J. M. *Geophys. Res. Lett.* **3**, 739–742 (1976).
4. Brice, K. A., Eggleton, A. E. J. & Penkett, S. A. *Nature* **268**, 127–129 (1977).

5. Freney, J. R., Denmead, O. T. & Simpson, J. R. *Nature* **273**, 530–532 (1978).
6. Bremner, J. M. & Blackmer, A. M. *Science* **199**, 295–296 (1978).
7. Hahn, J. *Tellus* **26**, 160–168 (1974).
8. McElroy, M. B., Elkins, J. W., Wofsy, S. C. & Yung, Y. L. *Rev. Geophys. Space Phys.* **14**, 143–150 (1976).
9. Yoshinari, T. *Mar. Chem.* **4**, 189–202 (1976).
10. Hahn, J. & Junge, C. *Z. Naturforsch.* **32a**, 190–214 (1977).
11. Elkins, J. W., Wofsy, S. C., McElroy, M. B., Kolb, C. E. & Kaplan, W. A. *Nature* **275**, 602–606 (1978).
12. Cohen, Y. & Gordon, L. I. *Deep Sea Res.* **25**, 509–524 (1978).
13. Cohen, Y. & Gordon, L. I. *J. geophys. Res.* **84**, 347–353 (1979).
14. Codispoti, L. A. thesis, Univ. Washington (1973).
15. Codispoti, L. A. & Packard, T. T. *J. mar. Res.* **38**, 453–477 (1980).
16. Pierotti, D. & Rasmussen, R. A. *Tellus* **32**, 56–72 (1980).
17. Yoshida, N. & Matsuo, S. *Geochem. J.* (in the press).
18. Yoshida, T. & Alexander, M. *Proc. Soil Sci. Soc. Am.* **34**, 880–882 (1970).
19. Goreau, T. J. *et al. Appl. envir. Microbiol.* **40**, 526–532 (1980).
20. Lipschultz, F. *et al. Nature* **294**, 641–643 (1981).
21. Hattori, A. & Tomoda, Y. *Preliminary Report of* Hakuho Maru *Cruise KH-82-5* (Ocean Research Institute, University of Tokyo, in the press).
22. Weiss, R. F. & Price, B. A. *Mar. Chem.* **8**, 347–359 (1980).
23. Cline, J. D. & Kaplan, I. R. *Mar. Chem.* **3**, 271–299 (1975).
24. Blackmer, A. M. & Bremner, J. M. *Soil Biol. Biochem.* **9**, 73–77 (1977).
25. Rasmussen, R. A. & Pierotti, D. *Geophys. Res. Lett.* **5**, 353–355 (1978).
26. Weiss, R. F., Keeling, C. D. & Craig, H. *J. geophys. Res.* **86**, 7197–7202 (1981).

Rare earth element fluxes and geochemical budget in the eastern equatorial Pacific

Kim Murphy & Jack Dymond

College of Oceanography, Oregon State University, Corvallis, Oregon 97331, USA

Rare earth element (REE) patterns and flux measurements of sediment traps, sediments, and nodules from the eastern tropical Pacific demonstrate the dominance of REE supply to the sea floor by particle settling. Quantitative partitioning of the particle flux reveals that biogenic debris is the major REE carrier in shallow depths; however, the labile particles are surpassed in importance by alumino-silicate particles at greater depths. Regeneration of biogenic-associated REE appears responsible for La/Ce variations in the particle flux with depth, and provides the primary source of REE accreted by nodules.

Studies of the fractionation of individual REE from one another have provided insight into the geochemical behaviour of REE in the marine environment[1–3]. Ce, because of its unique preference for the 4+ state in seawater, may be more readily adsorbed onto water column particles[4] and incorporated into deep-sea sediments and ferromanganese nodules[5]. Thus, the preferential adsorption of Ce may explain its depletion in seawater relative to the trivalent REE[6,7]. Seawater is also enriched in the heavy REE (HREE), possibly as a result of the greater stability of aqueous complexes formed by HREE, leaving seawater relatively enriched in these elements[6,7].

Although previous studies have used REE fractionation to emphasize the importance of a particle-derived source of REE to sediments and nodules (ref. 2 and others therein), ours are the first reported measurements of the particulate REE flux. We present the REE contents, patterns and fluxes of sedimentary samples from the Manganese Nodule Program (MANOP) site H (6.5° N, 93° W) in the eastern equatorial Pacific in order to establish the REE geochemical budget for this site. In addition, we partition particulate REE into biogenic and detrital components. Decomposition of organic matter and dissolution of opal and carbonate can release REE which are sorbed to other particle surfaces or lost to seawater. Since such material decomposes more readily than detrital phases, the associated REE are more available for uptake by nodules and other authigenic phases.

Samples for this study were analysed for La, Ce, Nd, Sm, Eu, Tb, Yb and Lu by instrumental neutron activation[8] (Table 1). Analytical precision for the REE was within 10% for all samples, except for Nd and Tb which have peak interferences. Interferences due to U were negligible.

Figure 1 shows the REE abundance patterns of site H samples normalized to that of world average shale[5]. Different sample types exhibit large variations in absolute concentration in the order: ferromanganese crust > nodule tops > sediment > nodule bottoms > sediment traps. Nonetheless, the general pattern for all samples is similar to that of site H bottom water (Fig. 1). All samples exhibit the Ce depletion and HREE enrichment typical of seawater. This does not, however, necessarily imply accretion by precipitation from seawater. Site H ferromanganese crust, dredged with basalts and isolated from sediment contact, is thought to form by direct precipitation from bottom water (hydrogenous accretion)[9]. This crust has the smallest Ce anomaly and least resembles the seawater pattern of all the site H samples (Fig. 1). In contrast, similar crusts of probable hydrogenous origin have large positive Ce anomalies[10].

The REE patterns of site H nodules are unlike those of most deep-ocean nodules, which have positive Ce anomalies[1,2]. Nodules from the Bauer Basin of the south equatorial Pacific, however, have negative Ce anomalies[11,12] which are believed to result primarily from hydrothermal iron oxyhydroxides possessing a seawater-like REE pattern[12]. The negative Ce anomalies of site H nodules may also indicate hydrothermal influence, since site H and the Bauer Basin are both within 1,000 km of the East Pacific Rise.

Accumulation rates (mass added per unit time per area) were calculated for site H crusts, nodule tops and bottoms, sediments, and trap material in order to provide a more complete understanding of REE cycling (Fig. 2). Distinctions between nodule tops and bottoms were based on texture and major element composition[9]. Results of site H flux calculations for Oregon State University single cone[13] and Soutar double cone[14] sediment traps deployed from September 1980 to October 1981 reveal increases in the total particle flux[15] with depth. In addition, the increase in the particulate REE concentration with depth (Fig. 1) results in a substantial increase in REE flux with

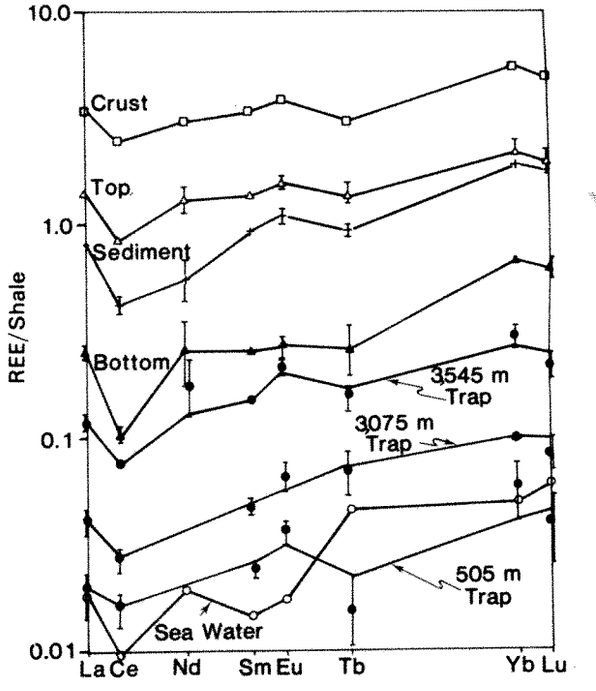


Fig. 1 REE abundances in ferromanganese crust, nodule top and bottom, sediment, sediment traps and bottom water normalized to the world average shale[1]. Seawater data are for deep water from the Guatemala Basin near site H[20] and are multiplied by a factor of 10^3. Values without error bars have errors within symbol size. Values not shown were below the detection limit or had associated errors >50%.

0028-0836/84/050444—04$01.00

© 1984 Macmillan Journals Ltd

Table 1 Site H sample REE compositions (INAA data) (in p.p.m.)

Accession no.	Box core	La	Ce	Nd	Sm	Eu	Tb	Yb	Lu
Tops									
MP2827	I26-1	56.0	68.0	48.0	9.8	2.43	1.61	7.4	1.14
MP2880	I26-4	48.0	52.0	42.0	9.5	2.22	1.55	6.9	1.05
MP2830	I27-1	35.0	37.0	29.0	6.8	1.71	1.13	6.0	0.92
MP2833	I27-3	16.0	10.0	14.0	3.0	0.76	0.60	2.9	0.47
MP2835	I34-1	29.0	40.0	26.0	5.6	1.41	0.87	5.0	0.76
MP2836	I34-2	35.0	42.0	32.0	7.1	1.67	1.19	5.4	0.82
MP2886	I34-4	22.0	17.0	22.0	4.4	1.20	0.78	3.7	0.55
MP2840	I36-1	34.0	37.0	30.0	6.3	1.58	1.01	5.4	0.82
MP2842	I36-2	46.0	60.0	35.0	7.9	2.17	1.30	6.3	0.93
MP4837	V37-1	46.0	58.0	40.9	8.9	2.12	1.51	7.3	1.04
MP5344	V52-1	33.0	48.0	35.0	6.8	2.09	1.01	5.9	0.82
Bottoms									
MP2826	I26-1	10.0	10.0	11.0	2.1	0.43	0.26	1.7	0.28
MP2828	I26-2	14.0	8.0	13.0	2.6	0.57	0.43	2.3	0.36
MP2879	I26-4	11.0	10.0	10.0	2.1	0.43	0.19	1.7	0.26
MP2829	I27-1	9.0	11.0	9.0	2.1	0.40	0.37	1.7	0.25
MP2832	I27-3	10.0	13.0	10.0	2.0	0.44	0.21	1.6	0.24
MP2882	I27-4	11.0	11.0	9.0	2.0	0.45	0.37	1.8	0.30
MP2834	I34-1	12.0	11.0	10.0	2.2	0.51	0.45	2.1	0.30
MP2837	I34-2	13.0	10.0	8.0	2.6	0.64	0.49	2.4	0.36
MP2885	I34-4	10.0	9.0	11.0	2.2	0.49	0.41	1.8	0.27
MP2839	I36-1	15.0	11.0	12.0	2.5	0.60	0.46	2.5	0.39
MP2841	I36-2	16.0	16.0	12.0	2.7	0.68	0.51	2.8	0.43
MP4836	V37-1	11.0	14.0	10.0	1.8	0.50	0.28	2.3	0.46
MP5345	V52-1	8.0	12.0	9.0	1.4	0.47	0.35	1.5	0.24
Whole nodules									
MP2881	I26-4	18.0	18.0	15.0	3.3	0.77	0.54	2.9	0.45
MP2831	I27-2	35.0	38.0	31.0	6.9	1.70	1.27	6.0	0.92
MP2883	I27-4	12.0	12.0	11.0	2.3	0.54	0.41	2.2	0.34
MP2838	I34-3	21.0	16.0	21.0	4.1	1.10	0.85	3.8	0.58
MP2884	I34-4	20.0	17.0	17.0	3.9	0.95	0.55	3.3	0.52
MP2887	I36-4	17.0	17.0	19.0	3.3	0.85	0.58	3.0	0.47
Crusts									
MP9843	P11-4	136.0	196.0	114.0	25.0	6.10	3.68	19.0	2.89
MP9844	P11-5	131.0	210.0	109.0	23.0	6.22	3.55	18.0	2.64
Sediments									
MP5301	BC37-1	33.0	35.0	29.0	7.0	1.8	1.3	6.3	1.1
MP5302	BC37-2	32.0	34.0	20.0	6.9	1.6	1.1	6.4	1.1
MP5304	BC37-4	38.0	38.0	44.0	7.2	1.8	1.3	6.8	1.1
Traps									
MPT9640	H1-U	0.83	1.4	—	0.16	0.059	—	0.22	0.031
MPT9525	H1-S	1.18	2.1	—	0.26	0.099	0.049	0.30	0.042
MPT9797	H1-L	1.66	2.1	3.8	0.35	0.10	0.084	0.32	0.049
MPT9527	H2-U	2.16	2.8	5.8	0.44	0.13	0.076	0.48	0.069
MPT9530	H2-S	2.77	3.5	2.5	0.63	0.17	0.11	0.45	0.076
MPT9531	H2-L	4.91	6.2	6.3	1.09	0.33	0.19	0.97	0.15

depth (Fig. 2). This may be caused by adsorption of REE onto particulate debris as it falls through the water column and/or additional sources of REE-rich particles at depth. Within the upper 1,500 m of the water column, there is a substantial increase in REE particulate flux with depth. Such an increase has been noted in other oceanic locations and may be due to lateral transport of material[16], particle aggregation[17], or zooplankton feeding dynamics[18]. Below 1,500 m REE flux increase is gradual until within approximately 500 m of the bottom. Below this, there is a sharp increase in apparent flux which is due to resuspension of bottom material[13]. Therefore, in calculating sediment trap REE fluxes to the sediment–water interface, an extrapolation of fluxes between 1,465 m and 3,075 m depths was used to avoid the influence of local resuspension which produces an artificial flux increase in near-bottom traps.

Comparison of the extrapolated trap flux to the elemental accumulation rate in the sediment reveals that the total REE particulate flux accounts for approximately 85–100% of the REE preserved in site H sediments (Fig. 2). Hydrogenous precipitation, presumably represented by accretion in ferromanganese crust, is another potential source of REE to the sediments. However, since REE accumulation in crust accounts for less than 7% and 1% of the REE accumulation rates in nodules and sediments, respectively (Fig. 2), the contribution from the hydrogenous source appears relatively insignificant, and particulate debris is apparently the major source of REE to site H nodules and sediments. Near-bottom enrichments of REE in the North Atlantic[3] suggest that REE are released by regeneration of labile biogenic particles at the sediment–water interface[17]. However, our geochemical balance (Fig. 2) implies that REE at H are strongly retained by sedimentary phases. Nonetheless, the range in bulk sedimentation rate (Fig. 2), the resuspension assumptions of the flux calculations, and uncertainties involved in comparing a single year of trap data with the sediment record, limit the precision of the extrapolated and observed flux comparison. For several refractory elements, such as Al, the agreement between sediment accumulation and extrapolated particle flux is within 10%, while labile elements, such as Cu, show 40–50% regeneration at the sediment–water interface[15]. Results for the REE clearly show that a particulate source is the major supply to site H sediments and that the fraction of REE regenerated from particles at the sea floor is small.

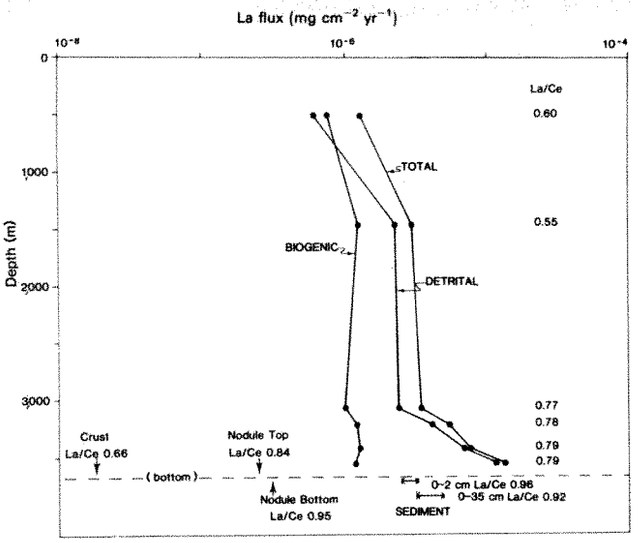


Fig. 2 La fluxes and La/Ce ratios of site H samples. Regression-derived REE concentrations and ratios: (1) biogenic: La = 0.5 p.p.m., Ce = 1.0 p.p.m., La/Ce = 0.50 ($r^2 = 0.99$); (2) detrital: La = 40.5 p.p.m., Ce = 43.0 p.p.m., La/Ce = 0.93 ($r^2 = 0.98$). Accumulation rates of REE in crusts, nodule tops, nodule bottoms, sediments and sediment traps were calculated using $A = SC\rho$; where A is the flux or accumulation rate of an element in a sample; S is the bulk growth rate, sedimentation rate, or flux applying to crusts/nodules, sediments and traps, respectively; C is the concentration of an element in a sample; and ρ is the dry bulk density of nodules, crusts or sediments. Biogenic and detrital fluxes were derived from linear programming. Crust growth rates are estimated at 1 mm per 10^3 yr (ref. 25). Nodule top/bottom growth rates were determined by refs 26 and 27. Sediment accumulation rate ranges are based on sedimentation rates of 0.45 cm per 10^3 yr (ref. 28) and 0.65 cm per 10^3 yr (ref. 29). Dry bulk density values of 1.35 g cm^{-3} (ref. 24) and 0.22 g cm^{-3} (K.M., unpublished data) were used for crust/nodules and sediment, respectively.

To clarify further the REE budget at site H, sediment trap and surface sediment samples were partitioned into end-member components using normative analysis. Weight fractions of carbonate, opal, organic matter and ferromanganese precipitates, which best account for the abundance of 13 elements (organic C, P, N, Al, Si, Ca, Ti, Mn, Fe, Cu, Ni, Zn and Co) in 27 annual and seasonal trap and surface sediment samples from MANOP sites H and M (9° N, 104° W), were estimated by linear programming[19]. The carbonate, opal and organic matter fractions were combined as 'biogenic material'. Regression lines were determined between the measured REE content and the percentage of biogenic or detrital component. Extrapolation of the line to 100% of each component allows estimation of the concentrations of pure end-members (Fig. 2). The REE flux from each source can be computed from the end-member fractions in each sample and the regression-derived REE end-member concentrations (Fig. 2). The model, of course, assumes biogenic and detrital components have a constant composition. Since the REE content of particles may result from scavenging, compositional variability with depth may occur which would add uncertainty to the normative analysis.

Table 2 shows that the sum of the regression-derived biogenic and detrital fluxes approximates the measured La flux within 10%, for five out of six traps. The biogenic and detrital fluxes account for nearly all the REE flux throughout the water column, while the ferrogmanganese end-member, possibly representing hydrothermal influence, is relatively insignificant in all traps (K.M. and J.D., unpublished data). The results demonstrate that the biogenic REE flux is dominant in the upper 750 m of the water column but is surpassed by the detrital flux below this depth (Table 2, Fig. 2). Within the bottom 500 m, resuspension of detrital material, which is relatively enriched in the

Table 2 Percentage of total measured La flux explained by end-members in annual traps

Trap depth (m)	Biogenic (%)	Detrital (%)	Difference between measured and calculated flux* (%)
505	60	48	8
1,465	41	76	17
3,075	29	70	1
3,225†	22	75	3
3,415†	17	91	8
3,545†	9	86	5

* Calculated flux = regression-derived biogenic + detrital fluxes.
† Resuspension influenced[13].

sediments, distorts the true flux of the two components. Nevertheless, the detrital REE flux increases gradually with depth, while the biogenic REE flux decreases slightly. This trend probably results from the decomposition and dissolution of biogenic debris as it falls through the water column, combined with an increase in the detrital fraction due to resuspension at depth. The observation that the biogenic REE flux comprises 30–60% of the total flux to site H sediment traps not influenced by resuspension (Table 2) is consistent with the suggestion that REE are associated with biogenic particles[3].

The La/Ce values suggest that some fractionation occurs between Ce and the trivalent REE. This ratio is a more direct means of comparison and has greater precision than the Ce anomaly, particularly for sediment traps which have low REE abundances and greater analytical errors for Nd (Fig. 1). The biogenic component of trap debris, with a La/Ce ratio of 0.50, is enriched in Ce relative to the detrital end-member, with a La/Ce of 0.93 (Fig. 2). Both components, however, are enriched in Ce relative to La, and although deep-ocean nodules have been claimed to be responsible for the REE pattern of seawater[1], a quantitative accounting of the Ce enrichments in these phases could also explain the negative Ce anomaly of seawater. The La/Ce ratio of Guatemala Basin bottom water near site H is approximately 2.0 (ref. 20), and North Atlantic waters below 200 m also have La/Ce ratios ranging between 1.0 and 2.4 (ref. 3). Therefore, the biogenic component at site H is enriched in Ce relative to La when compared with seawater. Elderfield and Greaves' study in the North Atlantic[3] revealed surface water enrichments of Ce that were high compared with the trivalent REE, presumably due to preferential diffusion of Ce from reducing continental margin sediments[21] and advection of margin waters. Since site H could also receive excess Ce by advection from the slope region, the extent of La/Ce fractionation by biogenous debris is uncertain without surface water REE data.

We find a consistently lower La/Ce ratio for traps from the upper 1,500 m (La/Ce ≈ 0.6) compared with La/Ce values of the near-bottom traps (La/Ce > 0.75) (Fig. 2). This trend may result from the aforementioned input of Ce mobilized from near-shore anoxic sediments. It is hypothesized that Ce, like Mn (refs 22, 23), is solubilized at locations where the oxygen minimum intersects the continental margin. The unusually high Mn content of site H trap particles[15] implies that near-shore reduction and subsequent remobilization of Mn influence the composition of particles at this location. Alternatively, the La/Ce increase with depth may be due to decomposition of Ce-enriched labile biogenic particles that release REE to seawater and increase the influence of the detrital fraction which is relatively depleted in Ce. Trapping of resuspended sediments in the deepest traps would also result in higher La/Ce, since the La/Ce of sediments is about 1.0. The 3,075 m trap, which is more than 500 m above the bottom, is believed to be free of resuspended material, however[24]. Since it, too, has elevated La/Ce compared with the near-surface traps, it appears that

resuspension is not the sole explanation for the La/Ce increase with depth. Moreover, the La/Ce of the deepest traps (0.8) does not match the La/Ce ratio of site H sediments (1.0). Fractionation of the REE, which apparently occurs during sediment diagenesis, may explain the increased La/Ce of site H sediments. During periodic, highly reducing conditions, Mn is remobilized within the sediments and conveyed to nodule bottoms[9]. Under such conditions, Ce may be reduced to its trivalent state and mobilized to nodules, and possibly into the bottom water[21]. Although the trivalent REE should also be mobilized under these conditions, enhanced Ce release could result if the Mn-rich phase that dissolves is also enriched in Ce relative to the other REE.

Thus, sediment trap material, sediments and nodules from the eastern tropical Pacific all have seawater-like REE patterns. Flux calculations imply that particles can account for most, if not all, of the REE supply to site H sediments and can easily account for REE accumulation rates in nodules. Breakdown of the particulate flux into end-member components discloses the importance of biogenic material as well as detrital particles in conveying REE to the ocean bottom at site H. The La/Ce ratios of the biogenic and detrital trap components and traps throughout the water column confirm the importance of both components in supplying REE to sediments and nodules and indicate the release of REE from labile biogenic components. Although biogenic and detrital material both contribute REE to sediment traps, it is the biogenic fraction which undergoes decomposition and dissolution reactions, that probably conveys the majority of REE to nodules.

We thank Dr D. Z. Piper for providing the nodule samples used in Fig. 1, Dr K. Fischer for the use of sediment trap data, Drs R. Schmitt, M. Lyle and R. Collier for their comments and constructive criticism of the manuscript, R. Conard for technical assistance, and N. Pisias' for help in computer-based data analysis techniques. This work was supported by NSF grant OCE-8026744.

Received 27 June; accepted 10 November 1983.

1. Piper, D. Z. *Geochim. cosmochim. Acta* **38,** 1007–1022 (1974).
2. Elderfield, H., Hawkesworth, C. J., Greaves, M. J. & Calvert, S. E. *Geochim. cosmochim. Acta* **45,** 513–528 (1981).
3. Elderfield, H. & Greaves, M. J. *Nature* **296,** 214–219 (1982).
4. Goldberg, E. D. *J. Geol.* **62,** 249–265 (1954).
5. Piper, D. Z. *Chem. Geol.* **17,** 285–301 (1974).
6. Goldberg, E. D., Koide, M., Schmitt, R. A. & Smith, R. H. *J. geophys. Res.* **68,** 4209–4217 (1963).
7. Høgdahl, O. T., Melson, S. & Bowen, V. T. *Adv. Chem. Ser.* **73,** 308–325 (1968).
8. Laul, J. C. *Atom. Energy Rev.* **17,** 603–695 (1979).
9. Dymond, J. *et al. Geochim. cosmochim. Acta* (in the press).
10. Calvert, S. E. & Price, N. B. *Mar. Chem.* **5,** 43–75 (1977).
11. Lyle, M. thesis, Oregon State Univ. (1978).
12. Elderfield, H. & Greaves, M. J. *Earth planet. Sci. Lett.* **55,** 163–170 (1981).
13. Fischer, K. thesis, Oregon State Univ. (1983).
14. Soutar, A., Kling, S. A., Crill, P. A., Duffrin, E. & Bruland, K. W. *Nature* **266,** 136–139 (1977).
15. Fischer, K., Dymond, J., Soutar, A. & Rau, S. *Geochim. cosmochim. Acta* (submitted).
16. Honjo, S., Spencer, D. W. & Farrington, J. W. *Science* **216,** 516–518 (1982).
17. Knauer, G. A. & Martin, J. M. in *Trace Metals in Seawater* (eds Wong, C. S. *et al.*) 447–465 (Plenum, New York, 1983).
18. Urrere, M. A. & Knauer, G. A. *J. Plankton Res.* **3,** 369–382 (1981).
19. Dymond, J. *Mem. geol. Soc. Am.* **154** (1981).
20. Høgdahl, O. *NATO Res. Grant 203, Prog. Rep.* **5,** 26 (1967).
21. deBaar, H. J. W., Bacon, M. P. & Brewer, P. G. *Nature* **31,** 324–327 (1983).
22. Klinkhammer, G. P. & Bender, M. L. *Earth planet. Sci. Lett.* **46,** 361–384 (1980).
23. Martin, J. H. & Knauer, G. A. *J. mar. Res.* **40,** 1213–1225 (1985).
24. Greenslate, J. *Mar. Min.* **1,** 125–148 (1977).
25. Moore, W. S. in *Inter-University Program of Research on Ferromanganese Deposits of the Ocean Floor, Phase I Report* (Seabed Assessment Program of IDOE, NSF, 1973).
26. Huh, C. A. thesis, Univ. Southern California (1982).
27. Finney, B., Heath, G. R. & Lyle, M. *Geochim. cosmochim. Acta* (submitted).
28. Kadko, D. C. thesis, Columbia Univ. (1981).
29. Kadko, D. & Heath, G. R. *J. geophys. Res.* (submitted).

Apparent changes in the climatic state of the deep North Atlantic Ocean

Dean Roemmich

Scripps Institution of Oceanography, La Jolla, California 92093, USA

Carl Wunsch

Center for Meteorology and Physical Oceanography, Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Determination of any long-term changes in the large-scale characteristics of the deep ocean circulation would be an important clue in understanding the climatic interactions of the ocean and atmosphere. In the summer of 1981, the RV *Atlantis II* reoccupied two transatlantic sections at nominal latitudes of 24°30′ N and 36°16′ N with a conductivity–temperature-depth instrument (CTD). One purpose of the work was to make a comparison with previous surveys conducted during the International Geophysical Year (IGY)[1], when sections were obtained in October 1957 and April–May 1959. We report here that significant warming occurred in an ocean-wide band from 700 m to 3,000 m with a maximum temperature difference of 0.2 °C. These changes are sufficient to expand the water column by several centimetres. The historical temperature–salinity curve was apparently unchanged. Interannual changes in local water mass characteristics have been proposed previously[2,3]. Perhaps it would be most surprising if no changes were seen to occur. What remains obscure is the significance of these changes and the extent to which they represent long-term climate trends, or merely the minor and random fluctuations to be expected in any complex fluid system.

To make a direct comparison of temperature in the two investigations, we have interpolated the data to common locations (excluding segments at the ends where the lines did not coincide). A fine mesh was generated (grid points over 40 m vertically and every 10 km horizontally) using the technique of objective mapping[4,5]. Subtraction of IGY temperatures from the 1981 temperatures gave temperature differences, but maps of temperature difference have strong mesoscale features that make it difficult to pick out large-scale trends. To suppress the small scales, a horizontal gaussian filter 1,000 km wide was applied to produce smoothed maps of temperature difference (Fig. 1*a* (24° N) and *b* (36° N)).

The most prominent feature of the temperature difference maps is an ocean-wide band of warmer water at middle depths of both latitudes. This band is much thicker and more intense in the western basin, where it extends from a depth of a few hundred metres to greater than 3,500 m. Relative to the IGY survey, mid-depth isotherms in the 1981 survey were lower overall and were tipped downward towards the west. Other strong features are seen in Fig. 1, such as the large area of negative temperature difference at 36° N, centred at about 500 m, just west of the mid-Atlantic ridge. However, these other features are not clearly similar at the two latitudes (they appear in different temperature ranges) and therefore cannot be said to be of ocean scale. Features high in the water column are likely to result from local processes while characteristics of the deep water are determined at remote source regions at high latitudes[6].

Figure 2 shows temperature difference averaged across the width of the ocean. The average warming begins at a depth of 500–700 m and extends to 3,000 m with a peak of about 0.2 °C at 1,000–1,500 m. By computing an average vertical temperature gradient, temperature difference was converted to an equivalent vertical displacement of isotherms (the vertical temperature gradient is much greater at 1,000 m than at 2,000 m). Vertical displacements were approximately 20 m and 100 m

0028-0836/84/050447-04$01.00

© 1984 Macmillan Journals Ltd

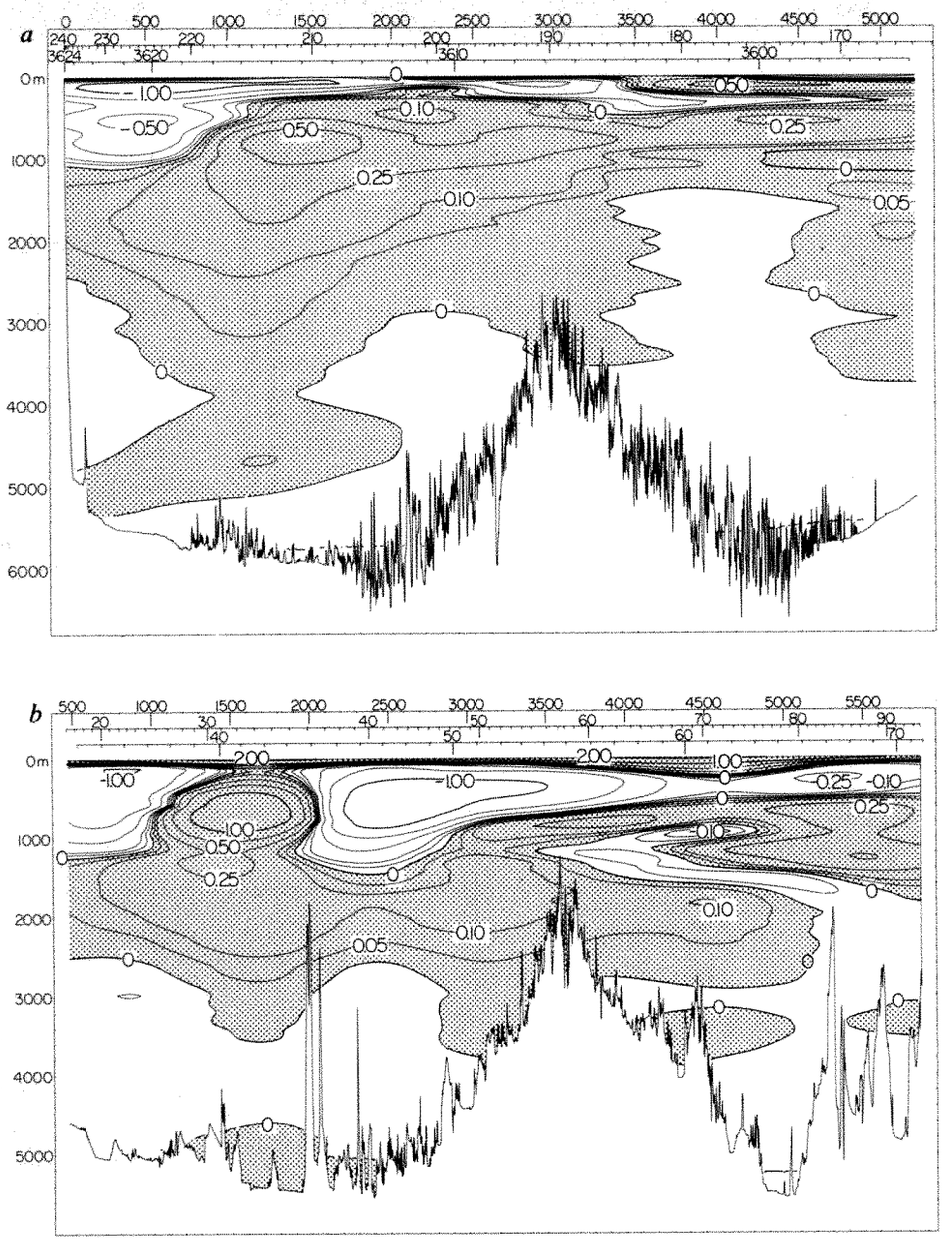


Fig. 1 *a*, Smoothed temperature difference section; IGY data subtracted from 1981 values, at 24° N. Distance scale at top in km, middle and bottom scales show 1981 and IGY station numbers respectively. *b*, Smoothed temperature difference, IGY data subtracted from 1981 values, at 36° N.

downward at 1,000 and 2,000 m respectively. The displacement at 2,000 m is large enough to be seen in a visual comparison of temperature maps from the 1981 and IGY sections, noting, for example, a drop of about 100 m in the 4 °C isotherm. Below 3,000 m, average temperature differences were small but tended to be negative. Above 700 m, a strong but thin band of negative difference is seen at 24° N at about 200 m and at 36° N a thicker band extends from 100 to 700 m. This upper-ocean cooling trend has been noted previously[7] at the Panuliris station (32° N, 64° W). At 24° N, the positive temperature difference at mid-depth dominates the vertical profile. Excluding the upper 100 m, where seasonal effects are important, the 1981 section at 24° N is 0.03 °C warmer than the IGY section. At 36° N, the positive differences are balanced by the negative ones and the total average temperature is nearly unchanged.

The tendency in salinity changes (not shown) was to maintain the historical temperature/salinity correlation; that is, positive temperature differences were usually accompanied by positive salinity differences. Because we observe greater vertical displacements in isotherms at some levels than at others, changes in the volumes occupied by different water masses must have occurred.

To study the expansion and contraction of water masses, the ocean was divided into six layers separated by surfaces of constant potential temperature. The six layers, chosen on the basis of earlier work[8], are shown in Table 1 with the area occupied by each of these water masses in each of the four sections. At both 24° N and 36° N there is an expansion in bottom water (<2 °C), a contraction in deep water (2–4 °C) and an expansion in middle and lower thermocline water (4–12 °C). Summing the change in deep and bottom water, the net contraction of water cooler than 4 °C lowers the isotherms of the middle and lower thermocline, consistent with the observation of warmer water at mid-depth.

In a discussion of error sources in the preceding calculations, measurement and mapping errors (negligible for present purposes) are included, as well as errors caused by the presence of short time scale eddies. The latter were found to be the largest error source, with a single intense eddy able to perturb the 1,000 m temperature by about 2 °C over 200 km, while the perturbation at 2,000 m is about 0.3 °C. Averaged over a 5,000 km basin, these estimates result in a bias of 0.08 °C at 1,000 m and 0.01 °C at 2,000 m. Because we observed the same pattern of change at both 24° and 36° N, and because the 0.1 °C

Table 1 Water mass census

Potential temperature class (°C)	Area (km²) 24° N 1957	Area (km²) 24° N 1981	Change 1981–1957	% Change	Area (km²) 36° N 1959	Area (km²) 36° N 1981	Change 1981–59	% Change
>17	1,751	1,753	2	0.1	936	998	62	6.6
12–17	1,451	1,502	51	3.5	2,124	1,890	−234	−11.0
7–12	1,716	1,778	62	3.6	3,108	3,277	169	5.4
4–7	4,008	4,238	230	5.7	3,413	3,748	335	9.8
2–4	12,795	12,107	−788	−6.2	11,425	10,870	−555	−4.9
<2	5,604	5,961	357	6.4	2,242	2,465	223	9.9

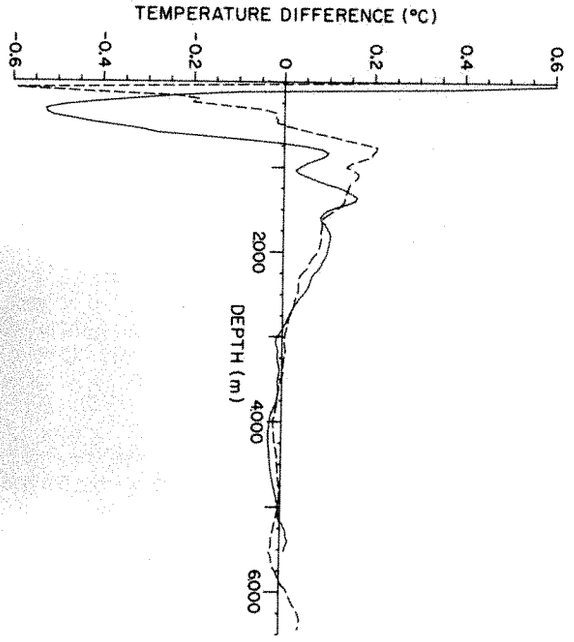


Fig. 2 Temperature difference, IGY data subtracted from 1981 values, averaged over the width of the ocean. Solid line, 36° N; dashed line, 24° N.

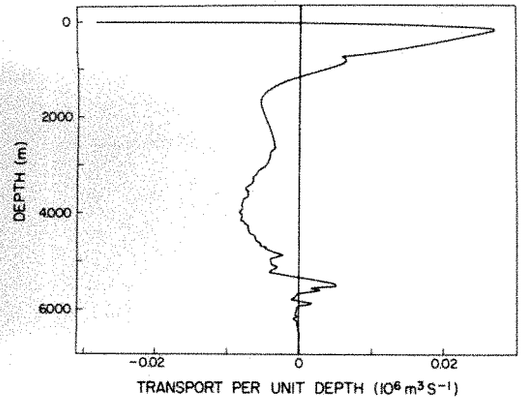


Fig. 3 Northward geostrophic transport per unit depth across 24° N (including the Straits of Florida).

signal at 2,000 m is much greater than the noise level, we see that the signal is not due to eddy noise. The only other error potentially of concern is in the depth inference; the IGY data depths are based upon reversing thermometers and those for the 1981 data upon a pressure sensor. The expected discrepancies can be shown to be less than about 10 m, well below the observed displacements.

The hydrographic station data can also be used to calculate geostrophic velocities and volume transports. Figure 3 shows horizontally-integrated transport per unit depth across 24° N (1981 data). Geostrophic shear together with mass conservation

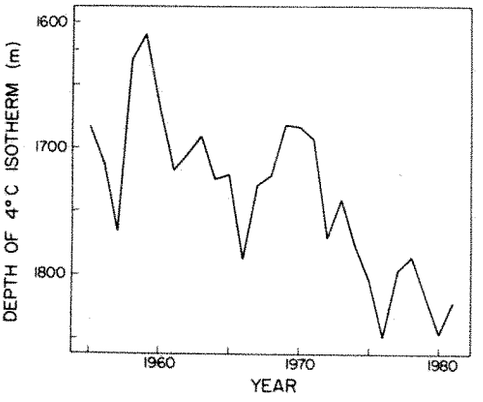


Fig. 4 Annual mean depth of 4 °C isotherm at the RV *Panuliris* station (32°10′ N, 64°30′ W).

constraints were used to estimate geostrophic velocity using an inverse method[9]. On the whole, surface and intermediate waters flow northward. Deep water flows southward in two lobes from its source regions in the Labrador and Greenland Seas and there is a small northward flow at the bottom. An interesting question is whether the observed water-mass shifts can be seen as the divergence of mass and/or heat transports. If the observed loss of mass in the 2–4 °C range was spread out evenly over the 23 yr between surveys, then a mass flux divergence of about 2×10^9 kg s^{-1} would be required. Relative to the transports of Fig. 3, which are of the order of 10^{10} kg s^{-1}, this value is too small to be detected reliably in geostrophic calculations. Similarly, the heat flux convergence required to add 0.1 °C to a layer 2,000 m thick is about 1×10^{13} W, which is only about 1% of the total northward heat flux across 24° N.

What is the proper time scale of the hydrographic changes we have described? Some deep water masses are thought to form only sporadically[3], with new formation being absent for years or longer. Are the differences we observe due to a 'sloshing' in the North Atlantic, measured in years, or are they part of a long term trend of expansion and contraction of water masses?

A simple north–south displacement of isotherms can be ruled out as the cause of the observed changes. The 4 °C isotherm depth is near its maximum value at 24° N. One can see this from meridional sections of temperature along 50° W and 66° W[1]. No north–south displacement could increase the 4 °C depth by 100 m, as was observed in 1981.

Unfortunately, there are very few long time-series of measurements with which to address the time scale problem. The Panuliris station has been occupied regularly to depths of about 2,000 m since 1955. We analysed these data by interpolating each station to locate the depth of the 4 °C isotherm and then averaging all stations in a year. The 4 °C isotherm was chosen because of its large observed displacement in our study. The annual means of 4 °C isotherm depth (Fig. 4) show a clear trend, increasing by the order of 100 m over 25 yr. This evidence supports the hypothesis of a long time scale in our observations. At temperatures warmer than 4 °C, up to the waters where a long-term cooling has been previously described[7], any possible

trends were obscured by eddy noise, which is large in this part of the Atlantic.

The present study is limited to the subtropical North Atlantic Ocean. Nevertheless, the questions that it raises are global. (1) Is the average temperature of the oceans increasing? Climate models which couple an atmosphere with a stratified ocean[10] show ocean warming in response to the global increase of atmospheric carbon dioxide, but warming of the model oceans is concentrated near the surface, in contrast to our results. (2) Can the global rise in sea level, reported to be 10 cm per century[11], be attributed to thermal expansion of the deep ocean? The thermal expansion coefficient of seawater is about 1.6×10^{-4} per °C at 5 °C, 35‰ in salinity, and 200 bar[12]. Thus, a column of water 1,000 m thick, heated by 0.1 °C gains nearly 2 cm. In the 1981 investigation, the water from 70 to 300 bar was thicker than in the IGY data by an average of 3 cm at 24° N and 2 cm at 36° N. Whether such changes occurred elsewhere in the world oceans is unknown.

CTD data were collected by the Woods Hole CTD group under the supervision of Dr M. McCartney. Captain Jorgensen and the crew of the RV *Atlantis* were most helpful. The work was supported by the NSF under grant OCE-80-185140 to the MIT and grant OCE-81-21262 to the University of California, San Diego.

Received 20 September; accepted 25 November 1983.

1. Fuglister, F. C. *Atlantic Ocean Atlas* (Woods Hole Oceanographic Institution, Massachusetts, 1960).
2. Jenkins, W. J. *J. mar. Res.* **40,** Suppl. 256–290 (1982).
3. Lazier, J. R. *Atmos. Ocean.* **18,** 227–238 (1980).
4. Bretherton, F. P., Davis, R. E. & Fandry, C. B. *Deep Sea Res.* **23,** 559–581 (1976).
5. Roemmich, D. *J. phys. Oceanogr.* **13,** 1544–1549 (1983).
6. Warren, B. A. in *Evolution of Physical Oceanography* (eds Warren, B. A. & Wunsch, C.) 6–41 (MIT Press, 1981).
7. Pocklington, R. *J. geophys. Res.* **77,** 6604–6607 (1972).
8. Worthington, L. V. *On the North Atlantic Circulation* (Johns Hopkins Press, Baltimore, 1976).
9. Wunsch, C. *Rev. Geophys. Space Phys.* **16,** 583–620 (1978).
10. Bryan, K., Komro, F. G., Manabe, S. & Spelman, M. J. *Science* **215,** 56–58 (1982).
11. Gornitz, V., Lebedeff, S. & Hansen, J. *Science* **215,** 1611–1614 (1982).
12. Sverdrup, H. U., Johnson, M. W. & Fleming, R. H. *The Oceans* (Prentice-Hall, Englewood Cliffs, 1942).

Suspended particulate organic material from hydrothermal vent waters at 21° N

Paul B. Comita & Robert B. Gagosian*

Department of Chemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

Peter M. Williams

Scripps Institution of Oceanography, University of California, La Jolla, California 92037, USA

Ocean floor spreading centres described recently consist of several sites with chemical and biological features unlike any oceanic sites previously known[1–4]. The high-pressure and warm-temperature regimes at these sites, coupled with dynamic hydrothermal water circulation, support biological communities that utilize a source of primary production which appears to be non-photosynthetic[5]. A detailed biochemical analysis of the suspended matter at a biologically-active site has been undertaken, with the goal of describing the relationship between microorganisms, benthic organisms and suspended particulate material within these hydrothermal systems. Suspended particulate matter from warm hydrothermal vent waters (5–38 °C) of the East Pacific Rise[1–4] (21° N, 106° W) was sampled on the OASIS expedition with an *in situ* pumping system in April 1982. Particulate organic carbon (POC) and nitrogen (PON) as well as individual organic compound classes from the lipid fraction were analysed to ascertain the sources and composition of the organic matter in this highly productive area. We show here that the particulate organic material (POM) of the vent water was both quantitatively and qualitatively different from the non-vent bottom water. The POC and PON measured within the vent and non-vent waters vary by a factor of 2–5, the higher values being in warm water. The organic matter in the non-vent water was found to be more resistant towards degradation or remineralization than in the vent water, as shown by the C:N ratio and the total lipid:POC ratio. Contributions to the lipid class compounds were assessed using well-known organic compound source biomarkers such as hydrocarbons, fatty alcohols, sterols, triacylglycerols and steryl and wax esters.

The hydrothermal vent sampling site was ~50 m^2, located adjacent to a collapsed lava lake. Twin peaks of 'black smoker' vents ($T \sim 270$ °C), a large 'white smoker' vent ($T \sim 10$–44 °C), and several other features were located in this area. Suspended particulate material from this site was sampled with an *in situ* pumping system. Four of the six filters were direct samples of warm (15–40 °C) hydrothermal vent water, and were from the following sites: (1) two from a hole located in the pillow basalt (dives 1212 and 1214) and (2) two from the base of a dense growth of vestimentiferan worms (*Riftia*) located adjacent to the white smoker (dives 1223 and 1220). One sample was of water intermediate in location and temperature between the warm vent water and non-vent bottom water (5 °C, above a bed of *Calyptogena magnifica*, dive 1212). The final sample was of non-vent bottom water at the vent site (2 °C, dive 1228). This sample will be referred to as the non-vent water sample. The *in situ* pumping system used to sample the particulate material consisted of a peristaltic pump, three solenoid valves, a polyethylene water bag, a five-in-one filter holder, a stainless steel single filter holder, and a computerized controller with associated electronics and power supplies. The pumping system was deployed from DSRV *Alvin* and sampled the hydrothermal vent water by directing the nozzle into a warm water source. Temperature readings of the water were made using a thermistor probe on *Alvin*. The vent water was pumped through Gelman type A/E glass fibre filters (GFF) at a rate of 1.6 l min^{-1}. The average sample size was ~150 l. The filters were previously combusted at 450 °C and Soxhlet extracted with 1:1 v/v toluene/methanol (MeOH) for 12 h. These filters retain >95% of particles of >1.0 μm diameter[6]. As previous studies indicate

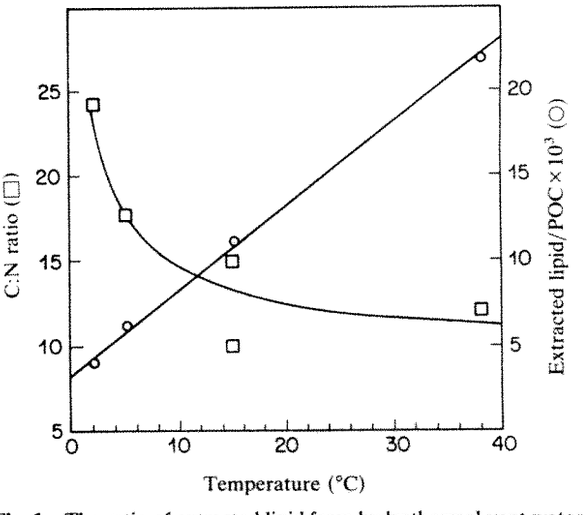


Fig. 1 The ratio of extracted lipid from hydrothermal vent water particulate material (μg l^{-1}) to the particulate organic carbon (μg l^{-1}) from the same sample, plotted as a function of water temperature, and the ratio of particulate organic carbon (μg l^{-1}) to particulate organic nitrogen (μg l^{-1}) plotted as a function of water temperature.

* To whom reprint requests should be addressed.

0028-0836/84/050450—04$01.00

© 1984 Macmillan Journals Ltd



nature is keeping ahead.

Are you being left behind?

New research results, the very latest international science news, key papers by the world's leading scientists and speed of publication ensure *Nature* remains one of the foremost weekly scientific journals in the world.

But if you're tied in to a circulation list at work, all this vital weekly information will undoubtedly reach you late, often incomplete and sometimes not at all.

With your own personal copy, you solve the problem. Every week you'll have all the latest research results and science news in your hands within days of publication:

News reports from throughout the world of science and technology.

A **News and Views** section, where new ideas are analysed and discussed by leading researchers.

Articles and Letters in which key investigators disclose their work for the first time.

Book Reviews – Eminent figures offer expert judgments on a wide variety of recent publications.

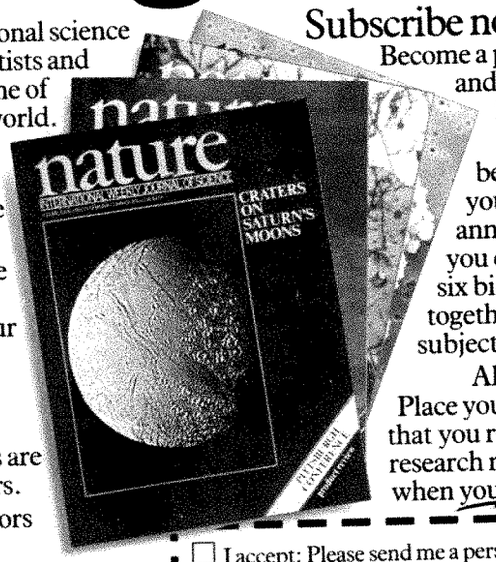
Special Issues providing an in-depth treatment of topics of importance.

Product Reviews – Detailed descriptions of laboratory equipment newly available.

Classified Advertisements for a wide variety of positions all over the world.

You will no longer be dependent upon other people passing down the 'list' copy, and because you will automatically receive every issue, you won't risk missing a single copy through absence from work. Moreover, you keep all this key information for permanent reference.

Through your door every week



Subscribe now – at Half Price

Become a personal subscriber now and you will receive a discount of 50% off the full rate.

All 51 weekly issues will be delivered to the address of your choice. You can start your annual subscription with any issue you choose. And you will receive six bi-monthly author indexes, together with the annual index of subject and author.

All this for just 94p a week.

Place your order now and make certain that you receive the scientific news and research results crucial to your work when you want them.

☐ I accept: Please send me a personal subscription to *Nature* for one year (51 issues) at the special half-price rate of £49.00.

☐ I enclose my personal cheque for £49.00 made payable to *Nature*. (Note: Institutional cheques cannot be accepted at this special rate).

☐ Please charge to my credit card account.
Access/American Express/Visa/Diners Club.

Account No._____

Card Expiry Date_____

Signature_____

Name_____

Address_____

_____

_____

_____

Date_____ 7B18

Please return this form to:
Circulation Manager
Nature
Brunel Road, Houndmills
Basingstoke
Hants RG21 2XS

This offer applies to UK and North America only. Please allow approximately 4 weeks for delivery of the first issue in your subscription year.

nature

Circle No.90 on Reader Service Card

MICRO '84

a meeting for scientists and technologists interested in a wide range of microscopical instrumentation and applications, will be held at the Bloomsbury Crest Hotel and the London School of Pharmacy from 9th-13th July, 1984.

ORGANISATION OF MICRO

Every two years, the Royal Microscopical Society holds its main scientific conference associated with a large trade exhibition. When drawing up the programme, the organisers have to bear in mind the multi-disciplinary needs of the fellowship, all of whom use microscopy as an essential tool. In order to achieve this, a lot of time is spent deciding on these areas where development is taking place most rapidly such as the introduction of new equipment or expansion of existing areas by development of new methodology. Parallel with this is the need to provide strong themes passing through the week to provide continuity for those scientists who wish to attend for the whole week.

INPUT FROM THE FELLOWSHIP

The Society's activities can be sub-divided into five main sections. These comprise electron microscopy, light microscopy, materials science, histochemistry and subjects of common interest such as the use of computers. The interests of the biologists as well as the material scientists are catered for in several of the areas mentioned above. Each section has a committee which runs the meetings and courses during the year and which also has a distinct input into the Micro programme. In this way, it is ensured that the whole spectrum of microscopical instrumentation and applications is considered.

CHOICE OF THE VENUE

The choice of the venue is inevitably a compromise between the needs of the participants to have a number of lecture theatres on the same site, easy to reach from most parts of the country and allowing free interchange between symposia. In addition, the choice of the Exhibition area has to be based on size, cost and again proximity to the lecture theatres to allow ready access. The timing of the programmes includes a long luinch break for visits to the Exhibition between the sessions. The Bloomsbury Crest Hotel has been used by the Society several times and is now well known to most of the delegates.

Circle No.17 on Reader Service Card.

INSTRUMENTATION

So what does Micro '84 have to offer to the participant? First and foremost, there is a good coverage of instrumentation including established and novel designs. For the electron microscopist there are several sessions including both SEM and TEM where the state of the art will be covered either as lectures or in the form of tutorials which are being introduced for the first time. This should encourage discussion on design, usage, testing performance and obtaining the best results. Data capture in the microscope and analysis are discussed by a number of experts, as are associated techniques such as X-ray analysis and EELS.

Other microscopical systems of interest are X-ray microscopy, laser doppler microscopy, acoustic microscopy, scanning optical microscopy and cryo-microscopy. X-ray microscopy is a rapidly developing field as is acoustic microscopy, both benefitting from the introduction of new and improved manufacturing methods and sources of X-rays such as the synchrotron. The Society has been fortunate to obtain speakers in these fields who have been foremost in pioneering the introduction of these novel systems.

COMPUTER-ASSISTED DATA HANDLING

The use of computers to assist the microscopist has grown rapidly as in other scientific fields and they are used either in association with collection of data within the microscope itself, as a data-storage component such as with X-ray microanalysis or as a means of analysing such data either collected directly or after taking data from micrographs to give quantitated results. All of these systems have been used both to speed up data collection and analysis and to obtain more meaningful results from the data so analysed.

PRACTICAL APPLICATION

Whereas a proportion of the people attending Micro '84 will be interested in developments in instrumentation, another significant population will be scientists wishing to refresh their ideas as to the many applications of microscopy. As this covers a wide range of knowledge and background, some broadly based lectures and complete sessions have been included such as methods for handling specimens, or the two days devoted to immunocytochemistry, a vital tool for the immunologist. Industrial users have been considered whether they are interested in semiconductors, crystallography or pharmaceutical applications. The academic scientist, on the other hand, will find many lectures pertinent to a wide range of subjects.

THE IMPORTANCE OF THE EXHIBITION . . . HELD IN ASSOCIATION WITH NATURE

All in all, this Micro promises to be of a high scientific standard and wide interest. To a large extent this is due not only to the enthusiasm and interest of the scientists responsible for putting together the programme and 'persuading' the experts to come and share their knowledge, but also the very loyal support and help of the Trade Committee which helps to co-ordinate what is the world's most comprehensive exhibition of microscopes and accessory equipment. As a result, the Society can direct any would-be user or buyer to the appropriate group of manufacturers; in return the income from the exhibitors' fees (there is no entrance fee) assists speakers to travel from all over the world to produce the best programme possible for the Society and its supporters.

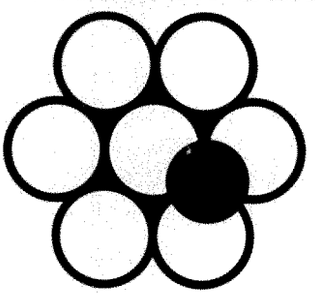
Further details available from:

The Administrator,
The Royal Microscopical Society,
37-38 St Clement's,
Oxford OX4 1AJ

The British Journal of Cancer

Published monthly on behalf of the

Cancer Research Campaign



Editor: M. Moore, Paterson Laboratories, Manchester
Chairman of Editorial Board: D.G. Harnden

THE BRITISH JOURNAL OF CANCER is one of the leading cancer journals in the world, publishing articles in all the major scientific disciplines relevant to the subject.

* **papers and short communications submitted by scientists throughout the world**

* **proceedings of the meetings of the British Association for Cancer Research regularly published**

* **other selected national and international meetings also covered**

* **up-to-date book reviews in each issue**

Please subscribe to:
David R. Guthrie
Scientific and Medical Division
The Macmillan Press Ltd., Houndmills,
Basingstoke, Hampshire RG21 2XS, UK.

Subscription rates:
£99 UK and Eire;
$240 (Airmail $285) USA and Canada;
£124 (Airmail £160) Rest of World;

☐ Please enter my subcription to the **British Journal of Cancer** commencing with the _____ (month) issue.

☐ I enclose a cheque for _____ made payable to *Macmillan Journals Ltd.*

☐ I authorise you to charge _____
to my VISA/ACCESS/AMERICAN EXPRESS
(delete as approriate)
Credit Card Account No

Expiry date _____ Signature _____

☐ Please send me a sample copy of the **British Journal of Cancer**

Name _____
Address _____

Postal/zip code _____

SCIENTIFIC & MEDICAL | M | MACMILLAN PRESS LTD

Table 1 *In situ* pump sample description and bulk chemical properties

Dive no.	1228	1221	1212	1214	1223	1220
Description site	Pillow basalt	Clam bed	Hole in basalt	Hole in basalt	Base of white smoker	Base of white smoker
Water temperature (°C)	2	5	12–20	12–20	~38	—
Volume filtered (l)	160	164	—	157	158	34
POC ($\mu g\,C\,l^{-1}$)	91.1	139	194	218	163	219
PON ($\mu g\,N\,l^{-1}$)	4.4	9.2	15	25	16	22
C:N (atom)*	24.2	17.6	15.1	10.2	11.9	11.6
Total lipid extract (TLE) ($\mu g\,l^{-1}$)	0.37	0.85	—	2.4	3.6	11.5
TLE/POC × 10^3	4.1	6.1	—	11.1	22.1	52.5
Filter description	Few macroscopic particles	Some macroscopic particles	—	24 copepods, few particles	30 large amphipods, many particles	Densely covered with inorganic sulphide particles

* C/N (weight) 1.167 = C/N (atom).

that >60% of the ATP biomass resides on particles >12 μm in hydrothermal vent waters[5], the filters should sample a large fraction of the particulate organic material from this source.

Particulate organic carbon and nitrogen were analysed by filtering several litres of the vent water (collected in polyethylene bags) through a stainless steel filter holder containing two 25-mm Gelman type A/E GFFs which had been previously combusted at 500 °C. The POC and PON determinations were accomplished using a CHN analyser.

The particulate material was also analysed for lipid class compounds using methods previously described[7]. The filters were weighed and ~25% of each was sonicated and extracted with methylene chloride (CH_2Cl_2)/MeOH/H_2O. The lipid extract was then partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 extract was evaporated to dryness under vacuum at <25 °C. Hexane was then added and elemental sulphur was removed on a short column of activated copper particles (105–250 μm diameter)[8]. The sulphur-free extracts were separated into individual compound classes by adsorption chromatography. Elution with a series of solvents of increasing polarity separated the lipids into alkane and alkene, polyunsaturated alkene, steryl and wax ester, triacylglycerol, fatty alcohol, and steroid alcohol fractions. The alcohols were converted to their respective acetates with acetic anhydride in pyridine. The individual fractions were spiked with appropriate external standards for quantitative determinations and analysed by high resolution glass capillary gas chromatography (GC) using a deactivated, cross-linked SE-52 coated (25.5 m × 0.32 mm i.d.) column. Compound structures were identified by comparison of GC retention times (alkanes, wax esters, and triacylglycerols) and mass spectra with authentic standards (fatty alcohols and steroid alcohols)[9]. Procedural blanks performed concurrently with these analyses indicated <1% contamination in each fraction.

The bulk chemical properties from the *in situ* pump samples are listed in Table 1. Water temperature roughly increased from the pillow basalt sample (dive 1228) of 2 °C non-vent water at the vent site, to 5 °C for the sample collected above the clam bed (dive 1221), to 15–38 °C for the directly sampled warm vent waters. The concentration of both POC and PON increased with increasing water temperature, but not in the same proportion. The total lipid extract (TLE) also increased with increasing temperature (Table 1). Similarly, the ratio of (TLE:POC) × 10^3 ranged from a value of 4 in the non-vent bottom water to values of 11–50 in the hydrothermal vent waters (Fig. 1). A comparison of these POC and PON values with other values found in the world's ocean is informative. Western North Atlantic surface coastal POC values are ~100 $\mu g\,l^{-1}$, whereas open ocean POC values are ~50 $\mu g\,l^{-1}$ (ref. 9). Wangersky found POC values for surface waters (0–100 m) of 54 $\mu g\,l^{-1}$ for the South Atlantic and 30 $\mu g\,l^{-1}$ for the Pacific Ocean[10]. At depths >3,000 m the POC value was ~5 $\mu g\,l^{-1}$ (refs 10, 11) for both the South Atlantic and Pacific Oceans. In the central North Pacific gyre, POC values range from 2 to 4 $\mu g\,l^{-1}$ (ref. 11). Off the coast of Peru, a productive upwelling site, POC values range from 100 to 700 $\mu g\,l^{-1}$ in surface waters[12]. The values for warm vent water POC are in the region of 200 $\mu g\,l^{-1}$ indicative of a relatively productive site.

The C:N ratio ranged from a value of 24.3 for non-vent bottom water to values of 10–15 in the warm vent water samples (Fig. 1). The values for the vent water C:N ratio are higher than expected for normal marine bacteria or phytoplankton (~5–6)[13], but similar to deep sea suspended POM. The C:N ratio for hydrothermal vent organisms, however, is not known. Values for dissolved organic carbon (DOC) for the warm vent water samples range from 0.64 to 0.85 $mg\,C\,l^{-1}$, somewhat lower than non-vent water (1.01 $mg\,C\,l^{-1}$).

Each filter analysed for lipids was qualitatively different. In general, the samples collected from lower temperature water contained less macroscopic particular material than samples from higher temperature water. The three warm vent water filters were each qualitatively different from one another. Two had numerous large zooplankton, copepods (Spinocalanidae new genus and species[14]) on filter 1214, and amphipods (genus *Onesimus* sp. (C. Ingram and R. Hessler, personal communication) on filter 1223. These large zooplankton were removed before analysis, as their lipids would dominate those present in the particulate material, masking any potential information in the particulate matter lipid fingerprint. Microscopic zooplankton and parts of zooplankton may still have been present on the filter when analysed, however, and may have contributed to the organic material on the filters. The filter with the highest amount of lipid had no large zooplankton or microscopic organisms when viewed with a ×50 magnification dissecting microscope. This filter had a relatively small amount of water pumped through it apparently because it became plugged due to a large amount of inorganic particulate material.

Five individual compound classes are listed in Table 2 for the five filters analysed for lipids. In general, the lipid fraction of vent samples was dominated by sterols, triacylglycerols, and wax esters (>90% average). The concentrations of all lipid classes increased as the temperature of the water sampled increased. The ratio of an individual compound class to POC was lower in the non-vent water than in the warm vent water (see dive 1228, Table 2), as was noted previously for the ratios of total lipids to POC.

The aliphatic hydrocarbons make up, on average, <3% of the total lipid classes analysed. The major components typically centred around C_{19} or C_{20} (Table 2). The *n*-alkanes dominate this compound class without any marked odd/even carbon predominance. A large unresolved complex mixture indicative of weathered organic material was present in every sample[15]. There

Table 2 Particulate organic compound classes

	Dive no.	1228	1221	1214	1223	1220
Aliphatic hydrocarbons						
C_{18}–C_{38} (ng l^{-1})		1.40	3.32	5.09	1.14	89.5
CPI*		1.1	1.1	1.0	1.1	1.0
Total/POC($\times 10^3$)		15.4	23.9	23.3	7.02	408.4
Major components†		25, 21, 22	19, 20, 21	19, 20, 29	19, 20, 18	19, 25, 20
Fatty alcohols						
C_{12}–C_{30} (ng l^{-1})		3.04	12.1	38.5	7.7	57.6
Total/POC($\times 10^3$)		33.4	86.8	176.3	47.4	263.1
Major components†		18, 16	16, 18:1	18:1, 16	18, 16	18:1, 16
Steryl and wax esters						
C_{30}–C_{44} total (ng l^{-1})		3.17	182.9	375.2	12.0	332.7
Total/POC($\times 10^3$)		34.8	1,242	1,719	73.8	151.9
Major components†		34:1, 32:1	40:1, 34:1	34:1, 32	34:1	34:1, 32
Triacylglycerols						
C_{42}–C_{56} total (ng l^{-1})		5.34	93.5	500.8	33.8	1,263
Total/POC($\times 10^3$)		58.6	672.0	2,294	208.4	5,767
Major components†		50, 52	50	50, 52	50, 52	50, 52
Sterols						
Total (ng l^{-1})		6.77	23.5	70.0	205.3	1,703
Total/POC($\times 10^3$)		74.3	168.7	320.5	1,264	7,775
Major components‡		1, 3	1, 2	1, 2	1, 3	1, 2

* CPI, carbon preference index. Odd/even for aliphatic hydrocarbons.

† The numbers indicate carbon number in order of decreasing abundance. Where the numbers are in the form $x:y$, x = number of carbons and y = number of double bonds.

‡ 1 is cholest-5-en-3β-ol, 2 is cholesta-5,24-dien-3β-ol, and 3 is 24-methylcholesta-5,24(28)-dien-3β-ol.

appears to be no clear distinction between the *n*-alkane distribution in the non-vent and vent water samples that would allow a distinction to be made about the source of the alkanes. The large amounts of aromatic hydrocarbons found in sediments at Guaymas Basin (23–32% of lipid extract)[16] were not present in the particulate material from warm vent waters at 21 °N. This observation, and the large amounts of what appear to be locally biosynthesized lipid compounds (see below), indicate little rapid thermal alteration of organic matter on the suspended particulate material at this vent site. We do not mean to imply that thermal alteration of organic matter does not occur at the 21 °N hydrothermal vents. However, the contribution of thermally altered organic compounds (in the form of aliphatic and polycyclic aromatic hydrocarbons) to the suspended particulate material is in fact very small. Markers for microbial activity in this compound class, such as hopanes[17], isoprenoid hydrocarbons[18], or sterenes[19], were also not present within our limits of detectability.

Free fatty alcohols also make a relatively small contribution to the total lipid (5% average), and were dominated by typical marine biomarker C_{16}, C_{18}, and $C_{18:1}$ alcohols (>74% average)[20]. The minor components were typically C_{14}, $C_{16:1}$, $C_{20:1}$ and C_{24} and on average comprised 12% of the total fatty alcohols. The free fatty alcohols exhibited a high even-to-odd carbon preference (>50).

Both the wax ester and triacylglycerol compound classes make up a large portion of the lipid fraction of organic material (average 56%). The major contribution to the wax ester fraction, $C_{34:1}$ and C_{32}, are typical marine zooplankton wax esters[21]. The high level of these compounds is not surprising considering the large number of macroscopic zooplankton collected from the warm vent water samples. These high values may reflect zooplankton input to the POM or may simply be parts of macroscopic zooplankton or microscopic zooplankton present on the filters that were not picked off. We are unable to distinguish between these two possibilities with our methodology. The concentration of wax esters in open ocean particulate matter, determined with the Woods Hole *In Situ* Pump (WHISP), is 14 ng l^{-1} (S. G. Wakeham, personal communication) for surface waters from 18° N, 108° W, a moderately productive environment. By comparison, the average of the three vent water values was 240 ng l^{-1}; the non-vent water sample was ~3 ng l^{-1}. Clearly, the vent particulate matter samples contain over an order of magnitude higher concentration of wax esters than moderately productive surface waters. The warm vent water samples contained both wax and steryl esters, whereas the non-vent and intermediate water filters 1228 and 1221 contained only wax esters, suggesting a warm water source for the steryl esters. This source may be a different species of organism contributing to POM sampled in the warm vent waters.

The steroid alcohols also comprised a major fraction of the total lipid (average 35%). The vent waters were dominated by three sterols (96% total sterols), cholest-5-en-3β-ol (cholesterol), cholesta-5,24-dien-3β-ol (desmosterol), and 24-methylcholesta-5,24(28)-dien-3β-ol (24-methylenecholesterol). Non-vent water samples contained mainly cholesterol, typical of other oceanic bottom waters[22]. The simple distribution of sterols in the vent water samples is not typical of seawater samples previously studied[9,22]. Pure cultures or single species of organisms usually biosynthesize several major sterols[23]. The simple pattern found for the vent samples most probably indicates a select number of single sterol sources. The higher concentration of sterols in warm water relative to non-vent water samples may indicate either an organism source solely living in the warm water or a gradation of productivity between vent and non-vent water. The distribution of the vent water sterols is very similar to that found for molluscs[24], suggesting that the clam *Calyptogena magnifica* may be the source. The sterols of *Calyptogena magnifica* are cholesterol, cholestanol and three minor sterols (not identified). This distribution clearly does not match that of the POM, but the sterol composition of the vestimentiferan worm matches extremely well. The major three sterols of the vestimentiferan worm are cholesterol, desmosterol, and 24-methylenecholesterol. 24-methylcholesta-5,22E-dien-3β-ol and 24-ethylcholesta-5,24(28)Z-dien-3β-ol, sterols known to have a phytoplankton source, were detected in extremely small amounts relative to other sterols.

The initial results from our studies have uncovered several interesting features. All of the vent water samples that we have analysed showed an enrichment of POM relative to non-vent bottom waters. This organic material was both quantitatively and qualitatively different between the non-vent water and vent water, that is the C:N ratio decreased from non-vent to vent

water indicating that the organic material in the non-vent water is more resistant, and represents the less-labile, carbon-rich fraction of the vent POM. The ratio of total lipid/POC also confirmed this conclusion. The lipid class compounds, one of the more labile forms of organic matter, make a larger contribution to the vent water organic carbon than to the non-vent water organic carbon.

The change in concentration of particulate organic matter at this site appears to be a very localized event. In this investigation all samples, including the non-vent sample of bottom water, were taken within an area that was actively emitting warm water and sustaining a large population of benthic organisms. The POC and PON levels measured within the warm waters and in the non-vent water at the vent site vary, in general, by at least a factor of 2–5, the higher values being in warm water. Observations from *Alvin* indicate that the vent organism at 21 °N live in or extremely near to a source of warm vent water, a feature common to other vent sites as well. Our analyses reflect this phenomenon and indicate that primary sources for organic carbon are localized in or near the warm vent water site[25].

The relative contribution to the POM by microbial versus animal carbon sources may be partially assessed by using our data on the organic compound source biomarkers. The wax esters, triacylglycerols, and sterols dominate the lipid fraction. The wax ester, triacyglycerol, and fatty alcohol compound classes that were present in the suspended particulate material are typical of marine zooplankton. Indeed, many amphipods and copepods were found in vent waters and collected in our *in situ* pump filters. The sterol distribution on the particulate material matches that of the *Riftia*. The microbial component of the lipid fraction cannot be completely resolved with our data set. Microbially produced lipids, such as hopanols and isoprenoid hydrocarbons, were not found on the filters. Fatty acids, and the more polar phospho- and glycolipids are in the process of being analysed, and these compound classes may contain a large fraction of microbial source markers. Another interesting feature of these analyses is that the typical markers for microbial transformation of sterols such as sterenes, stanones, or stanols, were not present within the limits of our detectability. Thus, we would conclude on the basis of this data set that the zooplankton and larger benthic organisms appear to be making the major contribution to the suspended particulate organic matter in the warm vent waters at 21 °N.

Further verification of both microbial and larger benthic organism contributions and analyses of other source markers will be needed to confirm these initial conclusions from the filtered vent water. Analyses of sediments, bacterial isolates[26], and larger organisms from 21° N are underway to ascertain more specifically the quantitative contributions of sources to the vent water organic matter.

We thank the NSF for support (OCE 80-24256) as part of the OASIS project. General support for the OASIS expedition was through NSF grant OCE 80-24895 to K. L. Smith. We gratefully acknowledge the assistance of expedition leader K. L. Smith, and R. Baldwin, G. Niles, and chief scientist of RV *New Horizon*, G. Somero. We also thank K. Doherty and H. Jannasch for design and construction of the *in situ* pumps; G. Nigrelli, K. Doherty, D. Nelson, and C. Wirsen for assistance with collecting samples; S. Wakeham, E. Peltzer, N. Frew and K. Robertson for analytical assistance; R. Hessler for communicating results before publication; and the captains and crews of the RV *New Horizon*, RV *Lulu*, DSRV *Alvin*, and RV *Melville*. J. Farrington, C. Lee, S. Wakeham, E. Peltzer, and C. Taylor made constructive comments to the manuscript. P.B.C. acknowledges a National Research Service Award in the form of a postdoctoral fellowship from NIH and a postdoctoral scholarship from Woods Hole Oceanographic Institution (1982–83). Woods Hole Oceanographic Institution contribution no. 5446.

Received 2 August; accepted 2 November 1983.

1. Edmond, J. M. *Nature* **290**, 87–88 (1981).
2. Ballard, R. D., Francheteau, J., Juteau, T., Rangan, C. & Normark, W. *Earth planet. Sci. Lett.* **55**, 1–10 (1981).
3. CYAMEX Scientific Team, *Nature* **277**, 523–528 (1979).
4. Rise Project Group *Science* **207**, 1421–1433 (1980).
5. Karl, D. M., Wirsen, C. O. & Jannasch, H. W. *Science* **207**, 1345–1347 (1980).
6. *Gelman Sciences Laboratory Filtration Catalog* PB-600, 21 (1982).
7. Peltzer, E. T., Alford, J. B. & Gagosian, R. B. *Sampling, Preparation, and Quantitative Identification of Lipids in Remote Marine Aerosols* (Woods Hole Ocean. Inst. Tech. Rep., in the press).
8. Blumer, M. *Analyt. Chem.* **29**, 1039–1041 (1957).
9. Gagosian, R. B. & Nigrelli, G. E. *Limnol. Oceanogr.* **24**, 838–849 (1979).
10. Wangersky, P. J. *Deep-Sea Res.* **23**, 457–465 (1976).
11. Williams, P. M., Carlucci, A. F. & Olson, R. *Oceanol. Acta* **3**, 471–476 (1980).
12. Gagosian, R. B., Loder, T., Nigrelli, G. & Love, J. *Woods Hole Oceanogr. Inst. Tech. Rep.* 83–5 (1983).
13. Parsons, T. R. in *Chemical Oceanography* Vol. 2, 365–383 (Academic, New York, 1976).
14. Fleminger, A. *Proc. Biol. Soc. Wash.* (in the press).
15. Farrington, J. W. & Meyers, P. A. in *Environmental Chemistry* Vol. 1 (ed. Eglinton, G.) 109–136 (The Universities Press, Belfast, 1975).
16. Simoneit, B. R. T. & Lonsdale, P. F. *Nature* **295**, 198–202 (1982).
17. Forster, H. J., Biemann, K., Tattrie, N. H. & Colvin, J. R. *Biochem. J.* **135**, 133–143 (1973).
18. Brassell, S. C., Wardroper, A. M. K., Thompson, I. D., Maxwell, J. R. & Eglinton, G. *Nature* **290**, 693–696 (1981).
19. Gagosian, R. B. & Farrington, J. W. *Geochim. cosmochim. Acta* **42**, 1091–1101 (1978).
20. Sargent, J. R., Lee, R. F. & Nevenzel, J. C. in *Chemistry and Biochemistry of Plant Waxes* (ed. Kolattukudy, P. E.) 50–93 (Elsevier, New York, 1976).
21. Wakeham, S. G. *Geochim. cosmochim. Acta* **46**, 2239–2257 (1982).
22. Gagosian, R. B. *Geochim. cosmochim. Acta* **39**, 1443–1454 (1975).
23. Nes, W. R. & McKean, M. L. *Biochemistry of Steroids and Other Isopentenoids* Ch. 10 (University Park Press, Baltimore, 1977).
24. Idler, D. R., Khalil, M. W., Brooks, C. J. W., Edmonds, C. G. & Gilbert, J. D. *Comp. Biochem. Physiol.* **59B**, 163–167 (1978).
25. Williams, P. M., Smith, K. L., Druffel, E. M., & Linick, T. W. *Nature* **292**, 448–449 (1981).
26. Comita, P. B. & Gagosian, R. B. *Science* **222**, 1329–1331 (1983).

Experimental manipulation of a contact guidance system in amphibian gastrulation by mechanical tension

Norio Nakatsuji* & Kurt E. Johnson

Department of Anatomy, The George Washington University Medical Center, Washington DC, 20037, USA

Contact guidance[1,2] has been implied in various morphogenetic movements including neural crest cell migration[3], primordial germ cell migration[4] and guidance of axonal growth cone[5]. In urodele gastrulae, we reported[6] the presence of an aligned network of extracellular fibrils on the inside of the ectodermal layer and suggested that it guides the migration of the presumptive mesodermal cells from the blastopore towards the animal pole. We also reported[7] *in vitro* experiments in which the fibril network of the ectodermal layer was transferred onto the surface of a coverslip. Dissociated mesodermal cells attach to and locomote actively on such conditioned surfaces in an oriented fashion along the blastopore–animal pole axis (bp–ap axis) of the ectodermal layer that conditioned the surface. Recent reports[8,18] suggest that these fibrils contain fibronectin. We now report that the fibril network on the conditioned surface can be artificially aligned in any orientation by exerting mechanical tension on the ectodermal layer during the conditioning. Such prepared surfaces cause cell movements aligned along the tension axis, even when the tension axis is perpendicular to the natural axis of alignment along the bp–ap axis. These results suggest that the extracellular matrix fibrils aligned by the mechanical stress that arises in embryos during development can orient cell migration by the contact guidance, in a similar manner to that reported in the collagen gel and fibroblasts system[9,10].

We used *Ambystoma maculatum* eggs collected in North Carolina and Virginia. Developmental stages were determined according to Harrison stages[11]. Stage 10– represents very early gastrulae in which the mesodermal cells have not yet started their migration away from the blastopore. In more advanced early gastrulae (stage 10), cell migration is under way. Our previous study[7] showed that conditioning by stage 10 ectodermal layer causes substantial alignment of mesodermal cell movement, while the conditioning by a stage 10– ectodermal layer

* Present address: MRC Mammalian Development Unit, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK.

0028-0836/84/050453—03$01.00

© 1984 Macmillan Journals Ltd

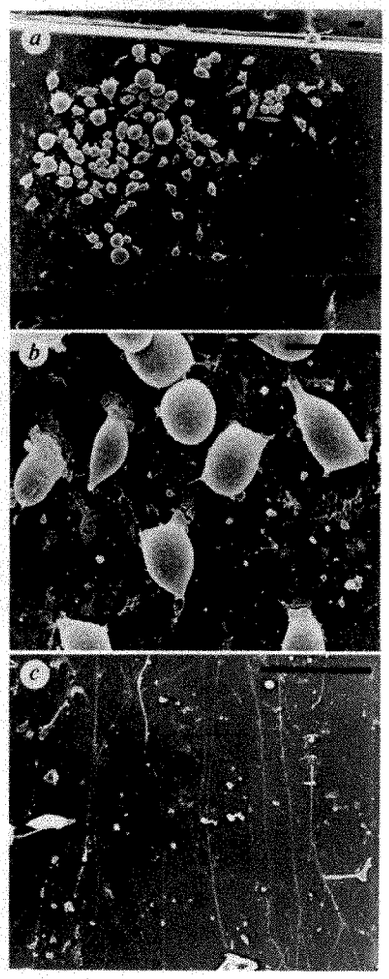


Fig. 1 Scanning electron micrographs of mesodermal cells attached to the conditioned coverslip strip. *a*, A low magnification view. Scale bar, 0.1 mm. *b*, Central part of *a* at higher magnification. Scale bar, 100 μm. *c*, Extracellular fibrils deposited on the coverslip surface. They are apparently aligned along the axis of tension (vertical axis). Scale bar, 10 μm. Strips were cut out from plastic coverslips for tissue culture (Thermanox coverslips, Miles) with a sharp steel blade. The composition of media used in this study is the same as used in the previous conditioning experiments[7]. The basic salt solution is a modified Stearns' solution (MSS)[15,16] buffered with 5 mM HEPES (Sigma). Mesodermal cells were dissociated in 0.02 M sodium citrate[17] in MSS lacking Ca^{2+} and Mg^{2+}. For conditioning of substrata with explants of the ectodermal layer, we used MSS with a *p*H of 8.0, a Ca^{2+} concentration of 110 μM, and containing 0.5% bovine serum albumin (BSA) (Sigma). This Ca^{2+} concentration is just high enough to prevent tissue dissociation and yet low enough to prevent curling of the ectodermal fragments, thus it allows the ectodermal fragments to adhere to the substratum and remain flat. The dissociated mesodermal cells were cultured in MSS *p*H 8.0, a Ca^{2+} concentration of 100 μM, and containing 0.5% BSA, which we have found to be optimal for cell locomotion.

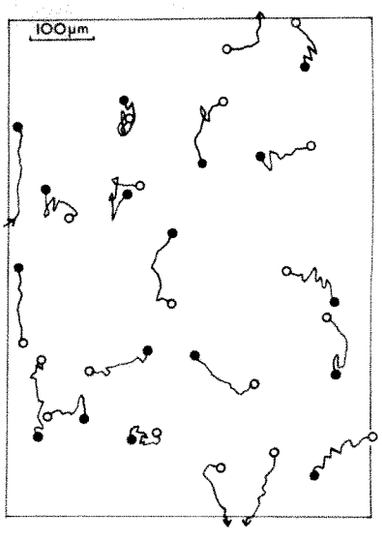


Fig. 2 Cell trails drawn from a time-lapse film of the tilted conditioning. ○, Starting points; ●, finishing points after 1 h. They are apparently aligned along the axis of tension (vertical axis). The overall *R* value calculated for all the cell trails here is +0.47. Time-lapse 16-mm films were taken with ×10 phase contrast lens at 8-s intervals. At the end of filming, the coverslip was fixed and processed for scanning electron microscopy[7]. We drew cell trails by projecting the time-lapse films and marking the centre of cell body at 4-min intervals[15].

gives only very weak alignment. This difference may be caused by the alignment of the fibril network by the expansion and stretching of the ectodermal layer along the bp–ap axis during gastrulation[12].

Rectangular pieces of the ectodermal cell layer were cut out from the whole blastocoelic roof of early gastrulae (stage 10−, or 10) using fine forceps and hair loops. The pieces were explanted on the coverslip strips (2 mm × 10 mm), which were set on top of two small fragments of glass capillary (outer diameter, 2 mm) like a bridge, so that the explant could be hung with both ends drooping free from the edges of the strip, thus producing constant mechanical tension perpendicular to the longer axis of the strip of coverslip. The inner surface of the ectodermal layer adhered to the coverslip surface. The strip of coverslip had been set horizontally or tilted by approximately 30° by applying a small amount of Vaseline between the glass capillary and the strip. The tilting allowed the mechanical tension to be maintained throughout the explant during the conditioning. In the horizontal setting, both edges of the coverslip held the ectodermal layer tightly and prevented the tension from transmitting to the central part of the explant. On the other hand, with tilting, there was one free edge so that the tension and stretching generated by the weight of the explant could be transmitted across the entire sheet. Explants from stage 10 embryos were placed on the coverslip with their bp–ap axis parallel to the longer axis of the strip. Explants from stage 10− embryos were oriented randomly, because they have not yet developed their natural alignment along the bp–ap axis[7].

After 5 h of culture at room temperature (22–24 °C), explants were removed from the coverslip by flushing them away with a Pasteur pipette. The coverslip strips were rinsed with culture medium, and placed horizontally in a plastic Petri dish. They were seeded with dissociated mesodermal cells from middle gastrulae (stage 10+) using a Pasteur pipette. The cells attach to the conditioned surface and start to migrate actively within 30 min. The mesodermal cells show no attachment outside the conditioned area. Figure 1*a* shows a low magnification view of a coverslip strip and attached cells. The cells frequently have a leading end with a large lamellipodium and associated filopodia and a slender or rounded trailing end (Fig. 1*b*). The conditioned surface is covered with fragments of cell-surface structures such as filopodia and a network of extracellular fibrils that can be observed only at higher magnifications (Fig. 1*c*). The network was apparently aligned along the axis of tension (Fig. 1*c*). In fact, when scanning electron micrographs of 10 randomly selected areas from the tilted conditioning were analysed by the microcomputer system (see below), they gave positive *R* values; mean and standard deviation were $+0.74 \pm 0.52$.

Cell trails from the tilted conditioning showed an apparent alignment along the axis of tension (Fig. 2). No alignment was noticeable when we used the horizontal conditioning. We further analysed the cell trails by using an image-analysing system with a microcomputer (Apple II Plus Computer and Graphics Tablet)[7]. The computer programs[7] we used enable us to determine

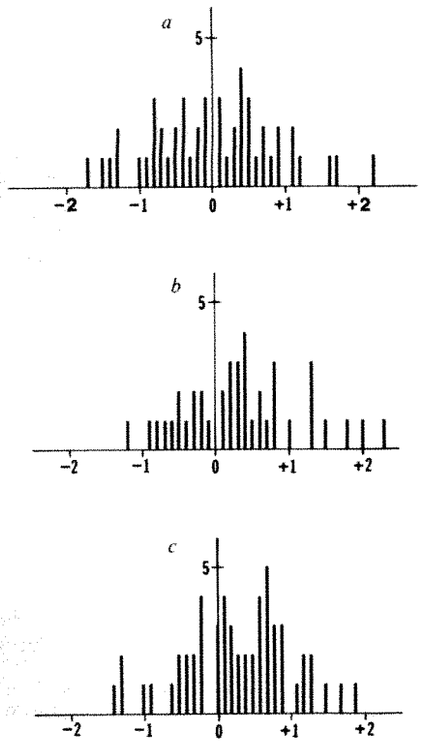


Fig. 3 Histograms showing distribution of cell trails according to their R values. Horizontal axis, R value; vertical axis, number of cell trails. *a*, Data from the horizontal conditioning, showing nearly normal distribution centred at zero (mean −0.01 and s.d. ±0.90, 52 cells). *b*, Tilted conditioning using the stage 10− ectodermal layer. The distribution is clearly shifted to the right (mean +0.36, s.d. ±0.78, 40 cells). The shift of mean is significant ($P < 0.005$). *c*, Tilted conditioning using the stage 10 ectodermal layer. The shift to the right (mean +0.29, s.d. ±0.73, 55 cells) is significant ($P < 0.005$).

whether the cell trails are aligned along the axis of the mechanical tension during the conditioning. Also, they were used to analyse alignment of the fibril network deposited on the conditioned surface. The programs calculate displacement (Δy and Δx) along the y-axis (axis of tension) and along the perpendicular x-axis in each short segment of the cell trail, and give sums of $|\Delta y|$ and $|\Delta x|$ separately for the whole cell trail. Then, it gives R values, $R = \log_2(\Sigma|\Delta y|/\Sigma|\Delta x|)$, as a parameter of the alignment; a zero value for non-aligned random movement, positive values of R for movement aligned along the axis of tension, and negative values for movement aligned perpendicular to the axis of tension. Figure 3 shows histograms of the distribution of cell trails according to their R values. When horizontal conditioning was used (Fig. 3*a*) we observed nearly normal distribution centred at zero, showing that the cell trails are not aligned. On the other hand, when we used tilted conditioning (Fig. 3*b*, *c*) we observed a distribution that was shifted to the right (positive values). The shift of the mean (+0.36 and +0.29) is larger than that of the natural alignment along the bp–ap axis in the stage 10 ectoderm conditioning (+0.22)[7]. Substratum conditioning using stage 10− ectodermal layers (Fig. 3*b*) gives stronger alignment than that using stage 10 ectoderm (Fig. 3*c*). The most likely explanation for this is that the stage 10 explant was oriented on the coverslip strip in such a way that its natural alignment along the bp–ap axis must be cancelled before any appearance of the alignment along the axis of tension.

Contact inhibition of cell movement is another mechanism that can direct cell migration from the blastopore with a high population of migratory cells towards the animal pole where the fibril network on the inner surface of the ectodermal layer offers an adequate substratum for cell attachment and locomotion. This possibility has been suggested[6,7,13,14], and the gastrula mesodermal cells indeed show contact paralysis against each other when cultured *in vitro*[13,15].

This work was supported by NIH grant HD11634 and 13419. We thank Dr A. K. Harris for sending us salamander eggs. The manuscript was prepared while N.N. was staying at the MRC Mammalian Development Unit and supported by the Meiji Institute of Health Science.

Received 12 September; accepted 25 November 1983.

1. Weiss, P. *J. exp. Zool.* **100**, 353–386 (1945).
2. Dunn, G. A. in *Cell Behaviour*, 247–280 (Cambridge University Press, 1982).
3. Löfberg, J., Ahlfors, K. & Fällström, C. *Devl Biol.* **75**, 148–167 (1980).
4. Heasman, J., Hynes, R. O., Swan, A. P., Thomas, V. & Wylie, C. C. *Cell* **27**, 437–447 (1981).
5. Silver, J. & Ogawa, M. Y. *Science* **220**, 1067–1069 (1983).
6. Nakatsuji, N., Gould, A. C. & Johnson, K. E. *J. Cell Sci.* **56**, 207–222 (1982).
7. Nakatsuji, N. & Johnson, K. E. *J. Cell Sci.* **59**, 43–60 (1983).
8. Boucaut, J.-C. & Darribere, T. *Cell Differentiation* **12**, 77–83 (1983).
9. Harris, A. K., Stopak, D. & Wild, P. *Nature* **290**, 249–251 (1981).
10. Stopak, D. & Harris, A. K. *Devl Biol.* **90**, 383–398 (1982).
11. Rugh, R. *Experimental Embryology* 3rd edn (Burgess, Minneapolis, 1962).
12. Keller, R. E. *J. Embryol. exp. Morph.* **60**, 201–234 (1980).
13. Johnson, K. E. *Exp. Cell Res.* **101**, 71–77 (1976).
14. Kubota, H. Y. & Durston, A. J. *J. Embryol. exp. Morph.* **44**, 71–80 (1978).
15. Nakatsuji, N. & Johnson, K. E. *Cell Motility* **2**, 149–161 (1982).
16. Stearns, R. N. & Kostellow, A. B. in *The Chemical Basis of Development*, 448–457 (Johns Hopkins University Press, Baltimore, 1958).
17. Feldman, M. *J. Embryol. exp. Morph.* **3**, 251–255 (1955).
18. Boucaut, J. C. & Darribere, T. *Cell Tissue Res.* **234**, 135–146 (1983).

Forskolin activation of adenylate cyclase *in vivo* stimulates nerve regeneration

Suzanne L. Kilmer & Richard C. Carlsen

Department of Human Physiology, School of Medicine, University of California, Davis, California 95616, USA

The previous demonstration of an increase and redistribution of adenylate cyclase activity in injured peripheral nerve[1] suggests that an increase in neuronal cyclic AMP concentration could play a role in peripheral nerve regeneration. We report our finding that accumulating adenylate cyclase activity was translated into a twofold increase in cyclic AMP concentration in the regenerating nerve stump, coincident with the initiation and elongation of regenerative nerve sprouts. We sought to magnify the role of cyclic AMP in regeneration by using forskolin, a robust activator of adenylate cyclase[2], to produce an additional increase in neuronal cyclic AMP *in situ*. Forskolin *in vitro* produced an approximately 40-fold greater elevation in neuronal cyclic AMP than an equimolar (10^{-5}) concentration of isoprenaline. Moreover, the elevated cyclic AMP concentration persisted for at least 60 min in the continued presence of forskolin. Daily injection of forskolin into the dorsal lymph sac of *Rana pipiens*, or delivery of forskolin through an implanted osmotic pump produced a sustained 40% increase in the rate of sensory nerve regeneration in freeze-lesioned sciatic nerves. We conclude that an increase in cyclic AMP concentration and, presumably, the activation of appropriate protein kinases stimulates regenerative nerve growth following trauma.

Adenylate cyclase, the enzyme responsible for catalysing the formation of cyclic AMP from ATP, undergoes anterograde, but not retrograde axonal transport in normal frog sciatic nerve. If the nerve is subjected to crush or transection, however, adenylate cyclase activity accumulates proximal to the site of injury and is incorporated in the retrograde transport system for return toward the cell body[1]. While these observations provide the basis for a stimulated production of cyclic AMP in regenerating nerve, increased production does not necessarily lead to increased concentration. A parallel elevation of phosphodiesterase activity, an enzyme which also undergoes axonal transport[3], could prevent accumulation of the cyclic nucleotide. We measured cyclic AMP concentration in specific regions of axotomized frog sciatic nerve at 2, 4 and 7 days after a freeze-

0028-0836/84/050455-03$01.00
© 1984 Macmillan Journals Ltd

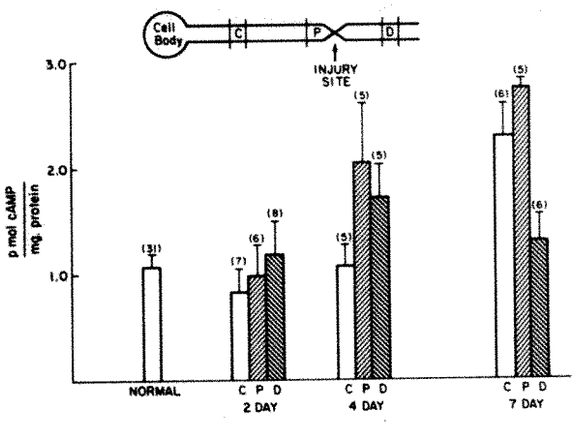


Fig. 1 The concentration of cyclic AMP in 3–4 mm desheathed nerve segments removed from indicated regions of normal and freeze-lesioned sciatic nerves at specified times following the injury. Numbers in parentheses represent the sample size for each measurement. Sample P was obtained just proximal to the injury and includes regenerative growth at 4 and 7 days. Samples C and D were removed from regions 1 cm proximal and 1 cm distal to the injury respectively.

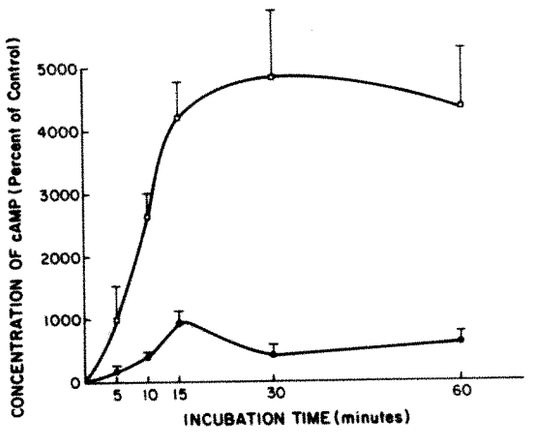


Fig. 2 Comparison of the relative stimulatory capabilities of equimolar concentrations (10^{-5} M) of forskolin (Calbiochem) (□) and isoprenaline (●) applied to desheathed 3–4 mm segments of normal sciatic nerve *in vitro*. Each time point represents the mean ± s.e.m. of four measurements. Nerve segments were preincubated for 1 h at room temperature in normal frog Ringer's solution through which a mixture 95% O_2:5% CO_2 was continuously bubbled. The preincubation solution was removed and fresh solution added for a second 15 min preincubation. The drug was then added for the specified period of time, at the end of which the nerve segment was frozen and cyclic AMP extracted for radioimmunoassay. Each stimulated segment was paired with an unstimulated control.

lesion of the nerve. These post-injury intervals were chosen to reflect the period prior to regenerative sprouting (2 days), the period of sprout initiation (4 days) and the early period of axon elongation (7 days). The results of these experiments are presented in Fig. 1. Freeze-lesioned or intact sciatic nerves were removed from northern *Rana pipiens* (30–50 g body weight, mixed sex, obtained from J. Hazen, Alburg, Vermont, USA, during Winter and Spring). The isolated nerve was placed in Ringer's solution and desheathed. Desheathed nerve segments, 3–4 mm in length, were obtained from the injury site (including regenerative sprouts) and from regions 1 cm proximal and 1 cm distal to the injury. Each segment was incubated in a separate flask containing Ringer's solution without a phosphodiesterase inhibitor for 1 h at room temperature (21–22 °C). The solution was continuously supplied with a mixture of 95% O_2 and 5% CO_2. At the end of the incubation period the segment was rapidly frozen in a mixture of ethanol:hydrochloric acid (60:1, wt/wt) cooled on dry ice (−70 °C). The frozen tissue was homogenized and prepared for analysis of cyclic AMP concentration by radioimmunoassay[4]. Protein concentration was determined using the Lowry assay. The mean concentration of cyclic AMP in segments from the selected locations were compared statistically to segments from similar regions of intact nerves using analysis of variance and Dunnett's *t*-test.

Cyclic AMP concentration was not significantly different from normal nerve in any of the three sample regions of axotomized nerve 2 days after injury. Thus, the early accumulation of adenylate cyclase activity at the injury site[1] was not translated into an equivalent increase in cyclic AMP concentration prior to the development of regenerative sprouting. After 4 days, however, cyclic AMP concentration was significantly ($P < 0.05$) increased in the region of regenerative growth, when regenerative sprouts are first appearing[3]. Concentration also increased in segments distal to the injury at 4 days, although the increase was not statistically significant. At 7 days, cyclic AMP concentration was significantly increased in nerve segments encompassing regenerative growth, and in segments 1 cm proximal to the injury ($P < 0.01$). Concentration in segments distal to the injury had returned to control values.

This pattern of change in cyclic AMP concentration supports our hypothesis that an increase in cyclic AMP concentration may play an integral role in nerve regeneration. Previous studies, however, in which dibutyryl cyclic AMP was injected into rats during the course of peripheral nerve regeneration offer mixed results[6–9]. The treatment-enhanced regeneration in two cases[6,7], but had no effect in two others[8,9]. None of these studies assessed the actual cyclic AMP concentration in the regenerating nerve, or the magnitude of the presumed increase resulting from the injection of dibutyryl cyclic AMP. We chose to explore the possible role of cyclic AMP in regeneration by using an extremely potent activator of adenylate cyclase, forskolin (Calbiochem), to increase endogenous levels of cyclic AMP in the injured nerve. Forskolin stimulates adenylate cyclase via the catalytic subunit of the enzyme[2], and produced an increase in activity two to three times that produced by sodium fluoride in frog nerve (unpublished observation). Incubation of desheathed nerve segments from normal frog nerve in media containing either 10^{-5} M forskolin or 10^{-5} M isoprenaline demonstrated that forskolin could produce an increase in cyclic AMP concentration 40-fold greater than that produced by isoprenaline (Fig. 2). Moreover, the increase in concentration was sustained for at least 60 min in the continued presence of forskolin.

The effect of forskolin on nerve regeneration was tested in frogs subjected to a unilateral freeze-lesion of the sciatic nerve at mid-thigh. Forskolin was provided either by daily injection of 0.5 ml of a 5×10^{-4} M solution into the dorsal lymph sac, or by implanting an osmotic pump (Alza) which delivered 0.55 μl per h (21 °C) of a 10^{-4} M solution in the vicinity of the injury. Animals with an implanted pump also received a single 'priming' injection of the 5×10^{-4} M forskolin solution (0.5 ml). Control animals received daily saline injections or had implanted osmotic pumps containing a 0.64% saline solution. Regeneration distance in both forskolin-treated and control animals was determined using axonal transport of H^3-leucine-labelled protein from lumbar dorsal root ganglia to determine the maximum extent of sensory nerve outgrowth[5,10]. Ganglia and attached spinal and sciatic nerves were removed and placed in a compartmented chamber[11]. The ganglia were contained in a centre compartment and the nerves passed through a silicone grease barrier into an outer compartment. The tissue was bathed in aerated Ringer's solution and 50 μCi ml^{-1} of ^{3}H-leucine added to the ganglia compartment. Following a 3–4 h incubation period the isotope was removed, the tissue washed and placed in a second chamber; axonal transport was then allowed to continue for an additional 16–18 h. At the end of the transport period the nerve was frozen and divided into 1- or 2-mm segments. Each segment was placed in 5% trichloroacetic acid (TCA)

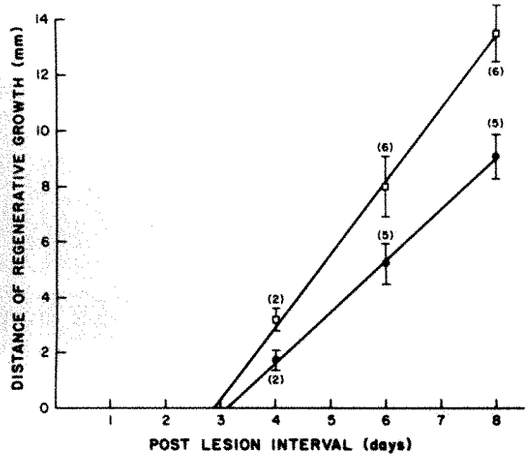


Fig. 3 Measurement of regeneration distance in axotomized sensory nerves from animals receiving forskolin and from saline-treated controls. Regeneration distance was determined at the indicated time points by assaying the distribution of axonally-transported radioactively labelled protein in the regenerating nerves. Distances at each time point represent the mean ± s.d. of the indicated number of measurements. Distance points were connected by linear regression lines using the method of least squares. The slope of each line indicates the regeneration rate in forskolin-treated and control, saline-treated sensory nerves. For treated nerves the slope is 2.6 mm per day, and for controls, 1.86 mm per day.

overnight to separate TCA-soluble and insoluble material. The soluble material was discarded and the amount of TCA-insoluble radioactivity determined by liquid scintillation spectroscopy. Regeneration distance was taken as the point at which sample counts declined to within two standard deviations of mean background activity[10]. Forskolin-treated frogs showed a 40% increase in the rate of sensory nerve regeneration without a statistically significant change in latency to the initiation of regenerative growth. Enhancement of regenerative growth was the same using either of the two modes of drug application (results are pooled in Fig. 3). Regeneration rate in forskolin-treated nerves was significantly greater ($P < 0.001$) than the rate in saline-treated animals, as determined by linear regression analysis[12]. Forskolin-stimulated enhancement of regeneration also exceeded the 12–17 percent enhancement obtained using the condition/test paradigm[9] in frogs at 25 °C[12].

Functional recovery following nerve injury requires both axonal regrowth and reconnection with appropriate target organs. The processes responsible for recovery are complex and require interaction between the injured axon, its cell body, periaxonal cells in the axon and, eventually, cells in the target organ[13]. In the present experiments we attempted to link an increase in cyclic AMP concentration in regenerating axons to the development of regenerative growth. We determined that forskolin, an agent which stimulates cyclic AMP production also enhances the rate of axon regeneration. The site of action of forskolin is currently unknown, but could involve processes in either or both the axonal injury site or the nerve cell body. Forskolin does not cross the blood–brain barrier[2], but dorsal root ganglia neurones are not contained within the barrier[14] and injected forskolin should be able to interact with injured sensory cell bodies. The role of cyclic AMP in regeneration is also unknown, although an increase in cyclic AMP might stimulate microtubule aggregation to enhance sprout formation[15] or provide a modulating influence on RNA and protein synthesis in the cell body[16]. While the rate of nerve regeneration is but one aspect of the recovery process, stimulation of rate, particularly in human nerve injuries located far proximal in a limb, might improve recovery by allowing re-innervation of target tissue before target organ degeneration has become extensive[17]. We are currently investigating the specific molecular role of cyclic AMP in injured nerve, but it appears clear that the nucleotide can play an important role in axonal growth.

Received 24 May; accepted 15 November 1983.

1. Carlsen, R. C. *Brain Res.* **232,** 413–424 (1982).
2. Seaman, K. B. & Daly, J. W. *J. Cyclic Nucleotide Res.* **7,** 201–224 (1981).
3. Bray, J. J., Kon, C. M. & Breckenridge, B. M. *Brain Res.* **26,** 385–395 (1971).
4. Carlsen, R. C. *Exp. Neurol.* **66,** 556–576 (1979).
5. Carlsen, R. C., Kiff, J. & Ryugo, K. *Brain Res.* **234,** 11–25 (1982).
6. Pichichero, M., Beer, B. & Clody, D. E. *Science* **182,** 724–725 (1973).
7. Gershenbaum, M. R. & Roisen, F. J. *Neuroscience* **5,** 1565–1580 (1980).
8. Black, M. M. & Lasek, R. J. *Exp. Neurol.* **63,** 108–119 (1979).
9. McQuarrie, I. G., Grafstein, B. & Gershon, M. D. *Brain Res.* **132,** 443–453 (1977).
10. Forman, D. S. & Berenberg, R. A. *Brain Res.* **156,** 213–225 (1978).
11. Dravid, A. R. & Hammerschlag, R. *J. Neurochem.* **24,** 711–718 (1975).
12. Carlsen, R. C. *Brain Res.* **279,** 9–18 (1983).
13. Liu, H. M. *Biology and Pathology of Nerve Growth* 161–235 (Academic, New York, 1981).
14. Jacobs, J. M., MacFarlane, R. M. & Cavanagh, J. B. *J. Neurol. Sci.* **29,** 95–107 (1976).
15. Roisen, F. J., Murphy, R. A., Pichichero, M. E. & Braden, W. G. *Science* **175,** 73–74 (1972).
16. Traugh, J. A. *Biochemical Action of Hormones,* Vol. 8, 167–208 (Academic, New York, 1981).
17. McQuarrie, I. G. in *Posttraumatic Peripheral Nerve Regeneration: Experimental Basis and Clinical Implications.* (eds A. Gorio *et al.*) 49–58 (Raven, New York, 1981).

Acceptors for botulinum neurotoxin reside on motor nerve terminals and mediate its internalization

J. Oliver Dolly*, Jennifer Black*, Richard S. Williams* & Jack Melling†

* Department of Biochemistry, Imperial College, London SW7 2AZ, UK
† Vaccine Research and Production Laboratory, Centre for Applied Microbiology and Research, Porton Down SP4 0JG, UK

Botulinum neurotoxin (BoNY) type A, a causative agent of botulism, is a di-chain protein (molecular weight 140,000) from *Clostridium botulinum*, and the most neurotoxic substance known[1]. Some cases of sudden infant cot deaths have been attributed to such a neuroparalytic condition[2]. BoNT inhibits irreversibly the release of acetylcholine from peripheral nerves in a highly selective manner[3–6]. Hence, it is potentially an invaluable probe for studying the mechanism of transmitter release[7,8]. Here we demonstrate specific labelling of murine motor nerve terminals with neurotoxic, ^{125}I-labelled BoNT (type A) by autoradiography. We observed saturable, temperature-sensitive binding of BoNT to sites which reside solely on the nerve terminal membrane; these were distributed on all unmyelinated areas, at an average density of 150–500 per μm^2 of membrane. The binding was mediated by the larger subunit of the toxin and was inhibited partially by tetanus toxin, another microbial protein[9]. No specific binding was detectable on any other cell types examined, including noradrenergic terminals. Following binding, internalization of radioactivity was observed; this process was energy-dependent as it could be prevented totally by azide or dinitrophenol (DNP). This direct demonstration of separable steps, including highly selective binding and acceptor-mediated internalization, is reconcilable with the unique potency and the multiphasic inhibitory action of BoNT on transmitter release, as shown electrophysiologically[8].

To facilitate characterization of BoNT-binding sites on neuronal membranes, with the long-term goal of elucidating the molecular basis of its action, BoNT type A was purified to homogeneity and ^{125}I-iodinated to high specific radioactivity (700–3,000 Ci mmol^{-1}). The resultant BoNT (^{125}I-BoNT) retained appreciable levels of neurotoxicity (60–85% of the original, equivalent to 2×10^8 mouse lethal doses per mg protein)[7,10] and was shown to bind saturably and with high affinity to a sialic acid-containing moiety on brain synaptic membranes[10]. Additional evidence of its biological activity was provided by the demonstration, using light microscope autoradiography, that it binds at low concentrations to a discrete

0028-0836/84/050457—04$01.00
© 1984 Macmillan Journals Ltd

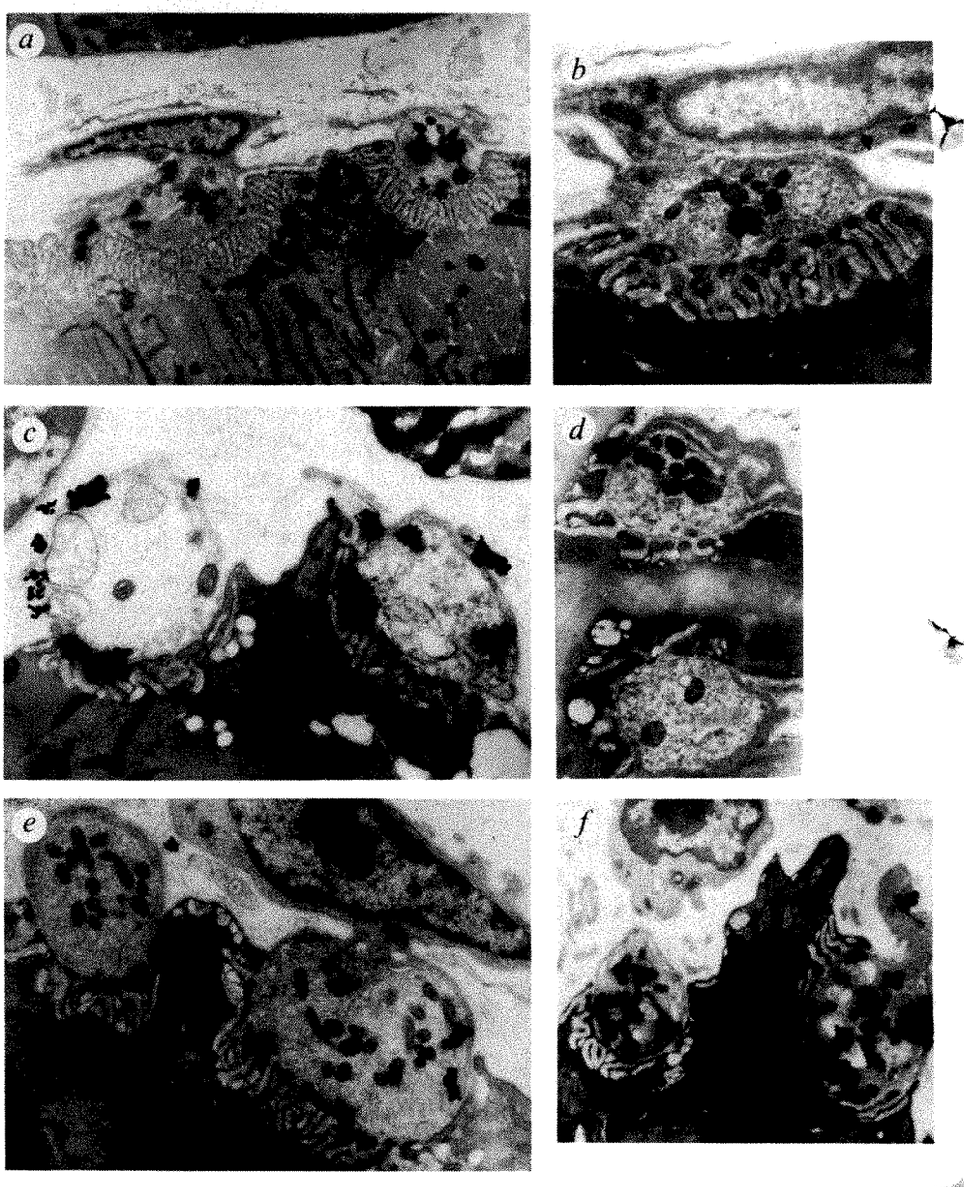


Fig. 1 Electron microscope autoradiograms of phrenic nerve muscle preparations labelled with ^{125}I-BoNT. *a*, *c*, *e*, With tetanus toxin; *b*, *d*, *f*, without. *a*, Sample treated with ^{125}I-BoNT alone. *b*, Control sample labelled with ^{125}I-BoNT and excess unlabelled toxin. *c*, Muscle preincubated at 22 °C for 20 min in Krebs–Ringer's containing 15 mM sodium azide and then labelled in the latter medium with ^{125}I-BoNT as in *a*. *d*, A control sample of tissue treated with sodium azide as in *c* then incubated with labelled and unlabelled BoNT as in *b*. *f*, Specimen labelled with ^{125}I-BoNT as in *a* except that the incubation period with toxin was 2 h. *e*, The preparation was preincubated with 0.9 μM tetanus toxin; ^{125}I-BoNT was then added and the incubation continued for a further 2 h.

Methods: Each dissected mouse hemidiaphragm was incubated with 11 nM ^{125}I-BoNT in 0.5 ml Krebs–Ringer's *p*H 7.4 at 22 °C for 1.5 h unless otherwise specified; use of a 3 ml incubation volume yielded similar results. Control samples were treated similarly except that 1.1 μM BoNT was added together with the radiolabelled toxin. Labelled tissues were washed repeatedly in Krebs–Ringer's for 30 min at 22 °C before fixation for 1 h with 2% glutaraldehyde in the latter solution. Endplate-containing regions of each sample were cut into 1-mm^3 pieces, treated with 1% OsO_4 for 1 h, dehydrated in ethanol and embedded in Spurr's resin. Pale gold or silver sections were cut using an LKB ultramicrotome and processed for autoradiography[13]; a monolayer of L4 emulsion was applied to the sample by dipping. Sections were exposed for 3 weeks, developed in Kodak D-19 developer, stained with 12% uranyl acetate, examined in a Hitachi electron microscope and photographed.

Table 1 Autoradiographic analysis of nerve terminals labelled with ^{125}I-BoNT

Incubations	Distribution of grains with respect to the plasma membrane (% of total)		Relative number of grains/unit area of plasma membrane (%)
	On*	Within	
Series I (28 nM ^{125}I-BoNT)			
No additives	62 (±16; $n = 15$)	38 (±16; $n = 15$)	100†
1.4 μM BoNT	—	—	4
$-Ca^{2+}/+2$ mM EGTA	61 (±24; $n = 21$)	39 (±24; $n = 21$)	74
Series II (11 nM ^{125}I-BoNT)			
No additives	61 (±17; $n = 22$)	39 (±17; $n = 22$)	100†
1.1 μM BoNT	—	—	5
0.5 μM BoNT large subunit	—	—	0
15 mM sodium azide	100 ($n = 50$)	—	85
0.9 μM tetanus toxin	61 (±25; $n = 23$)	39 (±25; $n = 23$)	31
Series III (1 nM ^{125}I-BoNT)			
No additives	54 (±24; $n = 6$)	46 (±24; $n = 6$)	100†
50 nM BoNT	—	—	0

Mouse hemidiaphragms were labelled with ^{125}I-BoNT for 1.5 h at 22 °C in 0.5 ml of Krebs–Ringer's solution in the conditions specified. Tissues were subsequently washed, fixed, embedded and pale gold sections were cut; autoradiograms of these were prepared and developed after 3 weeks' exposure, as detailed in Fig. 1. After examination in a Hitachi electron microscope, large numbers of nerve endings in several sections were photographed; the silver grains in each were counted and the mean values (±s.d., n = number of terminals counted) are expressed. The samples in each series of experiments were treated identically.

* 90% of these grains were found on the membrane itself; 10% were within 150 nm of the latter and can be attributed to expected scatter.

† The area of the terminal membrane in each section was calculated by digitization and the total number of grains observed on the membrane and enclosed within it were expressed per unit area of the membrane; these were taken to represent 100% in each case.

number of sites at murine motor endplates[7,11], following *in vivo* or *in vitro* labelling; a similar observation has been made for another preparation of ^{125}I-BoNT[12].

To locate the site of this binding, mouse hemidiaphragms were incubated with ^{125}I-BoNT, washed and processed for autoradiography[13] before examination by electron microscopy. To measure nonspecific binding of ^{125}I-BoNT, samples were treated similarly except that an excess of BoNT was included. After labelling of nerve–muscle preparations with ^{125}I-BoNT *in vitro*, in conditions which block neurotransmission[5], autoradiography showed a distinct deposition of silver grains at the motor nerve endings (Fig. 1*a*). This labelling was saturable as control samples treated with excess BoNT contained few, if any, silver grains (Fig. 1*b*; Table 1). All the sites were occupied when 50 nM ^{125}I-BoNT was used, in labelling conditions (1.5 h; 22 °C) that minimize structural damage to the tissue. Greatly reduced numbers of grains were seen when the incubation was done at 4 °C; we have been unable to ascertain how much changes in rate of diffusion contribute to this.

A similar qualitative deposition of grains was observed when low amounts (0.5 nM) of ^{125}I-BoNT were used, although fewer grains were apparent. This does not preclude the possibility that the acceptors have a high affinity for BoNT, as even higher concentrations (~1 μM) of α-bungarotoxin ($K_D \sim 10^{-11}$ M) are similarly required to overcome diffusion barriers in this tissue[14], within a similar incubation period. In all cases, the labelling with ^{125}I-BoNT was associated selectively with nerve terminals. Muscle, blood vessels, connective tissue, Schwann cells and myelin were virtually devoid of grains (Fig. 1); the number of grains observed was insignificant and these were distributed randomly, their presence being unaffected by excess unlabelled BoNT. Furthermore, it proved impossible to demonstrate any deposition of silver grains on noradrenergic terminals (Fig. 2) of mouse vas deferens[15], despite use of high ^{125}I-BoNT concentrations.

The saturable labelling of nerve terminals demonstrated with ^{125}I-BoNT was restricted to the presynaptic membrane (Fig. 1, Table 1); using the standard incubation conditions, most grains (62 ± 16% of the total) were located on the nerve membrane, the remainder being inside the terminal. Transfer of radioactivity across the plasma membrane was detectable after incubation with 11 nM toxin for 20 min, but the extent of internalization reached a maximum after 90 min; at present, we do not know whether the grains seen represent the intact toxin molecule or one of its subunits. However, when a homogeneous preparation of the non-toxic larger subunit of BoNT was included in the incubation medium, after separation[10] and renaturation (neurotoxic material can be reconstituted by the addition of the smaller subunit [55,000 molecular weight] to this preparation[16]), there was no ^{125}I-BoNT labelling (Table 1). This showed that the heavier subunit (97,000 molecular weight) is responsible for the observed binding, as is the case for binding to brain synaptosomal membranes[10]. The kinetics of the toxin's neuroparalytic action are consistent with the involvement of a target site within the terminal[17] and the findings presented here provide the first direct evidence for such internalization of BoNT. Lowering the temperature to 4 °C abolished internalization of radioactivity, as did inhibitors of energy production such as azide (Fig. 1*c*, Table 1) or DNP. This indicates that the overall process is energy requiring; we do not know which of the many possible mechanisms[18] is involved. In these conditions the labelling was saturable (Fig. 1*c* and *d*); although the total number of grains observed was reduced somewhat, they were now all deposited on the neuronal membrane (Table 1) or, at least, very close to it. Tetanus toxin was tested as a possible antagonist for the BoNT acceptor since its structure[8] is comparable and it has a related pharmacological action[9,19]. The labelling of nerve terminals was reduced significantly in the presence of tetanus toxin (Fig. 1*e* and *f*); however, the fraction of radioactivity internalized was unaffected (Table 1). Thus, the inhibitory action of tetanus toxin, together with that of the large BoNT subunit, on the labelling observed further supports the saturability of the BoNT acceptor. Collectively, the findings demonstrate the existence of at least two distinguishable and sequential steps—toxin binding and internalization.

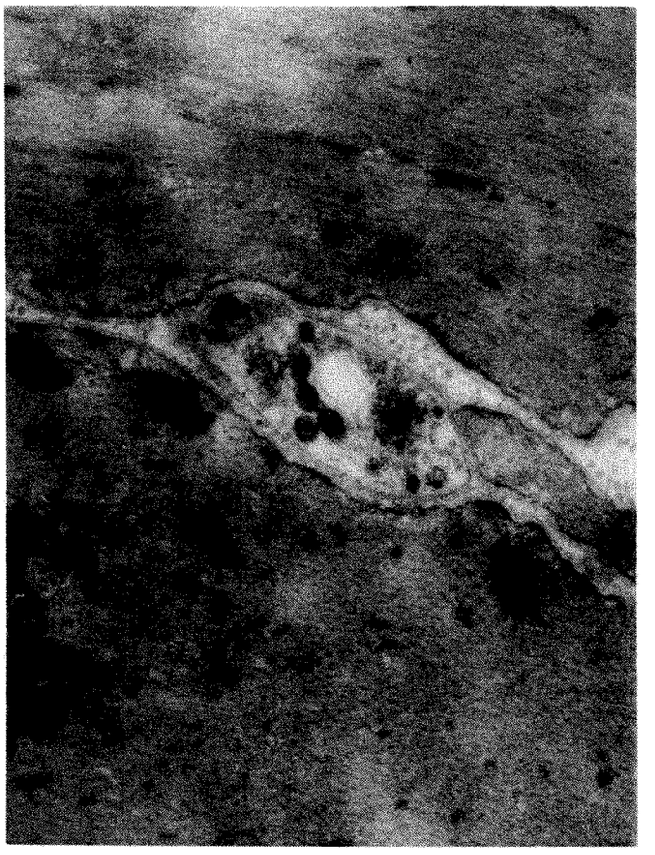


Fig. 2 Autoradiography of noradrenergic terminals from mouse vas deferens treated with ^{125}I-BoNT. The serosal coat of the dissected vas deferens was removed by gentle pulling and the remaining tissue incubated with 22 nM ^{125}I-BoNT in 0.5 ml Krebs–Ringer's for 90 min at 22 °C. The tissue was washed with Krebs–Ringer's for 30 min, then it was stretched and held in place with pins before being fixed for 90 min at 22 °C with 3% glutaraldehyde/2% paraformaldehyde/0.1 M sodium cacodylate buffer, *p*H 7.4. The sample was cut transversely into small segments and these were washed twice for 20 min in 5% sucrose/0.1 M sodium cacodylate buffer, *p*H 7.4 before treatment for 1 h at 22 °C with 2% OsO_4 in the latter buffer. This fixation method yields maximum preservation of dense core vesicles which facilitate identification of the noradrenergic terminals. After washing, the sample was dehydrated, embedded and prepared for autoradiography as described in Fig. 1 legend. The sections were developed after 3 weeks' exposure, viewed in the electron microscope and photographed. Dense core vesicles, which are characteristic of noradrenergic nerve terminals, were observed but no silver grains were seen in any area of the sections.

With toxin uptake inhibited, the content of binding sites could be quantified readily; using an established procedure[13], the average density was calculated to be 150–500 sites per μm^2 of membrane. Also, with azide present it was apparent that the binding sites are distributed uniformly over all the unmyelinated areas of the terminal membrane (Fig. 1*c*). Hence, in contrast to previous suggestions[20], it appears unlikely that the binding sites are involved directly in the process of transmitter release because their location is not restricted to the areas of membrane (active zones) opposing the postsynaptic folds, where release is thought to occur. It should be emphasized that with a low concentration of ^{125}I-BoNT (1 nM), in the presence of azide, the grain distribution obtained was similar; in fact, examination of autoradiograms of 21 endplates labelled in this manner showed that in 16, grains were not restricted to the active zone regions (as was seen also with higher toxin concentrations). Nevertheless, the apparent selective location (Figs 1 and 2) of

the binding site on cholinergic nerve membranes (at least in the periphery) indicates some, as yet unidentified, role. At least it seems clear from the de-energization experiments (Fig. 1*c*, Table 1) that the binding step is essential for translocation of the toxin into the nerve terminal. Furthermore, in the case of noradrenergic synapses[6] where BoNT is unable to inhibit neurotransmission at concentrations known to inhibit acetylcholine release at motor nerve terminals[21], the demonstrable absence of any binding sites for BoNT could render the transport of toxin across the plasma membrane to its site of action impossible. The minimal inhibitory effects on noradrenergic transmission in the anococcygeus muscle, seen after application of BoNT for long periods[6], may result from its inefficient uptake by another route. Similarly, absence of the toxin acceptor could explain why non-adrenergic, non-cholinergic transmission in guinea pig taenia coli is unaffected by BoNT[6]. It is likely that the intraterminal target may be common to several synapse types. Finally, the ability of tetanus toxin to antagonize BoNT binding emphasizes the importance of the toxin-binding step, as there are many similarities in the actions of these toxins at motor endplates[19].

Although all available pharmacological data[8] and the findings reported here support the contention that toxin translocation is essential for its neurotoxicity, additional evidence was sought using conditions known to interfere with the neuroparalytic action of BoNT, without perturbing its binding. In contrast to deductions from electrophysiological investigations[17], absence of Ca^{2+} from the incubation medium did not appreciably inhibit the internalization (or binding) of BoNT (Table 1). Similarly, lysosomotrophic agents (for example, chloroquine) which are effective antagonists of botulism[21] failed to prevent BoNT translocation (data not shown). Chloroquine is known to retard the expression of the activity of other microbial toxins[22], following their internalization by energy-dependent process(es) of the type described here. Thus, it is likely that this ability (which involves increasing the *p*H of lysosomes[22] or possibly of other compartments[23]), rather than its proposed effectiveness in inhibiting uptake[21], is implicated in delaying the onset of neuroparalysis by BoNT. With regard to the individual steps of botulinization, the resolution of autoradiographic techniques cannot exclude membrane sequestration of toxin following its binding to external sites. However, it is not likely that this could allow direct access of toxin to its pharmacological target because of the demonstrated internalization of toxin and its sensitivity to chloroquine. Thus, intoxication seems to depend on toxin uptake; the associated intraterminal events could facilitate a detrimental interaction of BoNT, or a fragment of it, with a component essential for transmitter release. This final target might reside within the active zones; in fact, it could be responsible for the presence of some silver grains in autoradiograms of terminals labelled with ^{125}I-BoNT in the absence of azide (Fig. 1*a* and *f*).

This work was supported by MRC (J.B) and SERC (R.S.W) studentships, and grants from the University of London Research Fund and Buitoni-Princess Foods Ltd are gratefully acknowledged.

Received 30 June; accepted 15 November 1983.

1. Sugiyama, H. *Microbiol. Rev.* **44,** 419–448 (1980).
2. Arnon, S. S., Midura, T. F., Damus, K., Wood, R. M. & Chin, J. *Lancet* **i,** 1273–1276 (1978).
3. Gundersen, C. B., Katz, B. & Miledi, R. *Proc. R. Soc. B* **216,** 369–376 (1982).
4. Sellin, L. C. & Thesleff, S. *J. Physiol., Lond.* **317,** 487–495 (1981).
5. Tse, C. K., Dolly, J. O., Hambleton, P., Wray, D. & Melling, J. *Eur. J. Biochem.* **122,** 493–500 (1982).
6. MacKenzie, I., Burnstock, G. & Dolly, J. O. *Neuroscience* **7,** 997–1006 (1982).
7. Dolly, J. O. *et al.* in *Biomedical Aspects of Botulism* (ed. Lewis, G.) 47–64 (Academic, New York, 1981).
8. Simpson, L. L. *Pharmac. Rev.* **33,** 155–188 (1981).
9. Wellhoner, H-H. *Rev. physiol. biochem. Pharmac.* **93,** 1–68 (1982).
10. Williams, R. S., Tse, C. K., Dolly, J. O., Hambleton, P. & Melling, J. *Eur. J. Biochem.* **131,** 437–445 (1983).
11. Dolly, J. O. *et al. Tox.* **20,** 141–148 (1982).
12. Hirokawa, N. & Kitamura, M. *Naunyn-Schmiedebergs Archs Pharmac.* **287,** 107–110 (1975).
13. Fertuck, H. C. & Salpeter, M. M. *J. Cell Biol.* **69,** 144–158 (1976).
14. Dolly, J. O., Albuquerque, E. X., Sarvey, J. M., Mallick, B. & Barnard, E. A. *Molec. Pharmac.* **13,** 1–14 (1977).
15. Burnstock, G. & Costa, M. in *Adrenergic Neurons* (Chapman and Hall, London, 1975).
16. Kozaki, S., Togashi, S. & Sakaguchi, G. *Japan. J. med. sci. Biol.* **34,** 61–68 (1981).
17. Simpson, L. L. *J. pharmac. exp. Ther.* **212,** 16–21 (1980).
18. Goldstein, J. L. *Nature* **279,** 679–685 (1979).
19. Habermann, E. in *Biomedical Aspects of Botulism* (ed. Lewis, G.) 129–141 (Academic, New York, 1981).
20. Hanig, J. P. & Lamanna, C. *J. theor. Biol.* **77,** 107–113 (1979).
21. Simpson, L. L. *J. Pharmac. exp. Ther.* **222,** 43–48 (1982).
22. Leppla, S. H., Dorland, R. B., & Middlebrook, J. L. *J. biol. Chem.* **255,** 2247–2250 (1980).
23. Hopkins, C. R. *Nature* **304,** 684–685 (1983).

L-Glutamate has higher affinity than other amino acids for [^{3}H]-D-AP5 binding sites in rat brain membranes

H. J. Olverman, A. W. Jones & J. C. Watkins

Department of Pharmacology, The Medical School, University Walk, Bristol BS8 1TD, UK

Electrophysiological studies indicate the existence of several types of receptors for excitatory amino acids[1]. Thus, responses induced by *N*-methyl-D-aspartate (NMDA) are potently and selectively blocked by D(−)-2-amino-5-phosphonopentanoic acid (D-AP5), while responses induced by such agonists as kainate and quisqualate are relatively resistant to this antagonist[2,3]. Evidence is mounting that excitatory amino acid receptors are involved in synaptic excitation in many regions of the central nervous system (see refs 1 and 4 for reviews). Although the identity of the transmitter(s) acting at these receptors remains uncertain[5], L-aspartate has been considered the most likely transmitter at NMDA receptors and L-glutamate at kainate/quisqualate receptors[1,4]. Other endogenous acidic amino acids proposed as possible transmitters include a range of sulphur-containing amino acids[6] and the tryptophan metabolite, quinolinic acid[7]. Ligand-binding studies offer a means not only of assessing receptor densities in different brain regions but also of comparing affinities of transmitter candidates for these receptors. However, to avoid difficulties of interpretation arising from the use of ligands which bind to more than one type of receptor, such as [^{3}H]-L-glutamate and [^{3}H]-L-aspartate (for example, refs 8–12), ligands with high receptor selectivity are required. Here, we report that [^{3}H]-D-AP5 binds specifically to rat brain membranes, that the hippocampus and cerebral cortex are enriched in these sites relative to other brain areas and that L-glutamate has higher affinity for these receptors than have all other transmitter candidates tested.

[^{3}H]-D-AP5 binding to rat cerebral cortical membranes reached equilibrium following 20 min incubation at 25 °C. Specific binding contributed about 40% to the total binding at 30 nM [^{3}H]-D-AP5. This binding was reversible, the bound ligand being completely displaced by 1 mM unlabelled ligand added at 25 min with a $t_{1/2}$ of 5 min. The kinetics of D-AP5 binding were investigated by incubating membranes with 17 nM [^{3}H]-D-AP5 in the presence of increasing concentrations of unlabelled D-AP5 (0.05–100 μM); computer fitting of the data revealed a single population of receptors (Fig. 1). Analysis of results from four such experiments gave K_D and B_{max} values of 0.47 ± 0.09 μM and 4.1 ± 0.7 pmol per mg protein respectively (mean values ± s.e.). D-AP5 binding sites were present in all areas of the CNS tested. There was, however, a marked enrichment especially in the hippocampus, but also in the cerebral cortex (Fig. 2). Specific binding was lowest in the cerebellum and in the medulla/pons, where the density of sites was one quarter that observed in the hippocampus.

To determine if the binding sites for [^{3}H]-D-AP5 were identical with NMDA receptors characterized in electrophysiological studies, a number of excitatory amino acid antagonists of known pharmacological profile were tested as inhibitors of binding. Within an homologous series of ω-phosphono-α-aminocarboxylates[2], both (±)-AP3 and (±)-AP4, which are the ω-phosphono analogues of aspartate and glutamate respectively, were relatively ineffective as inhibitors of binding, whereas the longer chain analogue, (±)-AP7, was almost equipotent with (±)-AP5.

0028–0836/84/050460—03$01.00

© 1984 Macmillan Journals Ltd

Table 1 Inhibition of [^{3}H]-D-AP5 binding to cortical membranes by excitatory amino acid agonists and antagonists

Antagonists	K_i (μM)
(±)-2-Amino-3-phosphonopropanoate (AP3)	560
(±)-2-Amino-4-phosphonobutanoate (AP4)	260
(±)-2-Amino-5-phosphonopentanoate (AP5)	1.7
(±)-2-Amino-6-phosphonohexanoate (AP6)	42
(±)-2-Amino-7-phosphonoheptanoate (AP7)	2.4
(±)-2-Amino-8-phosphono-octanoate (AP8)	640
D(−)-AP5	0.62
L(+)-AP5	55
γ-D-Glutamylglycine (γ-D-GG)	40
(±)-*cis*-2,3-Piperidine dicarboxylate (*cis*-2,3-PDA)	78
γ-D-Glutamylaminomethyl sulphonate (γ-D-GAMS)	770
Agonists	
NMDA	11
Quisqualate	250
Kainate	870
L-Aspartate	11
L-Cysteate	120
L-Cysteine sulphinate	13
L-Glutamate	0.9
L-Homocysteate	3.9
L-Homocysteine sulphinate	10
L-*S*-Sulphocysteine	2.1
Quinolinate	350
L-*O*-Sulphoserine	25
L-*O*-Phosphoserine	1,000

Membranes were incubated with [^{3}H]-D-AP5 (20 nM) as described in Fig. 1 in the presence of a range of concentrations of inhibitor (10 nM–1 mM). IC_{50} values were determined from log concentration/inhibition curves and K_i values calculated[16]. Results are mean values from two or three experiments.

The homologues (±)-AP6 and (±)-AP8 were of intermediate potency (Table 1). There was a close quantitative correlation between the potency of these compounds as inhibitors of [^{3}H]-D-AP5 binding and their potencies as antagonists of NMDA-induced depolarizations and of electrically evoked depolarizations in spinal cord preparations (Fig. 3). The effects of other amino acid antagonists on [^{3}H]-D-AP5 binding also paralleled their known potencies as NMDA antagonists[1–3]. Thus binding was stereoselective for the D-isomer[2,3], L(+)-AP5 being 80-fold weaker than D(−)-AP5. The slight activity of the L form may be due to incomplete resolution, consistent with the small difference in magnitude of the optical rotations of the two isomers. In addition, γ-D-GG and *cis*-2,3-PDA, which are less potent (and less selective) antagonists for NMDA receptors than are AP5 and AP7, were of intermediate potency. By contrast, γ-D-GAMS, which antagonizes neuronal depolarizations induced by kainate and quisqualate with little or no effect on NMDA-induced responses[13,14], was ineffective as an inhibitor of [^{3}H]-D-AP5 binding (Table 1). This strongly suggests that [^{3}H]-D-AP5 binds to the NMDA-type receptor identified in electrophysiological studies.

Depolarizations induced by agonists such as glutamate and aspartate are composite responses mediated by different types of receptors. This, coupled with other complications, such as differential rates of amino acid uptake, makes it difficult to compare relative potencies of agonists at particular excitatory receptors in electrophysiological studies. It was, therefore, of great interest to determine affinities of a range of agonists tested as inhibitors of [^{3}H]-D-AP5 binding to cerebral cortical membranes. Of the three amino acids normally considered to be selective receptor agonists, NMDA was the most potent being 25 and 80 times more potent than quisqualate and kainate, respectively. (The quisqualate activity may indeed be due to a trace of glutamate detected in our preparation by high-voltage paper electrophoresis.) The other agonists listed in Table 1 have all been found in brain and/or other body tissues or fluids and can thus be considered as possible neurotransmitters. Both

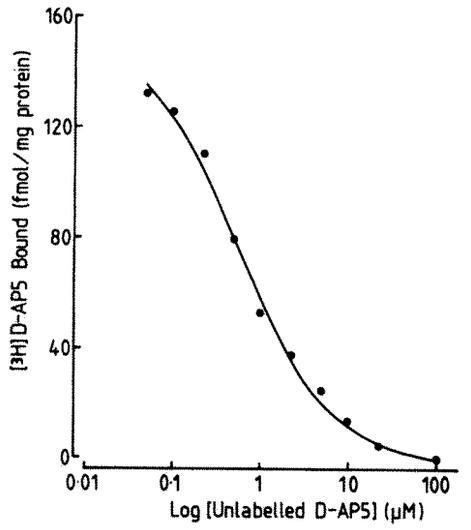


Fig. 1 Concentration dependence of [^{3}H]-D-AP5 binding to rat cerebral cortical membranes. Cerebral cortex from male Wistar rats was homogenized in 15 volumes ice-cold 0.32 M sucrose, *p*H 7, and the homogenate centrifuged at 1,000*g* for 10 min at 4 °C. The supernatant was recentrifuged (17,000*g*; 20 min) and the resultant P_2 pellet lysed with 40 volumes glass-distilled water (GDW). Following incubation at 35 °C for 30 min, the membranes were centrifuged at 50,000*g* for 10 min, then washed twice by resuspension in 40 volumes GDW and centrifugation (50,000*g*; 10 min). The final pellet was resuspended in 25 volumes 50 mM Tris-HCl buffer, *p*H 7.3. Binding of [^{3}H]-D-AP5 was measured by preincubating 0.4 ml aliquots of membrane suspension (0.3–0.5 mg protein) with 50 μl D-AP5 (final concentration 0.05–100 μM) or with buffer alone (control) for 3 min at 25 °C before addition of 50-μl [^{3}H]-D-AP5 (27.3 Ci mmol^{-1}; custom-labelled by Amersham International). Final concentration of [^{3}H]-ligand was 17 nM. Samples were centrifuged after 25-min incubation at 25 °C (20,000*g*; 1 min), the clear supernatant aspirated and the amount of radioactivity in the pellets measured by liquid scintillation counting (Packard Tricarb 460 CD). Specific binding of [^{3}H]-D-AP5 was determined by subtracting the nonspecific component (binding in the presence of 1 mM unlabelled D-AP5) from the total binding. Binding in control samples was 155 fmol per mg protein. Each point is the mean of three determinations. Protein was assayed as described by Lowry[17]. Results were fitted by least squares[18] to the logistic expression $Y = MX^p/(X^p + EC_{50}^p)$ and calculated values were *M*, 153 ± 9 fmol per mg protein; EC_{50}, 0.56 ± 0.11 μM and *P*, 0.9 ± 0.08.

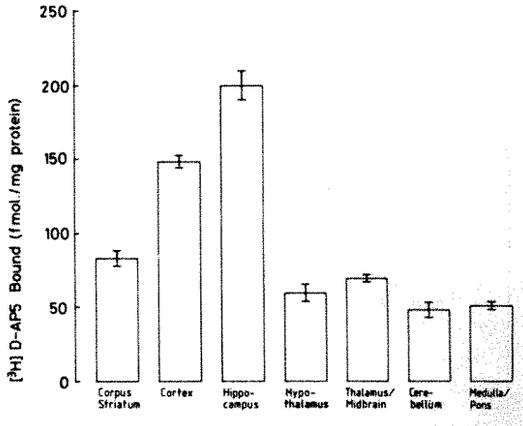


Fig. 2 Regional distribution of [^{3}H]-D-AP5 binding sites. Each brain area was carefully dissected as described by Glowinski and Iversen[20]. Membranes were prepared and specific binding of [^{3}H]-D-AP5 (16 nM) measured as in Fig. 1. Results are mean values ± s.e. ($n = 12$) from two separate experiments.

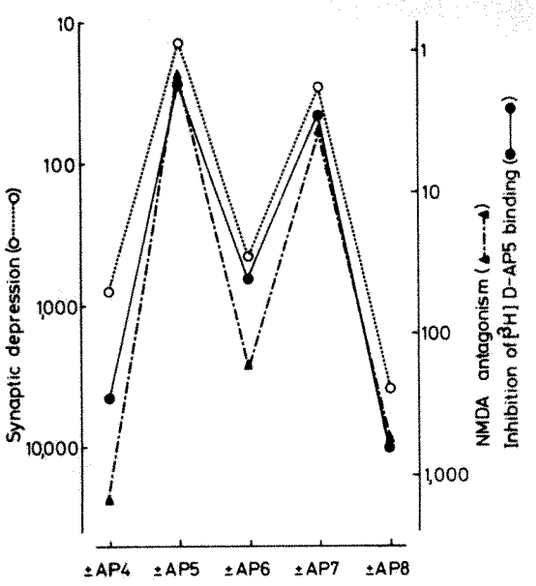


Fig. 3 Comparison of the effects of an homologous series of ω-phosphono-α-aminocarboxylates on electrically and NMDA-evoked responses recorded from ventral roots of frog spinal cord and on the binding of [^{3}H]-D-AP5 to rat cerebral cortical membranes. For details of electropharmacological measurements see ref. 2. [^{3}H]-D-AP5 binding was measured as described in Fig. 1, in the presence of concentrations of inhibitor between 10 nM and 1 mM. Left-hand ordinate scale and open circles indicate micromolar concentrations of phosphono-amino acid required to produce a 20% depression in amplitude of the pen recorded dorsal root-evoked ventral root potential. The right-hand ordinate scale and filled triangles indicate concentrations (μM) required to produce a dose ratio of two for antagonism of NMDA-evoked responses and filled circles the K_i value (μM) for inhibition of [^{3}H]-D-AP5 binding.

L-aspartate and L-Glutamate were more potent than their sulphinic and sulphonic acid analogues. In addition, all the amino acids of the glutamate chain length were more potent than aspartate and its analogues. L-Glutamate had the highest affinity of all agonists tested, being more than ten times as potent as L-aspartate, and having a K_i similar to that of D-AP5 itself. In contrast, quinolinate, a known selective NMDA type agonist[7], had only low affinity for the D-AP5 binding site.

The findings of this study may be compared with those of another investigation in which [^{3}H]-(±)-AP7 was used as the binding ligand[15]. Although there are several similarities in the results of the two studies, the conclusion[15] that [^{3}H]-AP7 binds predominantly to quisqualate-type receptors conflicts with our findings with [^{3}H]-D-AP5 and, indeed, is somewhat paradoxical. Like AP5, AP7 is considered highly selective for NMDA-type receptors in electrophysiological studies, and has little or no effect on quisqualate induced depolarizations[2]. It is possible that the nature of the AP7 binding site was transformed by the conditions used (notably the use of freeze-stored rather than fresh membranes). Alternatively, a misleading profile of binding inhibition may have been obtained by the use of the racemic form of [^{3}H]-AP7 rather than the pharmacologically active D(−) form of the substance[19].

The high affinity of L-glutamate for [^{3}H]-D-AP5 binding sites found in the present work contrasts with the apparent low sensitivity of L-glutamate-induced responses to such specific NMDA receptor antagonists[1]. However, the sensitivity of L-glutamate-induced responses to specific NMDA antagonists is probably masked by the actions of exogenous L-glutamate at other receptors[5], perhaps including extrasynaptic receptors not involved in transmission processes. A role of NMDA receptors in synaptic excitation is, however, well established[1].

The present results constitute the first evidence as to the relative affinities of endogenous (and likely endogenous) excitatory amino acids for NMDA receptors. While relative affinities do not necessarily identify a transmitter, clearly L-glutamate must be considered a strong candidate for the transmitter acting not only at kainate/quisqualate receptors, but at NMDA receptors as well.

We thank Dr R. B. Barlow for advice on computer fitting and Mr D. J. Oakes for technical assistance. This work was supported by the Wellcome Trust and the MRC.

Received 12 September; accepted 8 November 1983.

1. Watkins, J. C. & Evans, R. H. *A. Rev. Pharmac. Toxicol.* **21,** 165–204 (1981).
2. Evans, R. H., Francis, A. A., Jones, A. W., Smith, D. A. S. & Watkins, J. C. *Br. J. Pharmac.* **75,** 65–75 (1982).
3. Davies, J. & Watkins, J. C. *Brain Res.* **235,** 378–386 (1982).
4. Fagg, G. E. & Foster, A. C. *Neuroscience* **9,** 701–719 (1983).
5. Watkins, J. C. in *Amino Acid Neurotransmitters* (eds Mandel, P. & DeFeudis, F. V.) 205–212 (Raven, New York, 1981).
6. Mewett, K. N., Oakes, D. J., Olverman, H. J., Smith, D. A. S. & Watkins, J. C. in *CNS Receptors—From Molecular Pharmacology to Behaviour* (eds Mandel, P. & DeFeudis, F. V.) 163–174 (Raven, New York, 1983).
7. Stone, T. W. & Perkins, M. N. *Eur. J. Pharmac.* **72,** 411–412 (1981).
8. Baudry, M. & Lynch, G. *J. Neurochem.* **36,** 811–820 (1981).
9. Sharif, N. A. & Roberts, P. J. *Brain Res.* **211,** 293–303 (1981).
10. Di Lauro, A., Meek, J. L. & Costa, E. *J. Neurochem.* **38,** 1261–1267 (1982).
11. Werling, L. L. & Nadler, J. V. *J. Neurochem.* **38,** 1050–1062 (1982).
12. Fagg, G. E., Foster, A. C., Mena, E. E. & Cotman, C. W. *Eur. J. Pharmac.* **88,** 105–110 (1983).
13. Davies, J., Evans, R. H., Jones, A. W., Smith, D. A. S. & Watkins, J. C. *Comp. Biochem. Physiol.* **72C,** 211–224 (1982).
14. Davies, J. & Watkins, J. C. *J. Physiol. Lond.* **332,** 108–109P (1982).
15. Ferkany, J. W. & Coyle, J. T. *Life Sci.* **33,** 1295–1305 (1983).
16. Cheng, V. C. & Prusoff, W. H. *Biochem. Pharmac.* **22,** 3099–3108 (1973).
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randell, R. J. *J. biol. Chem.* **193,** 265–275 (1951).
18. Barlow, R. B. in *Biodata Handling with Microcomputers* 135 (Elsevier, Cambridge, 1983).
19. Perkins, M. N., Collins, J. F. & Stone, T. W. *Neurosci. Lett.* **32,** 65–68 (1982).
20. Glowinski, J. & Iversen, L. L. *J. Neurochem.* **13,** 655–669 (1966).

Magnesium gates glutamate-activated channels in mouse central neurones

L. Nowak, P. Bregestovski & P. Ascher

Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46, rue d'Ulm, 75230 Paris, Cedex 05, France

A. Herbet & A. Prochiantz

Laboratoire de Neuropharmacologie, Collège de France, 75231 Paris, Cedex 05, France

The responses of vertebrate neurones to glutamate involve at least three receptor types[1]. One of these, the NMDA receptor (so called because of its specific activation by *N*-methyl-D-aspartate), induces responses presenting a peculiar voltage sensitivity[2–6]. Above resting potential, the current induced by a given dose of glutamate (or NMDA) increases when the cell is depolarized[4–6]. This is contrary to what is observed at classical excitatory synapses, and recalls the properties of 'regenerative' systems like the Na^+ conductance of the action potential. Indeed, recent studies of L-glutamate, L-aspartate and NMDA-induced currents have indicated that the current–voltage (I–V) relationship can show a region of 'negative conductance' and that the application of these agonists can lead to a regenerative depolarization[4–6]. Furthermore, the NMDA response is greatly potentiated by reducing the extracellular Mg^{2+} concentration ($[Mg^{2+}]_o$) below the physiological level (~1 mM)[7,8]. By analysing the responses of mouse central neurones to glutamate using the patch-clamp technique[9], we have now found a link between voltage sensitivity and Mg^{2+} sensitivity. In Mg^{2+}-free solutions, L-glutamate, L-aspartate and NMDA open cation channels, the properties of which are voltage independent. In the presence of Mg^{2+}, the single-channel currents measured at resting potential are chopped in bursts and the probability of opening of the

0028–0836/84/050462—04$01.00

© 1984 Macmillan

channels is reduced. Both effects increase steeply with hyperpolarization, thereby accounting for the negative slope of the *I–V* relationship of the glutamate response. Thus, the voltage dependence of the NMDA receptor-linked conductance appears to be a consequence of the voltage dependence of the Mg^{2+} block and its interpretation does not require the implication of an intramembrane voltage-dependent 'gate'.

The experiments were performed on mesencephalic or striatal neurones from 13- or 15-day-old mouse embryos which were kept in culture for 6–10 days as described by Beaujouan *et al.*[10]. In the two patch-clamp configurations used ('whole-cell recording' and 'outside-out patches')[9], the pipette solution usually contained (mM): CsCl, 140; NaCl, 4; HEPES-K, 10 (*p*H 7.2); $CaCl_2$, 0.5; EGTA 5 (*p*Ca = 7.3), and the extracellular solution contained: NaCl, 140; KCl, 2.8; $CaCl_2$, 1.0; HEPES-Na, 10 (*p*H 7.2). Neurones used for whole-cell recordings were typically less than 12 μm in diameter with two or three fine processes. We can therefore assume that most of the cell membrane was adequately clamped in the whole-cell recording mode. The agonists, L-glutamate (referred to below as glutamate), L-aspartate, NMDA, quisqualate and kainate, were applied by bath perfusion.

In the whole-cell recording mode, when the membrane potential was held at −60 mV in a Mg^{2+}-free solution, glutamate (2–10 μM) elicited, in most neurones, an inward current which remained stable over periods of up to 10 min.

The effects of membrane potential and Mg^{2+} on the glutamate-activated current are illustrated in Fig. 1*a*. In the absence of extracellular Mg^{2+}, the glutamate-induced current varied nearly linearly with membrane potential. The reversal potential was 0 mV. The addition of extracellular Mg^{2+} (500 μM) produced little change in the outward currents, but markedly reduced the inward currents. The blocking effect of Mg^{2+} increased as the cell was hyperpolarized.

The effect of Mg^{2+} on the glutamate-induced current noise (Fig. 1*b*) was parallel to that observed on the total current. The power spectra of the glutamate-induced noise were well fitted by single lorentzians having a similar cut-off frequency at −40 and +40 mV in Mg^{2+}-free solution, and at +40 mV in Mg^{2+} (500 μM) (Fig. 1*c*). Under the usual assumptions made in the spectral analysis of transmitter-induced currents[11], this indicates that the mean open time of the channels opened by glutamate in Mg^{2+}-free solutions is independent of membrane potential. This open time is nearly 5 ms at 27 °C (Fig. 1*c*) and 3 ms at 30 °C (Fig. 1*b*). It is not altered by Mg^{2+} (1 mM) in the positive potential range.

We repeated the experiments described above on outside-out patches. In Mg^{2+}-free solutions, glutamate (1–10 μM) activated a homogeneous population of channels. With CsCl-containing pipettes, the elementary current varied linearly between −80 mV and +40 mV, inverting around 0 mV. The value of the single-channel conductance was 49 ± 8 pS ($n = 14$). When CsCl in the pipette was replaced by KCl, the analysis at potentials above −40 mV became difficult due to the currents flowing through the voltage-dependent K^+ channels. Between −80 mV and −40 mV, however, the glutamate-induced single-channel currents were identical to those measured with CsCl inside the pipette. In one experiment, external Cl^- was replaced by isethionate and the elementary current through the glutamate-activated channels was unchanged. These results suggest that the channels opened by glutamate are cation channels discriminating only slightly between monovalent cations, and

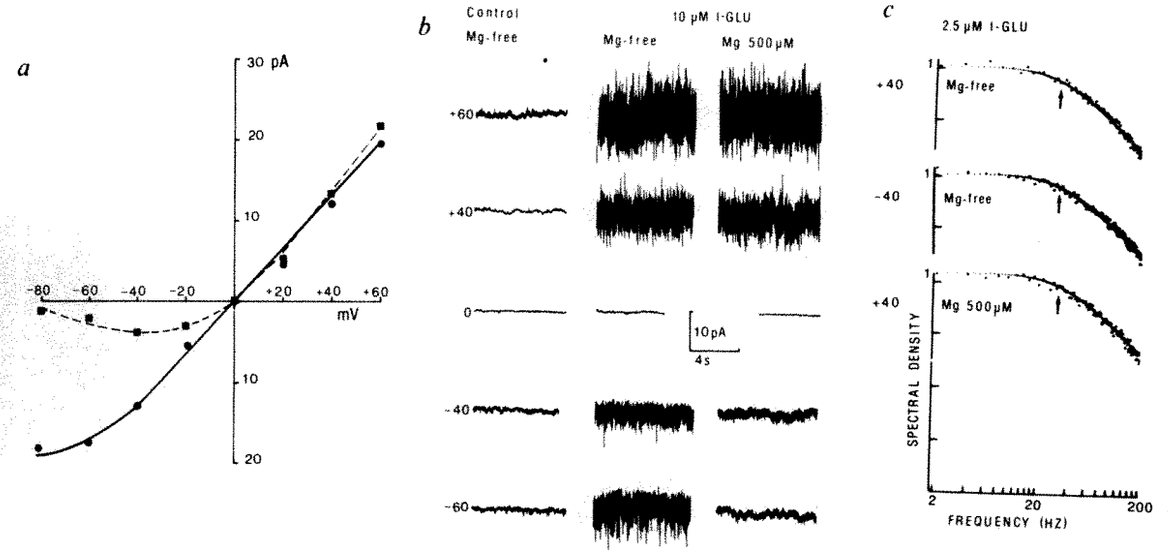


Fig. 1 Glutamate-induced currents in the whole-cell recording mode. ***a***, Voltage dependence of the glutamate-induced current. The glutamate-activated current was calculated at each potential as the difference between the current measured in control (Mg^{2+}-free or 500 μM Mg^{2+}) solutions and after addition of glutamate (10 μM). In a Mg^{2+}-free solution (●), the *I–V* relationship is approximately linear between +60 and −60 mV. The bend observed below −60 mV was not observed in all experiments, although in many cases the slope of the *I–V* relation was smaller in the negative potential range than in the positive range. In a solution containing Mg^{2+} (500 μM) (■), the currents are reduced at negative potentials, and the *I–V* relationship shows a 'negative resistance' region. In the positive potential range, no significant change is observed. ***b***, Glutamate-induced current noise. Same experiment as in *a*. The d.c. shift of the current is not illustrated. The control traces shown in the left-hand column were taken in Mg^{2+}-free solution in the absence of glutamate. The records of the central column were taken after the addition of glutamate (10 μM) (Mg^{2+}-free solution), and the records on the right were taken in a solution containing Mg^{2+} (500 μM) and glutamate (10 μM). The control records for 500 μM Mg^{2+} solutions (not shown) were identical to those shown for Mg^{2+}-free. ***c***, Power spectra of glutamate-induced current noise. In this experiment, the concentration of glutamate was 2.5 μM. Each spectrum was obtained by subtraction of control spectra from spectra recorded in the presence of glutamate. Spectral density: arbitrary units, log scale. The cut-off frequencies are: 34.5 Hz (upper record), 31.8 Hz (middle record) and 32.5 Hz (lower record). These frequencies correspond to respective time constants of 4.6, 5.0 and 4.9 ms. Temperature: 30 °C (*a*, *b*); 24 °C (*c*).

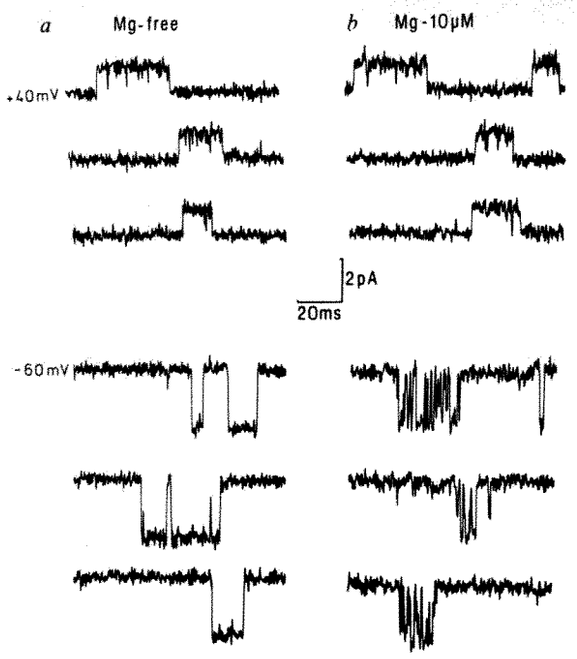


Fig. 2 Glutamate-induced single-channel currents in outside-out patches. In Mg^{2+}-free solution (*a*), the single-channel currents appear as simple events on both sides of the reversal potential (about −5 mV in this experiment). After addition of Mg^{2+} (10 μM) (*b*) the currents recorded at +40 mV are unchanged whereas the currents recorded at −60 mV appear as bursts. The size of the single-channel current is not altered by the addition of 10 μM Mg^{2+}. At higher Mg^{2+} concentrations (50–100 μM) an apparent reduction of the elementary current was observed (not shown), but this probably only reflects the inability of the recording system to display faithfully the transient openings of the channels. In this experiment, $MgCl_2$(2 mM) had been added to the internal solution. Note that the single-channel conductance is 51 pS at −60 mV, and only 33 pS at +40 mV. This discrepancy was observed in all experiments where Mg^{2+} (2 mM) was present in the internal solution but was not seen when Mg^{2+} was absent. Thus, when Mg^{2+} was absent both from the internal and external solutions, the *I*–*V* relationship of the glutamate-induced current was linear (between −60 and +60 mV) at the single-channel level as well as (Fig. 1) at the whole-cell level.

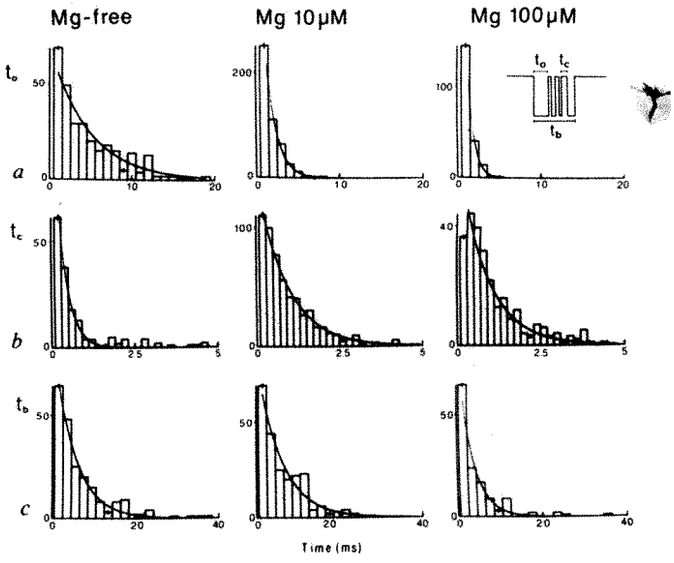


Fig. 3 Histograms of time intervals between open and closed states. Same experiment as in Fig. 2. Data recorded at −60 mV and analysed as described by Trautmann and Siegelbaum[23]. Bin length: 1 ms (*a*), 0.2 ms (*b*), 2 ms (*c*). Ordinate: number of observations. The insert (upper right) shows examples of open time (t_o), short closed time (t_c) and burst duration (t_b) used for analysis. *a*, Distribution of open times (t_o). The mean open time, $\bar{t}_o$, measured as the time constant of the exponential fitted on the histogram, was 4.7 ms in Mg^{2+}-free solution, 1.2 ms in 10 μm Mg^{2+}, and 0.7 ms in 100 μM Mg^{2+}. *b*, Distribution of short closed times (t_c). The values plotted are the durations of the short closures (gaps) inside the bursts illustrated in Fig. 2. The mean value, $\bar{t}_c$, was 0.3 ms in Mg^{2+}-free solution, 0.88 ms in 10 μM Mg^{2+}, and 0.84 ms in 100 μM Mg^{2+}. The last two values are comparable, as expected from a simple fast channel block model, in which they would imply a value close to $1{,}100\ s^{-1}(1/\bar{t}_c)$[14,18] for the rate constant of dissociation of Mg^{2+} from the channel. The $\bar{t}_c$ values in Mg^{2+}-free correspond to rare events, one of which is illustrated in Fig. 2*a*, middle record at −60 mV, Mg^{2+}-free. The gaps observed in such 'control' conditions were not analysed in detail, but their frequency and mean duration were similar at −60 mV and at +40 mV (not shown). *c*, Distribution of burst durations (t_b). The burst durations were measured by the same method as the open time, except that gaps of less than 5 ms (instead of gaps of 0.5 ms in the case of open times) were ignored. The time constant of the exponential, $\bar{t}_b$, was 5.7 ms in Mg^{2+}-free solution, 4.9 ms in 10 μM Mg^{2+}, 3.2 ms in 100 μM Mg^{2+}. The difference between $\bar{t}_o$ and $\bar{t}_b$ in Mg^{2+}-free solutions corresponds to the presence of the few gaps mentioned above.

resembling in this respect the glutamate-activated channels of the insect neuromuscular junction[12,13].

The open-time distribution of the single-channel currents could be reasonably well fitted by a single exponential with a voltage-independent time constant. Finally, the frequency of occurrence of the channel openings was (in a given patch) not noticeably affected by membrane potential. It thus appears that in Mg^{2+}-free solution, the three 'elementary' properties of the individual channels (elementary conductance, mean open time and frequency of opening) are, to a first approximation, voltage independent.

In the presence of extracellular Mg^{2+} (10 μM) the single-channel currents observed at positive potentials were identical to those recorded in the absence of Mg^{2+}, but at negative potentials major modifications were observed. The most striking change (Fig. 2) was that each channel opened in a burst, in a pattern similar to that first observed by Neher and Steinbach[14] in their study of the effects of local anaesthetics on acetylcholine-induced currents.

The appearance of such bursts suggested that extracellular Mg^{2+} may be acting by a 'fast channel block' mechanism[14–18]. We attempted to test some of the predictions of the simplest form of this scheme, in which Mg^{2+} ions block only the open state. If we call $\bar{t}_o$ the mean open time of a channel, $\bar{t}_b$ the mean duration of the group of openings constituting a burst, and $\bar{t}_c$ the mean value of the gap duration (blocked time) inside a burst[14,18] (see insert, Fig. 3), the fast channel block scheme predicts that as the Mg^{2+} concentration, $[Mg^{2+}]_o$, increases, $\bar{t}_o$ should decrease while $\bar{t}_c$ should remain constant. These two predictions were verified in the experiment illustrated in Fig. 3. However, the simple sequential scheme also predicts that, at low Mg^{2+} concentrations, the burst duration should increase when $[Mg^{2+}]_o$ increases, whereas the total charge carried during a burst should remain constant (see also ref. 19). This prediction was not verified. As illustrated in Fig. 3, the burst duration decreased when $[Mg^{2+}]_o$ was increased, and this effect was enhanced by hyperpolarization (not shown).

That a simple fast-channel block model cannot account for all the blocking effects of Mg^{2+} is further indicated by the fact (Fig. 4) that Mg^{2+} reduces the frequency of opening of the glutamate-induced channels. This effect and the reduction of burst duration (illustrated in Fig. 3) may well be the result of a common underlying mechanism, for example, slow channel block[20] or increased desensitization[21]. In general, it is premature to propose any specific scheme but it seems clear that the blocking effect of Mg^{2+} involves at least two voltage-dependent processes, one accounting for the fast flickering of the single-channel current, the other explaining the reduced frequency of

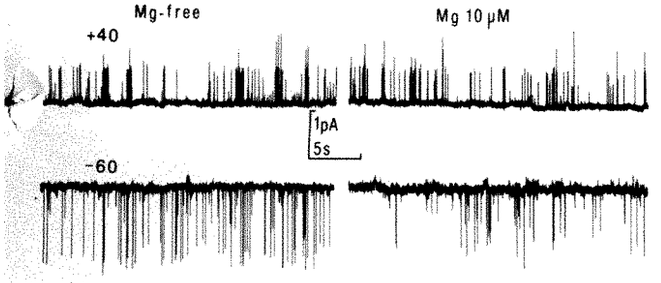


Fig. 4 Glutamate-induced currents in outside-out patches. Same experiment as in Fig. 2. The records, taken at slow speed on a chart recorder, illustrate the reduction in the frequency of channel openings produced by Mg^{2+} (10 μM) at −60 mV (lower records), whereas no change in frequency is observed at +40 mV (upper records). The small size of the elementary currents, and the apparent reduction of their size by Mg^{2+} (−60 mV) both result from the filtering introduced by the chart recorder and were not present when the currents were displayed on an oscilloscope (Fig. 2).

channel openings. A similar dual block has been recently proposed for the Na^+ block of some K^+ channels[22].

The hypothesis that the Mg^{2+}-sensitive glutamate-activated channels are of the NMDA type was supported by the finding that, both in the whole-cell recording mode and in outside-out patches, NMDA (2–10 μM) and L-aspartate (10 μM) elicited responses similar to those induced by L-glutamate, while kainate (up to 10 μM) and quisqualate (up to 5 μM) were ineffective.

Thus, our observations provide an explanation for the ionic mechanism of the glutamate response associated with NMDA receptors. Whereas early observations had suggested that glutamate may act through a K^+ conductance decrease[3], more recent work has indicated that L-glutamate, L-aspartate and NMDA all induce a voltage-dependent conductance increase[4–6]. Our data confirm this interpretation and further indicate that the channels opened by glutamate are permeant to both Na^+ and K^+. In addition, they suggest that the voltage dependence of the conductance does not result from a direct effect of the membrane potential on the receptor–channel complex but rather is the consequence of a voltage-dependent Mg^{2+} blockade.

This work was supported by grants from the CNRS (LA 04–295), MIR (81-E-1382) and Université Pierre et Marie Curie. L.N. is a NSF–CNRS fellow. P.B. was supported by the CNRS during his leave of absence from the USSR Cardiology Research Centre, Academy of Medical Sciences, Moscow, USSR.

Received 26 September; accepted 8 November 1983.

1. Watkins, J. C. & Evans, R. H. *A. Rev. Pharmac.* **21,** 165–205 (1981).
2. Shapovalov, A. I., Shiriaev, B. I. & Velumian, A. A. *J. Physiol., Lond.* **279,** 437–455 (1978).
3. Engberg, I., Flatman, J. A. & Lambert, J. D. C. *J. Physiol., Lond.* **288,** 227–261 (1969).
4. MacDonald, J. F., Porietis, A. V. & Wojtowicz, J. M. *Brain Res.* **237,** 248–253 (1982).
5. MacDonald, J. F. & Porietis, A. V. *Soc. Neurosci, Abstr.* **8,** 796 (1982).
6. Flatman, J. A., Schwindt, P. C., Crill, W. E. & Stafstrom, C. E. *Brain Res.* **266,** 169–173 (1983).
7. Ault, B., Evans, R. H., Francis, A. A., Oakes, D. J. & Watkins, J. C. *J. Physiol., Lond.* **307,** 413–428 (1980).
8. Scatton, B. & Lehmann, J. *Nature* **297,** 422–424 (1982).
9. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. *Pflügers Arch. ges. Physiol.* **391,** 85–100 (1981).
10. Beaujouan, J. C. *et al. Molec. Pharmac.* **22,** 48–55 (1982).
11. Neher, E. & Stevens, C. F. *A. Rev. Biophys. Bioengng* **6,** 48–381 (1977).
12. Jan, L. Y. & Jan, Y. N. *J. Physiol., Lond.* **262,** 215–236 (1976).
13. Anwyl, R. *J. Physiol., Lond.* **273,** 367–388 (1977).
14. Neher, E. & Steinbach, J. H. *J. Physiol., Lond.* **277,** 153–176 (1978).
15. Armstrong, C. M. *J. gen. Physiol.* **58,** 413–437 (1971).
16. Adams, P. R. *J. Physiol., Lond.* **260,** 531–552 (1976).
17. Fukushima, Y. *J. Physiol., Lond.* **331,** 311–331 (1982).
18. Colquhoun, D. & Hawkes, A. G. in *Single Channel Recording* (eds Sakmann, B. & Neher, E.) 135–175 (Plenum, New York, 1983).
19. Neher, E. *J. Physiol., Lond.* **339,** 663–678 (1983).
20. Ascher, P., Marty, A. & Neild, T-O. *J. Physiol., Lond.* **278,** 207–235 (1978).
21. Clapham, D. & Neher, E. *Naunyn-Schmiedebergs Archs Pharmak.* **322,** R62, 1983: *J. Physiol., Lond.* (in the press).
22. Marty, A. *Pflügers Arch. ges. Physiol.* **396,** 179–181 (1983).
23. Trautmann, A. & Siegelbaum, S. in *Single Channel Recording* (Sakmann, B. & Neher, E.) 473–480 (Plenum, New York, 1983).

Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis?

Thomas E. DeCoursey*, K. George Chandy†, Sudhir Gupta† & Michael D. Cahalan*

* Department of Physiology and Biophysics and † Division of Basic and Clinical Immunology, Department of Medicine, University of California, Irvine, California 92717, USA

Membrane receptors and ion transport mechanisms probably have an important role in lymphocyte activation leading to T-lymphocyte proliferation in the immune response. Here we have applied a gigaohm-seal patch clamp technique[1] to reveal the identity and properties of ion channels in human T lymphocytes. A voltage-dependent potassium channel bearing a resemblance to the delayed rectifier of nerve and muscle cells was found to be the predominant ion channel in these cells. In the whole cell recording conformation, the channels open with sigmoid kinetics during depolarizing voltage steps, reaching a maximum K^+ conductance of 3–5 nS. The current subsequently becomes almost completely inactivated during a long-lasting depolarization. Currents through single K^+ channels recorded in whole cell and outside-out patch recording conformations reveal a unitary channel conductance of about 16 pS in normal Ringer solution. Thus, the peak current corresponds to approximately 200–300 conducting K^+ channels per cell. Phytohaemagglutinin (PHA), at concentrations that produce mitogenesis, alters K^+ channel gating within 1 min of addition to the bathing solution, causing channels to open more rapidly and at more negative membrane potentials. ^{3}H-thymidine incorporation by T lymphocytes following PHA stimulation is inhibited by the 'classical' K^+ channel blockers tetraethylammonium and 4-aminopyridine, and also by quinine, at doses found to block the K^+ channel in voltage-clamped T lymphocytes, suggesting that K^+ channels may play a part in mitogenesis.

Figure 1 shows outward potassium currents recorded from a human T lymphocyte in the whole cell conformation. Depolarizing voltage steps up to −40 mV given from a holding potential of −80 mV produce very small leakage currents, representing an input resistance of greater than 20 GΩ for this cell. At −30 mV, outward current is just noticeable, and larger depolarizing pulses produce larger outward currents as more channels open. The current is carried by K^+ ions from the cytoplasm in diffusional equilibrium with the pipette solution. If the extracellular K^+ concentration is raised to the concentration within the pipette (not shown), we observe inward K^+ current below a well defined reversal potential near 0 mV. During a depolarizing pulse, the voltage-gated K^+ channels open after an initial delay and then slowly become inactivated. The inactivation process most closely resembles the K^+ channel inactivation described in molluscan nerve cell bodies[2] displaying 'cumulative inactivation', as shown in the inset of Fig. 1. Here, two identical voltage pulses to 0 mV are given, separated by 50 ms. The slow decline of current during the first pulse is interrupted by repolarization, and during the second pulse current rises to a lower level than at the end of the first pulse. This phenomenon has been modelled in a kinetic scheme with two separate inactivated states for the channel[3].

The K^+ channel inactivation process has two consequences for our experiments. First, because recovery from inactivation is very slow, pulses delivered faster than every 20 s result in decreasing current magnitude during a pulse series. We have found that replacement of chloride by fluoride in the pipette solution helps to provide the stability required at low pulse frequencies, allowing recording over a period of hours with little rundown. Second, because inactivation is nearly complete at depolarized potentials, it is possible to record current through single channels in the whole cell conformation, as illustrated in

© 1984 Macmillan Journals Ltd

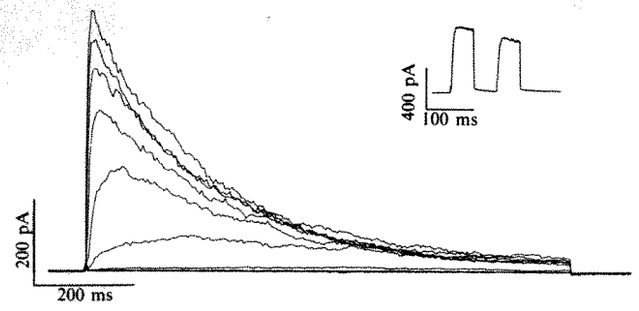


Fig. 1 Currents recorded from a T lymphocyte in the whole cell conformation. Peripheral blood was obtained from healthy volunteers (initials given in figure legends), and T lymphocytes were separated as described previously[29]. The membrane potential was held at −80 mV, and depolarizing pulses were given every 20 s in 10 mV steps up to +30 mV. Currents were recorded with a 10 GΩ feedback resistor, with active lowpass filtering at 2 kHz. Cell from S.C. Inset: a K^+ current record illustrating cumulative inactivation. Twin depolarizing pulses to 0 mV from a holding potential of −80 mV. Cell from S.G. T lymphocytes were suspended in medium RPMI-1640 supplemented with 10% human AB serum, 25 mM HEPES, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 2 mM L-glutamine (Gibco), and maintained at 37 °C in humidified 5% CO_2 atmosphere. In three separate determinations the cell population studied contained more than 90% T lymphocytes, estimated by Leu-1 (Pan T, Becton-Dickinson) monoclonal antibody binding and fluorescence activated cell sorter analysis. All voltage clamp measurements were made at room temperature (20–22 °C). The pipette input resistance, usually 3–6 MΩ, was measured with the pipette in the bath, and was used to set the series resistance compensation. The cells were bathed in a mammalian Ringer solution containing (in mM) 160 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES, at *p*H 7.4. The pipette contained an 'internal' solution consisting of 140 KF, 1 $CaCl_2$, 2 $MgCl_2$, 11 K_2EGTA and 10 HEPES, *p*H 7.2 (free Ca^{2+} concentration less than 2×10^{-9} M, based on a solubility product of 3.4×10^{-11} M^3 for CaF_2[30]. High resistance seals (often 50–200 GΩ) sometimes formed spontaneously on touching the pipette to the cell surface, but more often after application of a small negative pressure (usually <20 cm H_2O). Whole cell recording was achieved by applying strong negative pressure to the pipette interior (more than 100 cm H_2O) in order to disrupt the membrane patch within the pipette.

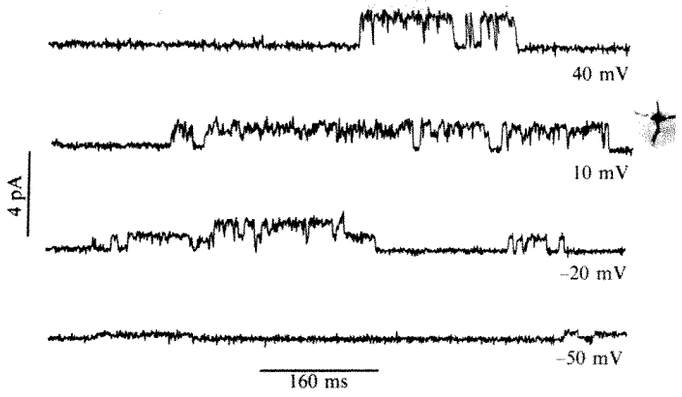


Fig. 2 Single K^+ channel currents recorded from a T lymphocyte in the whole cell conformation. Same bathing and pipette solutions as Fig. 1. Membrane potential held at potentials indicated. 800 Hz filter. Cell from S.G.

Table 1 Viability of T lymphocytes exposed to K^+ channel blockers

Expt	Treatment	% Viability (Trypan)	^{3}H-thymidine (c.p.m. ± s.d.)
I	Control	83	17,000 ± 5,000
	4AP + PHA	87	19,700 ± 9,100
II	Control	95	51,400 ± 2,700
	TEA + PHA	92	48,200 ± 4,800
III	Control	100	73,600 ± 3,400
	Quinine + PHA	94	53,400 ± 9,300

T lymphocytes (2×10^6 ml^{-1}) were incubated in sterile 12 × 75 mm tubes in medium described in Fig. 1 at 37 °C in humidified 5% CO_2 in the presence of 50 μg ml^{-1} PHA and 8.6 mM 4AP, 20 mM TEA or 100 μM quinine, for 48 h. Control T lymphocytes were suspended in medium alone for the same duration. Cells were then washed thrice in Hank's balanced salt solution supplemented with 25 mM HEPES, resuspended in media at 10^6 cells ml^{-1} and viability assessed by Trypan blue dye exclusion. The cells were then cultured in microtitre plates (10^5 cells per well) and incubated with 8 μg ml^{-1} PHA, and ^{3}H-thymidine uptake was measured 64 h later. Cells were from M.T. (I), N.W. (II) and K.C. (III). Viability in the presence of 4AP and TEA was assessed in three additional determinations by Trypan blue dye exclusion with similar results. All concentrations given are final concentrations.

Fig. 2. When the membrane potential is stepped and held at a depolarized level, K^+ current is first turned on and then becomes inactivated nearly to zero, with single channel events seen above the background noise. From records such as these, and from outside-out recordings in which the patch is excised from the cell (not shown), we estimate a single channel conductance of about 16 pS in normal Ringer. The single channel currents are 'flickery' and tend to become inactivated with rapid pulsing, whether recorded from intact cells on first making a seal, from whole cells as in Fig. 2, or in outside-out patches. Dividing the mean maximum whole cell conductance, 4 nS, by the single channel conductance, provides an estimate of at least 250 conducting K^+ channels per cell, although more channels may be present than those opening during a large depolarization.

In a small number of cells, whole cell recording revealed qualitatively different outward currents that slowly become activated at potentials above 0 mV and fail to become inactivated. This minority population of cells may represent contaminating non-T cells that are present in the T-lymphocyte-enriched population at a frequency of about 10%. In more than 90% of the cells studied, the voltage-gated K^+ channel described here was clearly dominant in the recordings. Our recording conditions, with very low free calcium concentration and fluoride in the pipette, may have prevented our seeing other channels that might be present in intact T lymphocytes, such as calcium channels reported to be present in a B-cell myeloma line[4] or calcium-activated channels. Calcium currents in snail neurones are abolished by internal perfusion with fluoride-containing solutions[5]. The K^+ channel described here differs from calcium-activated 'maxi-K^+' channels described previously in other tissues[6,7] in its calcium sensitivity, gating properties, single channel conductance and pharmacological sensitivity. The free Ca^{2+} in our pipette solution was only 2 nM (see Fig. 1 legend), whereas 1–10 μM internal Ca^{2+} is required to activate Ca^{2+}-dependent 'maxi-K^+' channels with the voltage dependence found in the present work[8–10]. We have not investigated the possible dependence of the K^+ channel on intracellular free Ca^{2+} concentration. Sigmoid activation kinetics and voltage-dependent inactivation are characteristic of delayed rectifier K^+ channels, but not Ca^{2+}-activated K^+ channels. The ubiquitous maxi-K Ca^{2+}-activated channels have a very large unitary conductance, 100–240 pS[6], although Ca^{2+}-activated K^+ channels of 20–50 pS, closer to the 16 pS found here, have been reported in *Helix* neurones[11,12] and in red blood cells[13], but have not yet been well characterized. Pharmacological experiments provide additional characterization of the voltage-gated K^+ channels. T-lymphocyte K^+ channels are blocked reversibly in a dose-dependent manner by tetraethylammonium (TEA), 4-aminopyridine (4AP) and quinine. Figure 3*a* illustrates dose–response curves for channel block by these three agents. The curves represent a one to one drug molecule–receptor relationship, fitted by eye to the measured fractional current remaining at various drug concentrations. Quinine is the most potent, with an inhibitory constant, K_i, of

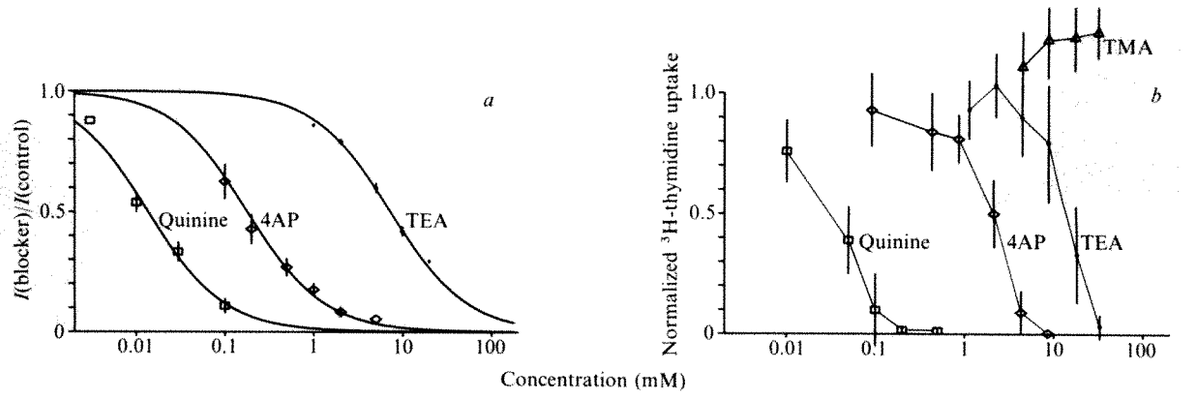


Fig. 3 Dose-response curve for blockade of the K^+ channel (*a*) and inhibition of mitogenesis (*b*) by quinine (□), 4AP (◇) and TEA (●) in T lymphocytes. Also shown in (*b*) are data for tetramethylammonium (TMA, △), which did not appreciably block K^+ currents. *a*, Data points show the mean K^+ current remaining in the presence of the indicated concentration of blocker added to Ringer solution. Cells from S.G., S.B., E.S. and M.T. PHA, at concentrations used for mitogenesis, seemed to have no effect on blocking by TEA or 4AP, and for these blockers data in the presence and absence of PHA have been pooled. s.e. bars are shown for means including data from three or four cells. In all experiments the membrane potential was held at −80 mV and stepped briefly to +20 mV every 30 s. After leak subtraction the peak current in the presence of blocker was normalized to the average of control currents recorded before addition and after washout of blocker. The bath temperature was monitored with a thermistor, and current records were temperature matched to within 0.2 °C. The preparation was allowed to equilibrate in each test solution until the peak current amplitude appeared to have reached steady-state. In 4AP-containing solutions both block and recovery progressed more slowly than in quinine or TEA-containing solutions, and about 5 min was allowed before recording. Recovery from the first application of 4AP to a cell was about 90% complete, so data from the first exposure to 4AP were not included. The curves express a bimolecular interaction between blocker and channel, with the fractional current remaining expressed as $1/(1+(B/K_i))$, where B is the drug concentration and K_i is the equilibrium dissociation constant. The K_i adjusted by eye to fit the data points was 0.014 mM for quinine, 0.19 mM for 4AP and 8 mM for TEA. *b*, Cells from S.C., N.W., G.C., S.G., K.L., S.B. and M.T. 10^5 T lymphocytes per well in medium (Fig. 1 legend) were plated in 96-well round-bottomed microtitre plates (Corning) and incubated in the presence of PHA at a final concentration of 8 μg ml^{-1}, with or without quinine, 4AP or TEA at 37 °C in humidified 5% CO_2 for 64 h. Cells were pulsed with ^{3}H-thymidine (1 μCi per well) for the final 16 h. Stock solutions of blockers in medium were titrated to *p*H 7.2 and added to the cells to the appropriate final concentration. All experiments were done in triplicate. The data points show the mean and s.d. from three or four experiments, of ^{3}H-thymidine incorporation normalized to that in PHA-treated cells in the absence of blocker. Background counts from cells with no PHA added were always less than 200 c.p.m.

about 14 μM, followed by 4AP at 190 μM and TEA at 8 mM. Quinine is believed to block Ca^{2+}-activated K^+ channels, but this block is not specific[14]. In neuroblastoma cells, for example, quinine blocks both Ca^{2+}-activated and voltage-gated K^+ channels[15]. TEA also blocks both types of K^+ channels, whereas block by aminopyridines appears to distinguish between voltage-gated and Ca^{2+}-activated K^+ channels[7]. Thus, the K^+ channel described here seems to be distinct from any Ca^{2+}-activated channel described to date, but closely resembles voltage-gated delayed rectifier K^+ channels.

Phytohaemagglutinin is a potent mitogen for T lymphocytes, stimulating cell division by mechanisms that have been intensively studied[16–19]. Involvement of ionic channels at an early stage of PHA activation is suggested by reports of membrane depolarization[20], hyperpolarization[20,21], increased ion fluxes across the membrane[22,23] and increased intracellular free calcium[21,24]. We therefore studied the effects of the three K^+ channel blockers described above and found them to inhibit PHA-induced mitogenesis in a dose-dependent manner (Fig. 3*b*), with the same potency sequence as for channel block (Fig. 3*a*). These data suggest that mitogenesis is suppressed when some fraction (perhaps 50%) of the K^+ channels do not function. The relative potency of 4AP for channel block compared with inhibition of mitogenesis seems to be greater than that of TEA or quinine. This result may be due to scatter in the data, or it might reflect relief of 4AP block by depolarization, as reported in other tissues[25,26], since the normal lymphocyte resting potential is substantially more positive than the −80 mV holding potential used here[20,21,27,28]. Complete replacement of the external sodium by 160 mM tetramethylammonium (TMA), an analogue of TEA, produced very little reduction of K^+ currents (data not shown). Up to 33 mM TMA added to the medium did not inhibit mitogenesis, showing that the inhibition by TEA was not due to the higher osmolarity of the medium. Table 1 shows that T lymphocytes incubated for 48 h in the presence of PHA and 4AP, TEA or 100 μM quinine were viable, as assessed by Trypan blue dye exclusion and by the ability to respond normally to added PHA after washing. Viability was reduced by 200 μM quinine. 4AP did not nonspecifically prevent ^{3}H-thymidine uptake and incorporation because 4AP added at the time of thymidine addition, 48 h after PHA activation, did not alter thymidine incorporation.

PHA may bind to cell-surface glycoproteins, producing effects by altering membrane transport properties. Since the voltage-gated K^+ channel described above is the predominant ion channel in our patch clamp experiments, we studied the influence of PHA on the kinetics of K^+ channel gating. Figure 4 illustrates the major immediate effects of PHA on the K^+ channel. In this cell before PHA addition (Fig. 4*a*), K^+ current was just noticeable during depolarizing pulses to −20 mV. At more positive potentials more channels open. Shortly after adding 3 μg ml^{-1} PHA (Fig. 4*b*), current was noticeable at −40 mV, about 20 mV more negative than the control. As shown in Fig. 4*c* the conductance–voltage relationship appears steeper and shifted towards hyperpolarized potentials after PHA treatment, with little or no change in the maximum conductance. Taking the potential required to activate half-maximal conductance as a measure and pooling the data for eight cells at PHA concentrations of 3–100 μg ml^{-1}, PHA produced a hyperpolarizing shift of −15 ± 3.5 mV (mean ± s.e.m.), with larger PHA concentrations tending to produce larger shifts. At a given potential the channels opened more rapidly following addition of PHA, causing a decrease in the time to peak current, also seen in Fig. 4. The effect of PHA was not readily reversed by washing with normal Ringer. The primary action of PHA may be to increase the voltage-dependent rate constants that lead to K^+ channel opening. If PHA acts by altering external surface charges 'sensed' by channel activation gates, our results indicate that the local surface potential would be more negative after PHA treatment. As a result of the alteration of K^+ channel gating by PHA more K^+ channels would be open on the average, possibly contributing to the hyperpolarization and increased K^+ fluxes previously

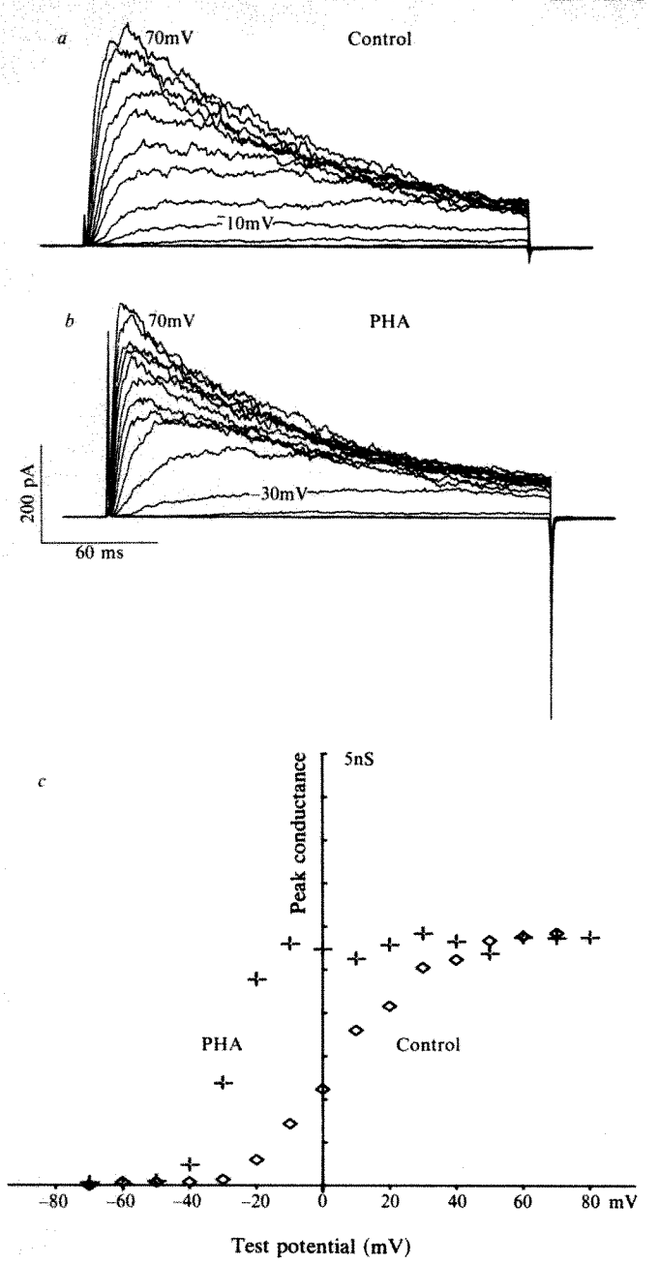


Fig. 4 *a* And *b* show whole cell currents recorded from a T lymphocyte from S.G. Same bathing and pipette solutions as previous figures, except 3 $\mu g\ ml^{-1}$ PHA (PHA-P, Difco) was added before recording currents in *b*. PHA solutions were mixed fresh daily from aliquots of frozen 10 $mg\ ml^{-1}$ stock solution. Same cell as Fig. 2. Depolarizing steps up to +70 mV in 10 mV increments were given every 20 s from the holding potential of −80 mV. *c*, The corresponding conductance–voltage relationships calculated by dividing the peak K^+ current at each depolarizing voltage step by the potential minus the measured reversal potential, −82 mV.

reported[21–24]. The rapid effect of PHA on K^+ channel gating, taken together with the inhibition of ^{3}H-thymidine incorporation by K^+ channel blockers, suggests that functioning K^+ channels altered by PHA may bring about a sequence of cellular and molecular events leading to DNA synthesis.

We thank Drs D. Corey, G. Yellen, R. Aldrich and V. Dionne for advice on patch clamp techniques, C. Rhodes and K. Lee for valuable technical assistance, and Dr J. Fernandez for advice on pipette solutions. This work was supported by grant NS14609 and by a Research Career Development Award to M.D.C. from the NIH, and a fellowship to K.G.C. from the American Diabetes Association, Southern California Affiliate.

0028-0836/84/050468—04$01.00

Received 24 June; accepted 3 October 1983.

1. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges Physiol.* **391,** 85–100 (1981).
2. Aldrich, R. W., Getting, P. A. & Thompson, S. H. *J. Physiol., Lond.* **291,** 507–530 (1979).
3. Aldrich, R. W. *Biophys. J.* **36,** 519–532 (1981).
4. Fukushima, Y. & Hagiwara, S. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2240–2242 (1983).
5. Kostyuk, P. G., Krishtal, O. A. & Pidoplichko, V. I. *Nature* **257,** 691–693 (1975).
6. Latorre, R. & Miller, C. *J. Membrane Biol.* **71,** 11–30 (1983).
7. Schwarz, W. & Passow, H. *A. Rev. Physiol.* **45,** 359–374 (1983).
8. Barrett, J. N., Magleby, K. L. & Pallotta, B. S. *J. Physiol., Lond.* **331,** 211–230 (1982).
9. Wong, B. S., Lecar, H. & Adler, M. *Biophys. J.* **39,** 313–317 (1982).
10. Methfessel, C. & Boheim, G. *Biophys. Struct. Mech.* **9,** 35–60 (1982).
11. Lux, H. D., Neher, E. & Marty, A. *Pflügers Arch. ges. Physiol.* **389,** 293–295 (1981).
12. Hermann, A. & Hartung, K. *Pflügers Arch. ges. Physiol.* **393,** 254–261 (1982).
13. Hamill, O. P. in *Single Channel Recording* (eds Sakmann, B. & Neher, E.) 451–471 (Plenum, New York, 1983).
14. Lew, J. Z. & Ferreira, H. G. *Curr. Topics Membrane Transport* **10,** 217–277 (1978).
15. Fishman, M. C. & Spector, I. *Proc. natn. Acad. Sci. U.S.A.* **78,** 5245–5249 (1981).
16. Naspitz, C. K. & Richter, M. *Prog. Allergy* **12,** 1–85 (1968).
17. Oppenheim, J. J. & Rosenstreich, D. L. *Prog. Allergy* **20,** 65–194 (1976).
18. Wedner, H. J. & Parker, C. W. *Prog. Allergy* **20,** 195–300 (1976).
19. Resch, K. & Kirchner, H. *Mechanisms of Lymphocyte Activation* (Elsevier, Amsterdam, 1981).
20. Kieffer, H., Blume, A. J. & Kaback, H. R. *Proc. natn. Acad. Sci. U.S.A.* **77,** 2200–2204 (1980).
21. Tsien, R. Y., Pozzan, T. & Rink, T. J. *Nature* **295,** 68–71 (1982).
22. Quastel, M. R. & Kaplan, J. G. *Expl. Cell Res.* **63,** 230–233 (1970).
23. Segel, G. B., Simon, W. & Lichtman, M. A. *J. clin. Invest.* **64,** 834–841 (1979).
24. Metcalfe, J. C., Pozzan, T., Smith, G. A. & Hesketh, T. R. *Biochem. Soc. Symp.* **45,** 1–26 (1980).
25. Yeh, J. Z., Oxford, G. S., Wu, C. H. & Narahashi, T. *J. gen. Physiol.* **68,** 519–535 (1976).
26. Hermann, A. & Gorman, A. L. F. *J. gen. Physiol.* **78,** 63–86 (1981).
27. Felber, S. M. & Brand, M. D. *Biochem. J.* **210,** 885–891 (1982).
28. Deutsch, C. J., Holian, A., Holian, S. K., Danielle, R. P. & Wilson, D. F. *J. cell. Physiol.* **99,** 79–94 (1979).
29. Gupta, S. & Good, R. A. *Cell. Immun.* **34,** 10–18 (1977).
30. Sillén, L. G. & Martel, A. E. *Chem. Soc. Spec. Publ.* **17** (1964).

K channels in T lymphocytes: a patch clamp study using monoclonal antibody adhesion

D. R. Matteson & C. Deutsch

Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA

Ionic fluxes are thought to be involved in mediating the proliferation of peripheral blood lymphocytes (PBLs) in response to mitogenic substances[1,2]. Among the earliest events occurring after the addition of mitogen to cultured lympocytes are changes in rates of cation transport[3–9]. We were interested, therefore, in the possible role of ion channels in mediating the lymphocyte proliferative response. The development of patch clamp techniques by Neher and colleagues[10–12] has made it possible to study membrane conductances in a variety of small cell types[12]. We have developed a method which uses monoclonal antibodies to make cells adhere to solid surfaces for two major reasons: (1) it is much easier to patch clamp stationary cells, and (2) the method can be used to selectively adhere a particular cell type from a heterogeneous population. We have used these techniques here to identify whole-cell potassium currents, in lymphocytes recognized by OKT11 monoclonal antibody, which are increased 1.9-fold by mitogenic stimulation.

Subclasses of human T lymphocytes, of ~7 μm diameter, were selectively adhered to solid surfaces (plastic Petri dishes and glass coverslips) using monoclonal antibodies of known specificity[13,14]. Human T-lymphocyte populations have been defined by the presence of antigenic determinants on the cell surface. Using flow cytometry we found that 75% of the cells in the monocyte-depleted PBL suspension were T lymphocytes recognized by monoclonal antibody OKT3 (Table 1). Of these T cells, 66% were predominantly helpers recognized by OKT4 and 30% were predominantly suppressor recognized by antibody OKT8.

To demonstrate selective adhesion, we used both indirect and direct immunofluorescence microscopy, the former being more sensitive than the latter. In all experiments, suspensions of PBLs

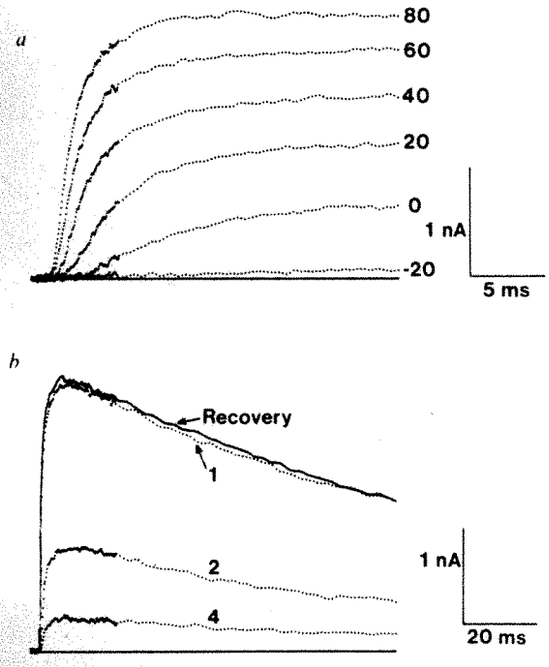


Fig. 1 Voltage-dependent outward currents in T lymphocytes. *a*, When lymphocytes were depolarized they generated an outward current. These currents were elicited by 25-ms step depolarizations to the indicated voltages from a holding potential of −70 mV. The currents in *b* were generated by 100-ms depolarizations to +100 mV. Dotted traces labelled 1, 2 and 4 resulted from first, second and fourth pulses, respectively, of a train delivered at 1.5 Hz, and the solid trace was recorded 74 s after the train. For this experiment, cells were attached with OKT11 and had been stimulated with succinyl concanavalin A for 20 h.

Methods: Patch electrodes, of 2–5 MΩ resistance, were made with borosilicate glass and filled with the following solution (mM): 140 KCl, 2 $MgCl_2$, 10 EGTA and 10 HEPES (*p*H 7.3). Just before recording, the solution bathing the antibody-adhered cells was changed to a protein-free solution of the following composition (in mM): 130 NaCl, 10 $CaCl_2$, 2 $MgCl_2$, 5 KCl and 10 HEPES (*p*H 7.3). After obtaining a gigaseal against the cell, further suction was applied to rupture the patch of membrane under the electrode tip. In these conditions, the electrode monitors current through the entire cell surface. The electrode was connected to a current-to-voltage (I–V) converter and the analog current signal was acquired with an LSI 11/23 computer at 50 kHz and stored for later analysis. Linear components of capactive and ionic current were subtracted out with a P/2 procedure[26]. Cells were voltage-clamped by controlling the positive input of the I–V converter.

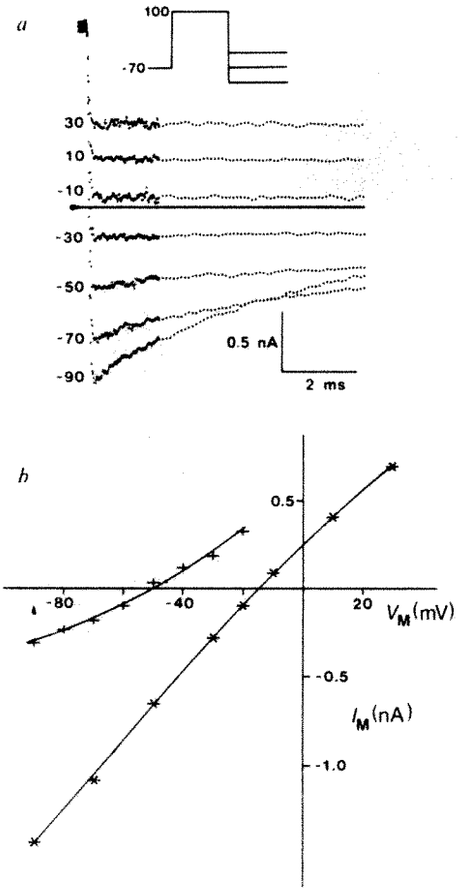


Fig. 2 K dependence of the reversal potential. The pulse protocol used to obtain the instantaneous current–voltage relationship is shown in the inset of *a*. The initial depolarization to +100 mV activated the outward current. The membrane was then stepped to various potentials and the resulting instantaneous change in current measured. For each trace shown in *a*, the first few points were taken before the pulse to provide a current baseline. Sampling was then stopped and restarted 200 μs before the second voltage step was applied. The voltage of the second step is indicated alongside each current trace. Pipettes were filled with the same solution as for Fig. 1 and the external solution had the following composition (mM): 85 NaCl, 60 KCl, 5 $CaCl_2$, 2 $MgCl_2$ and 10 HEPES. The magnitudes of the instantaneous currents shown in *a* (which were measured ~150 μs after the step because of the limited frequency response) are plotted in *b* (*); it can be seen that the current reverses direction at ~−15 mV. When the external solution contained only 15 mM K^+ (+) the reversal potential shifted to ~−50 mV. Cells were attached with OKT11 and had been stimulated with succinyl concanavalin A for 20 h.

were first depleted of monocytes by adhering these sticky cells to T150 tissue culture flasks (final monocyte content ~1%). For indirect immunofluorescence measurements, lymphocytes were incubated with either OKT3, OKT4 or OKT8 (Ortho Pharmaceutical Corp.), washed, made to adhere to goat anti-mouse IgG (Tago)-coated surfaces, then incubated with fluorescein-labelled Tago. In each case, almost all cells adhering to the surfaces were fluorescent. The following results were obtained in control experiments: (1) in the absence of antibodies, lymphocytes did not adhere to Tago-coated surfaces; (2) lymphocytes were not fluorescently labelled by incubation in suspension with fluorescein–Tago alone; and (3) Tago is necessary for adhesion of cells that have been labelled with monoclonal antibody. These results demonstrate that monoclonal antibody is necessary in order to observe fluorescence and adhesion, and therefore the specificity of the antibody imparts specificity to the cell attachment. Direct immunofluorescence measurements, in which adherent lymphocytes were incubated with OKT3, OKT4 or OKT8 (fluorescein-labelled) and then aliquoted onto Tago-coated surfaces, showed enhancement of the specific cell population on the surface, compared with its distribution in suspension (Table 1), again indicating selective adhesion. This adhesion method is accomplished by a simple protocol, is highly reproducible and could therefore be useful for a wide range of electrophysiological studies of specific cell types. In general, wherever cell types are distinguishable by their surface antigens, this method could be used to isolate particular cell types from heterogeneous populations.

We used this method to attach human lymphocytes for our patch clamp experiments. Cells were adhered with monoclonal antibody OKT3, 4 or 11. OKT11 is used for identification of human peripheral T lymphocytes; it binds to >95% of the cells that are E rosette-positive. The advantage of using OKT11 rather than OKT3 is that is is not mitogenic, and its use ensures that unstimulated cells are not activated by exposure to mitogenic antibody. We studied primarily OKT11-adhered cells, and in future studies we shall investigate those T-cell subsets predominantly recognized by OKT3, OKT4, OKT8 or other antibodies.

Table 1 Specificity of cell adhesion with monoclonal antibodies

Monoclonal antibody	% Positive in suspension	% Positive* bound to dish (direct immunofluorescence)	% Positive† bound to dish (indirect immunofluorescence)
OKT3	75.5	84	100
	74.9	85	
OKT4	49.9	64	100
OKT8	28.9	58	
OKT11			100

Human T lymphocytes were prepared from heparinized human venous blood which was collected from healthy donors, separated by gradient centrifugation using a modified Ficoll–Hypaque technique[25], suspended in minimal essential medium (MEM) or Hank's (Ca–Mg-containing medium) with 15% mixed human AB serum and incubated at 50×10^6 cells per flask (20 ml) in T-150 tissue culture flasks for 1.5 h. The supernatant was gently decanted, centrifuged at 1,500 r.p.m. for 10 min, resuspended in MEM and cultured in a 5% CO_2 incubator at 37 °C as described previously[25]. Goat anti-mouse IgG (Tago Inc., Burlingame, California; 1.5 ml of a 1:10 dilution in Tris buffer, *p*H 9.5) was added to a Falcon Petri dish (bacterial grade, 13×100 mm) at 0 °C and incubated for 40 min. After this time, the dishes were rinsed with cold Tris buffer then with cold phosphate-buffered saline (PBS, *p*H 7.2) and cells were poured onto the plate and placed in the cold (4 °C) for ⩾2 h. Coverslips (Corning) were treated in exactly the same way as the Falcon Petri dishes. Cells (1×10^6 ml^{-1}) suspended in 1 ml PBS/10% fetal calf serum (FCS) were incubated with fluorescein-labelled OKT3, 4 or 8 (0.5, 0.5 and 0.25 μg per sample, respectively) for 30 min, centrifuged and resuspended in 0.5 ml PBS. 0.25 ml was added to each coverslip and allowed to stand for 30 min at 0 °C. The coverslips were then washed in three successive rinse wells containing PBS and counted using a Zeiss fluorescence microscope (×40). In addition, 0.25-ml samples from the cell suspension were assayed using an Ortho-Spectrum III flow cytometer. Indirect immunofluorescence assays were carried out as described above, except that unlabelled OKT3, OKT4 and OKT11 (1 μg per 10^6 cells) were used. After incubation of the cell suspension on the coverslip for 30 min at 0 °C, the supernatant was removed and 100–200 μl of fluorescein–Tago (1:20 dilution) were added. The coverslips were then washed and the cells counted. All suspensions contained <1% monocytes. Those cells incubated with no antibody showed only weak autofluorescence.

* Fraction of labelled cells in 100–200 cells observed.

† No non-fluorescent cells were attached.

Macroscopic currents were recorded from human T lymphocytes using the whole-cell recording configuration of the patch clamp technique[12]. Gigaohm seals were routinely obtained on these cells with 2–5 MΩ borosilicate electrodes. When lymphocytes are depolarized, outward currents are generated (Fig. 1). Both the magnitude of the current and its activation kinetics increase as the membrane potential is made more positive. As a result of prolonged depolarization, the outward current inactivates (see Fig. 1*b*). Inactivation is manifested both as a decline in current during depolarization, and as a decrease in current magnitude during a train of voltage clamp steps.

Several lines of evidence indicate that K channels carry the outward current illustrated in Fig. 1. For example, we have shown that the reversal potential for the current is close to the K equilibrium potential. Reversal potentials were determined from instantaneous current–voltage relationships (see Fig. 2). With 60 mM K^+ outside and 140 mM K^+ inside, the current reversed at ~−15 mV whereas the K equilibrium potential predicted from the Nernst equation is −21 mV. When the external solution contained 15 mM K^+, the reversal potential shifted to −50 mV; the predicted shift is to −56 mV. Thus, the channel appears to be K-selective, but we have not conclusively identified the type(s) of K channels present. Agents that block K channels in other preparations block this conductance also. We have found that quinine (100 μM) reversibly blocks outward currents in human lymphocytes, which is consistent with previous reports that both the volume-induced and A23187-induced K and ^{86}Rb effluxes in these cells are blocked by quinine[15]. Cs^+ blocks K channels in several preparations[16,17], and we also find that the K currents in human T cells are abolished when K^+ in the patch electrode is replaced with Cs^+.

We were interested in comparing the K currents in mitogen-stimulated cells with those in unstimulated cells, because there are several precedents for mitogen-induced increases in plasma

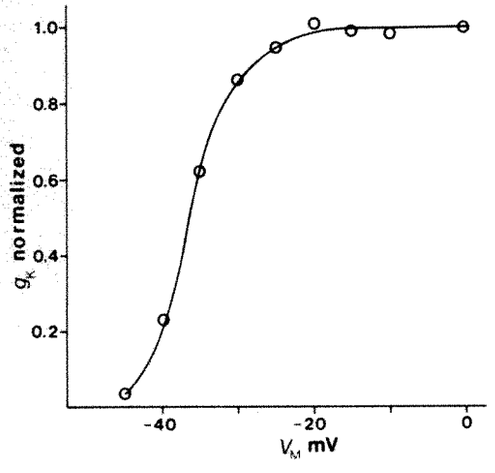


Fig. 3 Relationship of conductance to voltage. As the instantaneous *I–V* curve with 60 mM external K^+ is approximately linear, its slope was used as a measure of the K conductance activated during the initial depolarization (see also the pulse protocol in Fig. 2*a*). The activating voltage was varied in the range of membrane potentials indicated (V_M). The data points represent the average values obtained for three different cells 10–15 min after breaking into the cell. The midpoints of the individual conductance–voltage curves were at −35, −36 and −38 mV, indicating that they were in approximately the same position. The external solution contained (in mM): 85 NaCl, 60 KCl, 5 $CaCl_2$, 2 $MgCl_2$ and 10 HEPES (*p*H 7.3) and the internal solution contained (in mM): 100 KCl, 40 KF, 2 $MgCl_2$ and 10 HEPES (*p*H 7.3). Fluoride was included in the pipette solution because it increased the recording time in each cell. The K currents and the conductance–voltage relationship were not significantly different in the presence or absence of fluoride. The cells had been stimulated with succinyl concanavalin A for 24 h. Series resistance compensation was used as described previously[27].

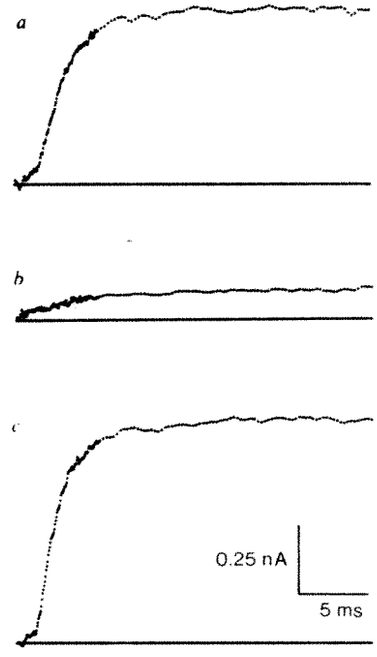


Fig. 4 External Ni^{2+} reduces K^+ currents in lymphocytes. The currents shown were generated by pulses to +50 mV and represent typical examples under the following conditions. *a*, Control current recorded in a solution of the following composition (in mM): 138 NaCl, 5 $CaCl_2$, 2 $MgCl_2$, 5 KCl and 10 HEPES (*p*H 7.3); *b*, after addition of 1 mM Ni^{2+} to the external medium; *c*, after returning to the control solution. Ni^{2+} clearly reduces the magnitude of the K current and the effect is reversible. The pipette solution was the same as for Fig. 3. Cells were stimulated with succinyl concanavalin A for 24 h.

membrane ion permeabilities[18–20]. Lymphocytes which had been stimulated with 50 μg ml^{-1} succinyl concanavalin A in culture for 20 h showed a 1.9-fold increase in K current. The current magnitude at +100 mV averaged 0.67 ± 0.31 nA (mean ±s.d.) in 15 unstimulated cells and 1.27 ± 0.66 nA in 21 stimulated cells, a difference that is significant ($P < 0.01$). It is not yet clear exactly what the relationship is between stimulated growth and increased K conductance. Additional studies are under way to determine whether the increase in conductance is due to an increase in number of channels or an increase in single-channel conductance.

To further characterize the K channels in mitogen-stimulated cells, we studied the relationship between membrane voltage and K conductance (g_K). Figure 3 shows that the threshold for g_K activation in human lymphocytes is ~ −45 mV, the midpoint of the curve is −36 mV and the conductance saturates at −20 mV. The maximum g_K averaged 11.8 ± 2.8 nS (mean ± s.d.) in 9 cells.

There is indirect evidence that the K conductance in human lymphocytes may, in part, be due to the presence of calcium-activated K channels. For example, in the presence of extracellular calcium, the Ca^{2+} ionophore A23187 causes a severalfold increase in ^{86}Rb efflux and uptake[15]. In addition, Tsien *et al.*[21] reported that in stimulated mouse thymocytes a rise in the concentration of intracellular free Ca was accompanied by cell hyperpolarization.

Therefore, we tried to obtain further evidence for the existence of Ca-activated K channels in human lymphocytes. One approach involved the use of Ni^{2+}, which is known to block Ca channels in other preparations[22]. Addition of 1 mM Ni^{2+} to the external medium reversibly reduced the K current, as illustrated in Fig. 4. This could reflect decreased Ca^{2+} entry which would result in a decreased activation of Ca-activated K channels. Alternatively, Ni^{2+} could be directly blocking the K channels, although we are not aware of any reports of this effect. Using Cs^+-filled electrodes to block the K conductance, we have been unable to observe any macroscopic Ca currents in these cells. Calcium channels may well be present in PBLs, but in numbers too small to detect as macroscopic current. By contrast, it is interesting that Fukushima and Hagiwara[23] found Ca channels in cultured myeloma cells; perhaps transformation is accompanied by an increased number of Ca channels[24].

In summary we have patch-clamped human T lymphocytes and identified potassium channels in both resting and mitogen-stimulated cells. Selective adhesion with monoclonal antibodies should allow us to characterize the membrane conductances of individual subsets of lymphocytes in a variety of conditions in which cellular and humoral immune responses occur.

This work was supported by NIH grant AM 27595, and by a research grant from the American Heart Association. C.D. is a recipient of a Research Career Development Award (AM 00838). We thank Dr J. Hoxie and Mr D. Mathews for their assistance with the immunofluorescence microscopy.

Received 24 June; accepted 8 December 1983.

1. Leffert, H. *Ann. N.Y. Acad. Sci.* **339,** 74–262 (1980).
2. Cameron, I. L. & Pool, T. B. (eds) *The Transformed Cell,* 1–420 (Academic, New York, 1981).
3. Kaplan, J. G. *A. Rev. Physiol.* **40,** 19–41 (1978).
4. Kaplan, J. G. & Owen, T. *Ann. N.Y. Acad. Sci.* **339,** 191–200 (1980).
5. Allwood, G., Asherton, G. L., Davey, M. J. & Goodford, P. J. *Immunology* **21,** 509–516 (1971).
6. Deutsch, C. & Price, M. *Biochim. biophys. Acta* **687,** 211–218 (1982).
7. Whitney, R. B. & Sutherland, R. M. *Cell. Immun.* **5,** 137–147 (1972).
8. Parker, C. W. *Biochem. biophys. Res. Commun.* **61,** 1180–1186 (1974).
9. Freedman, N. H., Raff, M. C. & Gomperts, B. *Nature* **255,** 378–382 (1975).
10. Neher, E. & Sakmann, B. *Nature* **260,** 799–802 (1976).
11. Neher, E., Sakmann, B. & Steinbach, J. H. *Pflugers Arch. ges. Physiol* **375,** 219–228 (1978).
12. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflugers Arch. ges. Physiol.* **391,** 85–100 (1981).
13. Reinherz, E. L. & Schlossman, S. F. *Immun. Today* **2,** 69–75 (1981).
14. Haynes, B. F. *Immun. Rev.* **57,** 127–161 (1981).
15. Grinstein, S., Dupre, A. & Rothstein, A. *J. gen. Physiol.* **79,** 849–868 (1982).
16. Bezanilla, F. & Armstrong, C. M. *J. gen. Physiol.* **60,** 588–608 (1972).
17. French, R. J. & Adelman, W. J. Jr *Curr. Topics Membranes Transport* **8,** 161–207 (1976).
18. Segel, G. B., Hollander, M. M., Gordon, B. R., Klemperer, M. R. & Lichtman, M. A. *J. cell. Physiol.* **86,** 327–336 (1975).
19. Hamilton, L. J. & Kaplan, J. G. *Can. J. Biochem.* **55,** 774–778 (1977).
20. Segel, G. B., Simon, W. & Lichtman, M. A. *J. clin. Invest.* **64,** 834–841 (1979).
21. Tsien, R. Y., Pozzan, T. & Rink, T. J. *Nature* **295,** 68–70 (1982).
22. Hagiwara, S. & Byerly, L. *A. Rev. Neurosci.* **4,** 69–125 (1981).
23. Fukushima, Y. & Hagiwara, S. *Biophys. J.* **41,** 292a (1983).
24. Moyer, M. P., Moyer, R. C. & Waite, M. R. F. in *Ions, Cell Proliferation and Cancer Symp.,* 53 (National Cancer Institute and W. Alton Jones Cell Science Center, Lake Placid, New York, 1982).
25. Deutsch, C., Price, M. & Johansson, C. *Expl Cell Res.* **136,** 359–369 (1981).
26. Armstrong, C. M. & Bezanilla, F. *J. gen. Physiol.* **63,** 533–552 (1974).
27. Matteson, D. R. & Armstrong, C. M. *J. gen. Physiol.* (in the press).

Retinoblastoma—origin from a primitive neuroectodermal cell?

A. P. Kyritsis*, M. Tsokos†, T. J. Triche† & G. J. Chader*

* Laboratory of Vision Research, National Eye Institute and
† Laboratory of Pathology, National Cancer Institute,
National Institutes of Health, Bethesda, Maryland 20205, USA

The histogenesis of retinoblastoma, the most common intraocular neoplasm of childhood[1], remains controversial. Previous studies have attributed the origin of the tumour to neuronal, glial or primitive stem cells of retina[2–7]. In the study described here we have used immunofluorescence to search for the presence of a neuronal marker, neurone-specific enolase (NSE)[8,9] and a glial marker, glial fibrillary acidic protein (GFAP)[10,11], in the cells of the human retinoblastoma line Y-79 (ref. 4), before and after successful differentiation into neuronal and glial-like cells. We found that all undifferentiated cells contain both NSE and GFAP, whereas the differentiating neuronal and glial-like cells gradually lose one marker and selectively express the marker that correlates with their morphology. Our results support the notion that retinoblastoma originates from a primitive bipotential (or multipotential) neuroectodermal cell.

Monolayers of Y-79 cells were obtained in dishes precoated with poly-D-lysine and fibronectin and kept in both serum-supplemented and serum-free defined media[12]. N^6,O^{12}-dibutyryl adenosine 3′,5′-cyclic monophosphate (dbcAMP) was added to some dishes with serum-supplemented media[12]. Cells growing in serum-supplemented medium retained their conventional morphology of small round cells[12] (Fig. 1*a*), whereas cultures growing in serum-free defined medium showed extensive neuronal differentiation, consisting of a gradual increase in cells with neuritic-type processes (up to 30%) and rosette formation (Fig. 1*b*). In contrast, dbcAMP caused a small but significant increase in flat, glial-like cells (up to 10%) (Fig. 1*c*).

Indirect immunofluorescence was done in order to investigate the presence of NSE and GFAP in these cells. Normal rabbit serum (1 : 50) was always used as a negative control. A human neuroblastoma line, SMS-SAN (ref. 13) and a human glioma line, U-251MG (ref. 14), were used as positive and negative controls for each marker; human normal fibroblasts maintained in culture for >1 yr were used as a negative control for both markers. The specificities of the antisera were further tested by primary tissue staining of 5-μm sections from paraffin blocks of formalin-fixed tissue, by using the immunoperoxidase (PAP) method of Sternberger *et al.*[15]. The tissues examined included both positive and negative controls: normal human brain and spinal cord (tested with both antisera); an olfactory neuroblastoma, a pineoblastoma, a medulloblastoma, a mixed ependymoma–oligodendroglioma, a spinal and a cerebral astrocytoma, and a mixed grade 4 astrocytoma–meningeal sarcoma (tested with anti-GFAP); and five neuroblastomas, five lymphomas, one medulloblastoma, five Ewing's sarcomas and four soft tissue sarcomas (tested with anti-NSE). The diagnoses for all the above cases had been documented by light microscopy and when necessary, by electron microscopy and by additional means such as study of surface markers for lymphomas.

0028-0836/84/050471-03$01.00

© 1984 Macmillan Journals Ltd

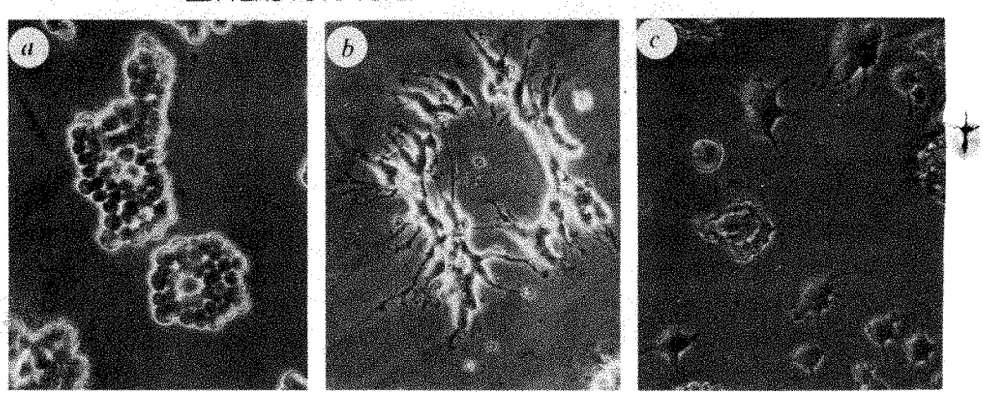


Fig. 1 Monolayers of the Y-79 cell line. *a*, Undifferentiated cells at 6 days in culture. Cells were seeded in 35-mm plastic dishes which had been pretreated with poly-D-lysine (0.2 mg ml^{-1}) at 23 °C for 6 min, washed once with minimum essential medium (MEM) containing 2 mM glutamine and 100 U ml^{-1} penicillin, and coated with fibronectin (5 μg ml^{-1}) at 37 °C for 30 min. The culture medium consisted of the medium described above, supplemented with 10% fetal bovine serum (×190). *b*, Cells demonstrating neuronal differentiation, at 22 days in serum-free, defined medium. Cells were seeded as for *a*, incubated for 2 h in serum-containing medium, and washed once with MEM. Finally, serum-free medium was added (supplemented with 5 μg ml^{-1} insulin, 10 μg ml^{-1} transferrin, 6.3 ng ml^{-1} progesterone, 8.8 ng ml^{-1} putrescine and 4 ng ml^{-1} sodium selinite) (×125). *c*, Flat cells with short cytoplasmic projections, resembling astrocytes (22 days in culture). In the centre there is a clump of small, closely packed undifferentiated cells. These cells were cultured in serum-containing medium; dibutyryl cyclic AMP (4 mM) was added at 3-day intervals beginning on day 8 of culture (five doses in total) (×155).

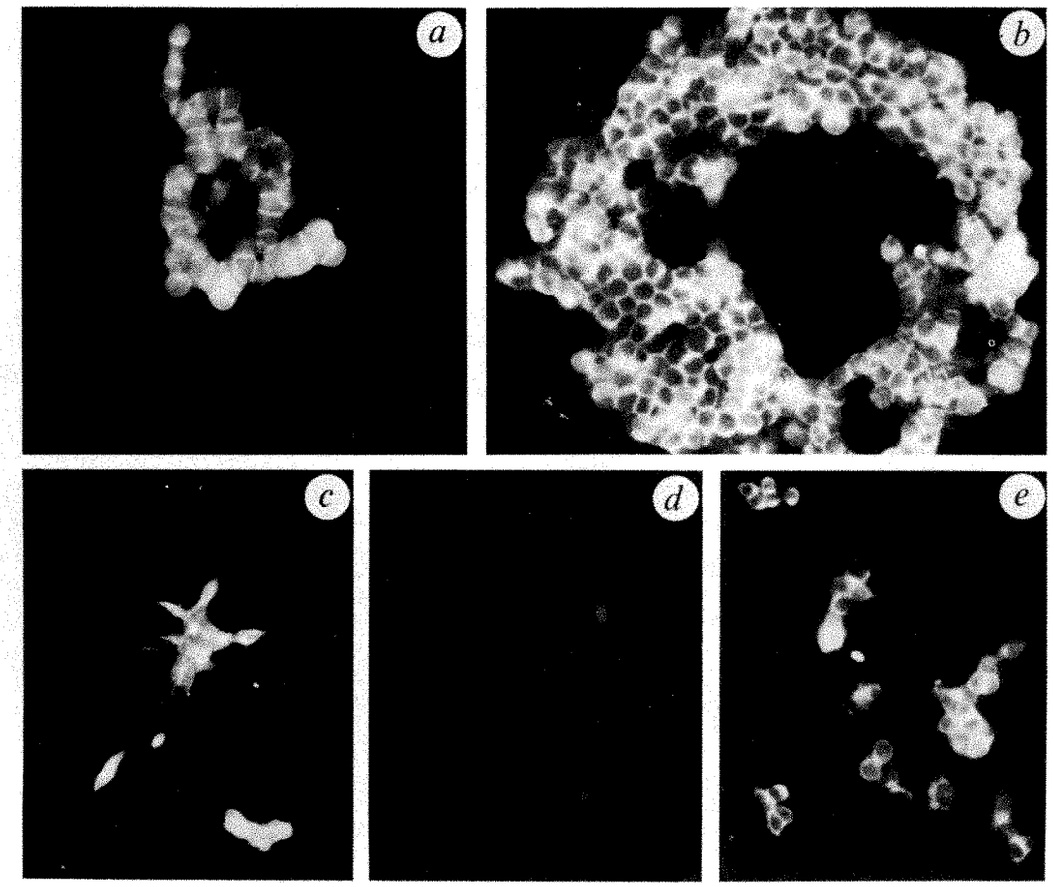


Fig. 2 Indirect immunofluorescence staining of Y-79 cells. Cells were fixed and incubated with antiserum as described in Table 1. *a*, Undifferentiated round cells positive for NSE (×257). *b*, Undifferentiated round cells positive for GFAP (×185). *c*, Cells with neuronal differentiation positive for NSE (×135). *d*, Flat glial-like cells negative for NSE. The flat cells are isolated; they are growing without forming clumps, chains or rosettes, in contrast to the undifferentiated or neuronal cells (×150). *e*, Flat cells positive for GFAP (×135).

The results of staining for the control and retinoblastoma cell lines before and after differentiation are given in Table 1. The pattern of staining for the control cell lines confirms the specificity of the antisera used in this study. Positive NSE staining was only observed in neuroblastoma cells. Anti-GFAP antiserum stained only glioma cells as reported previously[14–16], thereby obviating the possibility of cross-reactivity between GFAP and tubulin. Normal fibroblasts remained repeatedly negative for either marker, providing indirect evidence that our antisera did not cross-react with vimentin. A highly specific staining pattern was observed with immunoperoxidase also: that is, the neurones of the sections from brain and spinal cord as well as the cross-sectioned neuritic axons in the white matter of the spinal cord and adjacent spinal nerves were positive for NSE and negative for GFAP. In contrast, astrocytes in both tissues stained intensely positive for GFAP and remained negative for NSE. Among the other tissues examined, the olfactory neuroblastoma, pineoblastoma and the oligodendrocytic and sarcomatous components of the glial tumours, were negative for GFAP, whereas the astrocytomas and ependymoma were positive for GFAP, as reported previously[11]. The neuroblastomas were positive for NSE, whereas the lymphomas, Ewing's sarcoma and all soft tissue sarcomas were negative for NSE, as expected[17,18]. The medulloblastoma was generally negative for both NSE and GFAP, except for occasional GFAP-positive cells, previously interpreted as glial differentiation of the tumour[19].

All cells of the Y-79 human retinoblastoma line showed the simultaneous presence of NSE and GFAP before their differentiation into neuronal and glial-like (flat) cells respectively (Fig. 2*a*, *b*). The anti-GFAP antiserum gave a higher intensity of staining overall than the anti-NSE antiserum. One-third of the cells with neuronal differentiation in the defined medium were

Table 1 NSE and GFAP reactivity of retinoblastoma and control cells *in vitro*

Type of cell line	NSE reactivity	GFAP reactivity
(1) Retinoblastoma (Y-79)		
Undifferentiated	+	+
Differentiated*		
a Neuronal	+	+/−
b Glial-like	−	+
(2) Neuroblastoma (SMS-SAN)	+	−
(3) Glioma (U-251-MG)	−	+
(4) Normal fibroblasts	−	−

All cultures were fixed in absolute ethanol at −200 °C for 4 min and subsequently washed in phosphate-buffered saline (PBS). Primary antisera raised in rabbit against rat NSE (1:50; Polysciences, Warrington, Pennsylvania) and human GFAP (1:100; Accurate, Westbury, New York) were applied to all cultures at 4 °C for 24 h. The cultures were subsequently washed in PBS, incubated for 1 h with swine anti-rabbit IgG linked to fluorescein isothiocyanate (FITC) (1:20), washed again in PBS and observed under a Zeiss Standard microscope equipped with an epifluorescence illuminator and FITC narrow-band filter.

* The staining results for NSE and GFAP refer only to the differentiated cells. The cells which remained undifferentiated always retained both markers. One-third of the cells with neuronal differentiation became GFAP-negative, whereas all glial cells became NSE-negative.

GFAP-negative. All neuronal cells showed a higher intensity of NSE reactivity (Fig. 2*c*) than the undifferentiated cells. On the other hand, flat cells in dbcAMP-treated cultures were always negative for NSE (Fig. 2*d*), whereas most of them were positive for GFAP (Fig. 2*e*).

The simultaneous presence of NSE and GFAP in the retinoblastoma cells suggests that these cells originated from a primitive neuroectodermal cell capable of differentiation into neuronal and glial elements. Thus far, NSE and GFAP have not been detected simultaneously in another tumour either *in vivo* or *in vitro*. Morphological transformation of the retinoblastoma cells into neuronal and glial-like cells with specific staining patterns for NSE (neuronal) and GFAP (glial) reinforces the concept of the primitive bipotential nature of retinoblastoma. The fact that two-thirds of the cells in serum-free medium, despite their morphological differentiation into neuronal cells, retained their GFAP positivity suggests that they were in an earlier stage of biochemical differentiation. Both neuronal and glial cells have been described previously in retinoblastomas *in vivo* and *in vitro*[3,6,7,20]. However, the neoplastic nature of the glial cells in retinoblastoma has so far been a moot point. The present system obviates this problem, as it appears that the neoplastic cells differentiate into both neuronal and glial-like cell types and express biochemical patterns which correlate with the morphological phenotype of the differentiating cells.

The potential of the embryonal retinal cell to differentiate into neurones and glial cells has been shown by Ohnishi[21] and Fujisawa *et al.*[22] in dissociated neuroretinal cells of chick embryos. We have shown here that the same phenomenon can occur in the neoplastic counterpart of retinal cells—the retinoblastoma cell. An analogous situation exists in differentiating neuroblastoma (neural and Schwann cells)[23,24] and medulloblastoma (neuronal and glial cells)[19]. In both cases, the primitive tumour cells are presumed to have differentiated into neuronal and supporting cells. One can speculate that retinoblastoma is the result of exuberant proliferation of residual primitive retinal cells, or that dedifferentiation of mature retinal cells towards a more primitive neuroectodermal cell is necessary as a prelude to the neoplastic behaviour. It is interesting that the coexistence of neurofilaments (previously seen only in neurones) and vimentin (previously seen in some forms of glia) has been reported recently in the axonless horizontal cell of the outer plexiform layer in mouse retina, leading to the conclusion that this cell might represent an intermediate stage of differentiation between neuronal and glial cells[25].

This is the first time that *in vitro* differentiation of retinoblastoma has been achieved and studied morphologically and cytochemically. We believe that the present study will contribute to our knowledge of the origin and biological behaviour of retinoblastoma. It may also provide a system to assess the effects of various agents on tumour maturation *in vitro*, which may ultimately effect a potential cure *in vivo*.

We wish to thank Dr Alan Rabson for helpful comments during preparation of the manuscript.

Received 13 June; accepted 24 November 1983.

1. Puklin, J. E. in *Principles and Practice of Ophthalmology* (eds Puklin, J. E. & Apple, D. J.) 1136–1149 (Saunders, Philadelphia, 1980).
2. Virchow, R. *Die krankhaften Geschwuelste* Vol. 2, 151 (Hirschwald, Berlin, 1864).
3. Popoff, N. A. & Ellsworth, R. M. *Lab. Invest.* **25,** 389–402 (1971).
4. Reid, T. W. *et al. J. natn. Cancer Inst.* **53,** 347–360 (1974).
5. Ohnishi, Y. *Ophthalmologica* **174,** 129–136 (1977).
6. Taylor, H. R., Carroll, N., Jack, I. & Crock, G. W. *Br. J. Ophthal.* **63,** 551–559 (1979).
7. Lane, J. C. & Klintworth, G. K. *Am. J. Ophthal.* **95,** 197–207 (1983).
8. Schmechel, D., Marangos, P. J. & Brightman, M. *Nature* **276,** 834–836 (1978).
9. Marangos, P. J., Polak, J. M. & Pearse, A. G. E. *Trends neurol. Sci.* **5,** 193–196 (1982).
10. Eng, L. F. in *Proteins of the Nervous System* (eds Bradshaw, A. R. & Schneider, M. D.) 85–117 (Raven, New York, 1980).
11. Velasco, M. E., Dahl, D., Roessmann, U. & Gambetti, P. *Cancer* **45,** 484–494 (1980).
12. Kyritsis, A., Tsokos, M. & Chader, G. *Expl Eye Res.* (in the press).
13. Reynolds, P. C., Frenkel, P. E. & Smith, R. G. *Trans. Ass. Am. Phys* **93,** 203–211 (1980).
14. Bigner, D. D. *et al. J. Neuropath. exp. Neurol.* **40,** 201–229 (1981).
15. Sternberger, L. A., Hardy, P. H. Sr, Cuculis, J. J. & Meyer, G. *J. Histochem. Cytochem.* **18,** 315–333 (1970).
16. Paetau, A. *et al. Acta neuropath.* **47,** 71–74 (1979).
17. Tsokos, M., Linnoila, R. I., Triche, T. J. & Chandra, R. *Lab. Invest.* (Abstr.) **48,** 87A (1983).
18. Jaffe, R. & Yunis, E. J. *Lab. Invest.* (Abstr.) **48,** 7P (1983).
19. Palmer, J. O., Kasselberg, A. G. & Netsky, M. G. *J. Neurosurg.* **55,** 161–169 (1981).
20. Ts'o, M. O. *Int. Ophthal. Clinics* **20,** 191–210 (1980).
21. Ohnishi, Y. *Jap. J. Ophthal.* **18,** 394–402 (1974).
22. Fujisawa, H., Nakamura, H. & Chin, M. *J. Embryol. exp. Morph.* **31,** 139–149 (1974).
23. Romansky, S. G., Crocker, D. W. & Shaw, K. N. F. *Cancer* **42,** 2392–2398 (1978).
24. Taxy, J. B. *Archs Path.* **104,** 355–360 (1980).
25. Drager, U. C. *Nature* **303,** 169–172 (1983).

A novel 6:10 chromosomal translocation in the murine plasmacytoma NS-1

Roger M. Perlmutter, Joan L. Klotz*, Dimitrina Pravtcheva†, Frank Ruddle† & Leroy Hood

Division of Biology, California Institute of Technology, Pasadena, California 91125, USA
* City of Hope Medical Center, Duarte, California 91010, USA
† Department of Biology, Yale University, New Haven, Connecticut 06511, USA

Specific chromosomal abnormalities are regularly associated with many murine and human malignancies[1,2]. In particular, the majority of murine plasmacytomas and human Burkitt's lymphomas contain a characteristic translocation which results in the juxtaposition of a cellular oncogene, c-*myc*, with the immunoglobulin heavy-chain gene locus[3–7], and this rearranged c-*myc* directs the synthesis of qualitatively and quantitatively abnormal transcripts which may have an aetiological role in the development of the transformed state in lymphoid malignancies[8,9]. Similarly, rearrangement and abnormal expression of c-*myb* (ref. 10) and c-*mos* (ref. 11) has been reported in other murine lymphoid tumours. Here we describe a novel 6:10 chromosomal translocation in the murine plasmacytoma cell line NS-1 which juxtaposes the immunoglobulin C_κ gene with a single-copy sequence of unknown function. The NS-1 plasmacytoma is a frequently used fusion partner in hybridoma production[12] and is known to contain a rearranged c-*myc* gene[4,13] and a genetic element which transforms normal mouse fibroblasts in DNA-mediated transfection assays[14]. We conclude that individual B-cell tumours may contain multiple chromosomal translocations perhaps relevant to oncogenesis.

Phage clones containing C_κ sequences were isolated from a genomic library of hybridoma DNA prepared using the L47.1

© 1984 Macmillan Journals Ltd

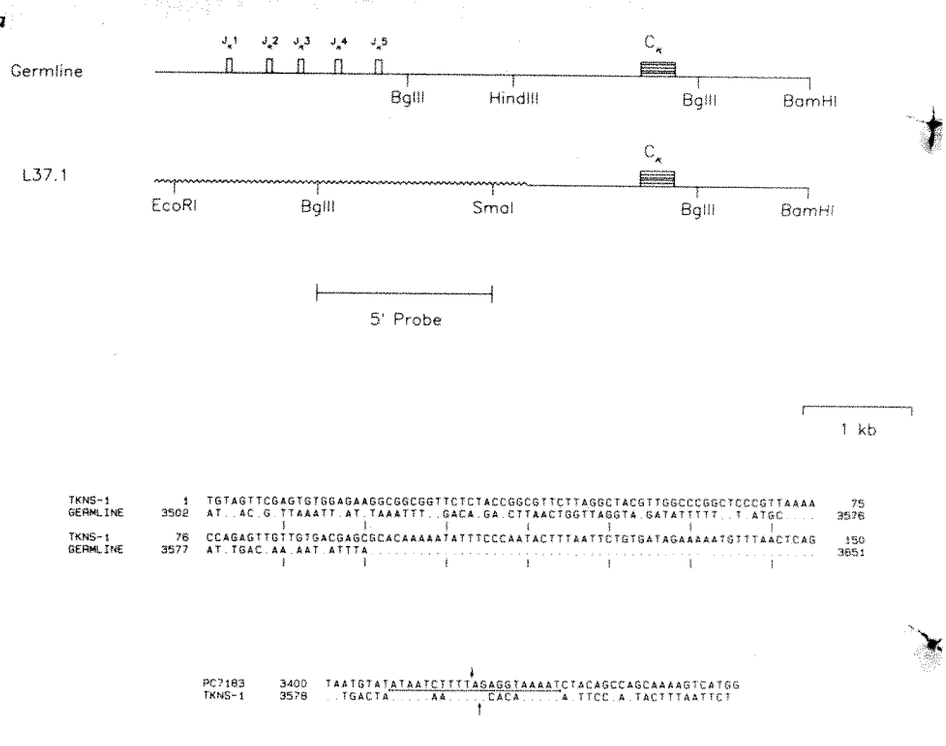


Fig. 1 *a*, An aberrantly rearranged C_κ gene. Genomic DNA was isolated from the HGAC9 hybridoma (secreting antibody to group A streptococcal carbohydrate[32]) and partially digested with *Mbo*I to allow construction of a library in L47.1 phage (see ref. 15). On screening with a murine C_κ gene probe, several clones were obtained which contained a novel sequence juxtaposed to C_κ. Restriction enzyme maps for the germ-line C_κ locus and for one of the rearranged clones, L37.1, are shown. The rearranged sequence begins just 3′ to the germ-line *Hin*dIII site and is denoted by a wavy line. A *Sma*I/*Bgl*II digest was used to generate a probe for this sequence (5′ probe) which was subcloned into M13mp8 (BRL). *b*, DNA sequence at the site of the T_κNS-1 translocation site. The T_κNS-1 sequence was determined by the chain termination method of Sanger[33]. The germ-line sequence is from ref. 34. Numbering of the T_κNS-1 sequence is arbitrary; numbering of the germ-line sequence reflects ref. 34. Homologous bases are indicated with dots. The remainder of the T_κNS-1 sequence extending towards the C_κ gene agrees precisely with the published sequence for this region[34]. *c*, Two potential target sites for translocation in the C_κ intervening sequence. The points of translocation for PC7183[20] and for T_κNS-1 (this paper) within the germ-line C_κ intervening sequence[34] are compared (arrows). The solid line denotes a 21-bp sequence, straddling the recombination points, which is closely homologous for both translocation sites.

phage vector[15]. Several overlapping clones were obtained which contained the C_κ gene and an aberrant sequence joined 1 kilobase (kb) 5′ to the C_κ gene (Fig. 1*a*). A 1.6-kb fragment derived entirely from this novel 5′ sequence was prepared by digestion with *Sma*I and *Bgl*II endonucleases (Fig. 1*a*) and used to probe Southern blots of BALB/c sperm DNA and DNA from various cell lines. Figure 2 shows that this probe identifies a single-copy sequence in germ-line (sperm) DNA but that both the hybridoma cell line from which the library was prepared (HGAC9) and the parent line NS-1 contain a rearranged copy of this sequence which results from the juxtaposition of C_κ (Fig. 1*a*). Thus, the C_κ rearrangement which we cloned from a specific hybridoma was present in the common parent cell line.

To identify the chromosomal location of the single-copy sequence aberrantly joined to the C_κ gene in NS-1 cells, we used mouse–hamster somatic cell fusion lines which retain limited numbers of different murine chromosomes[16]. Southern blot analysis of DNA from a number of such lines using our 1.6-kb probe unambiguously localized this sequence to chromosome 10 (Fig. 3). Thus, a 6:10 translocation in the NS-1 plasmacytoma fuses the C_κ gene on chromosome 6 to a single copy element from chromosome 10. We call this element T_κNS-1.

Because of the recent demonstration of translocations involving c-*myc* and immunoglobulin genes in murine plasmacytomas, we sought to identify a retroviral oncogene within the T_κNS-1 sequence. Using cloned v-*onc* probes, we were unable to identify any sequence homologous to *src*, *myb*, *myc*, *abl*, *ras*^{H}, *ras*^{K}, *fes*, *yes*, *sis*, *mos*, *rel*, *bas*, *erb*, *fms* or *fos* in the ~8 kb of DNA 5′ to C_κ in this cloned translocation (refs 17, 18 and data not shown). Similarly, the T_κNS-1 probe did not detect any rearranged sequences in NIH3T3 fibroblast transformants generated using murine plasmacytoma DNA which therefore contain the previously described plasmacytoma transforming element known to be present in NS-1 cells (ref. 14 and R.M.P. and M. A. Lane, unpublished observation). We conclude that T_κNS-1 probably does not include a previously described oncogene sequence.

As 90% of BALB/c plasmacytomas appear to contain a 12:15 chromosomal translocation[3-5,9,19], we examined 10 different B-cell tumours using Southern blotting and the T_κNS-1 probe to assess the frequency of this specific rearrangement. No other examples of this translocation event were identified. In this sense the T_κNS-1 6:10 translocation resembles a complex 6:12:15 translocation recently characterized by Van Ness *et al.*[20] which was also localized to the C_κ intervening sequence and which is also exceedingly infrequent in murine plasmacytomas (M. Weigert, personal communication). Thus, if these translocations predispose to malignant transformation, they are clearly not essential for the development of B-cell neoplasia. In this regard, we may speculate that the C_k intervening sequence could be particularly susceptible to chromosomal rearrangements.

DNA sequence analysis of T_κNS-1 at the translocation breakpoint reveals little homology between the C_κ intervening sequence and the novel element derived from chromosome 10 (Fig. 1*b*). Involvement of immunoglobulin switch sequences in the aberrant rearrangement of c-*myc* has suggested that normal B-cell differentiation mechanism may be involved in the genesis of the 12:15 translocation in plasmacytomas[4-6]. This explanation cannot apply to either the T_κNS-1 translocation or the 6:12:15 translocation described in PC7183[20] because both occur in a region of the C_κ intervening sequence which does not ordinarily undergo DNA rearrangements.

Interestingly, careful comparison of the translocation points within the C_κ intervening sequence found in NS-1 and PC7183, which are separated by 180 bases, reveals a region of homology (15/21 base pairs, bp) straddling these sites (Fig. 1*c*). A search of the entire C_κ locus sequence, nearly 6 kb, identified these as the only two regions containing a sequence homologous to the T_κNS-1 target site. This raises the possibility that some translocations occur via a site-specific mechanism.

Three properties distinguish the region immediately 5′ to the C_κ gene. First, heteroduplex analysis has established an area of conserved sequence between human and mouse DNA about 700 bp 5′ to the C_κ gene[21]. In addition, this region contains a DNase-I hypersensitive site, felt to reflect gene activation for increased expression[22], in approximately the same position as the man–mouse sequence homology[23-25]. Finally, the C_κ inter-

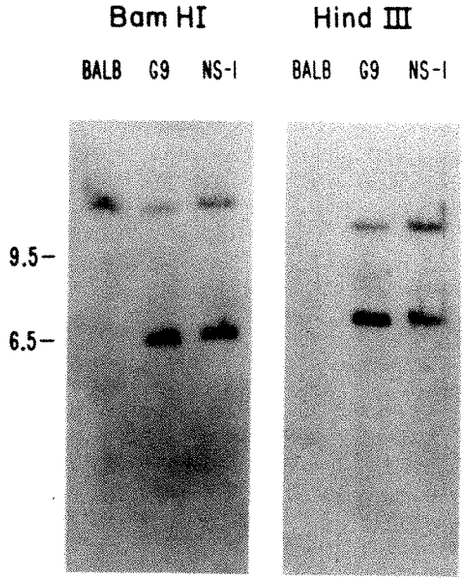


Fig. 2 The L37.1 rearranged C_κ gene is derived from the NS-1 hybridoma parent. Southern blots of 10 μg genomic DNA from BALB/c sperm, HGAC9 hybridoma cells or NS-1 plasmacytoma cells digested with *Bam*HI (left panel) or *Hin*dIII (right panel) were performed using the 1.6-kb *Sma*I/*Bgl*II 5′ probe shown in Fig. 1 labelled to 10^8 d.p.m. with ^{32}P-nucleotide triphosphates by primer extension[35]. After electrophoresis in 0.7% agarose, fragments were blotted onto nitrocellulose and hybridization was performed in 50% formamide at 42 °C as described previously[36]. The blots were washed in 0.015 M sodium citrate, 0.15 M sodium chloride, 0.1% SDS at 68 °C for 2 h before autoradiography overnight at −70 °C using Kodak XAR-5 film (Eastman Kodak) and an intensifying screen. The positions of standard size markers derived from *Hin*dIII fragments of phage λ DNA are noted in kb at the left.

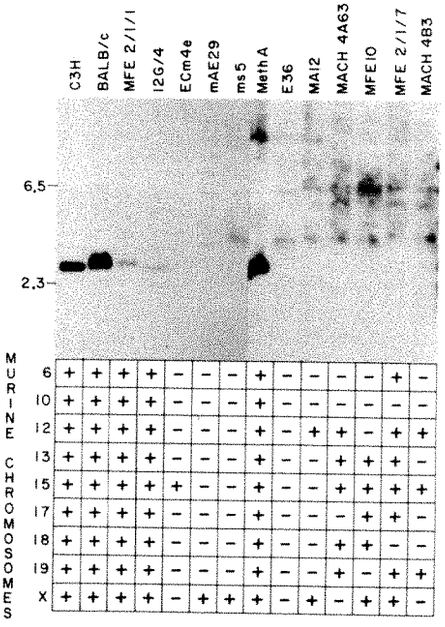


Fig. 3 Chromosomal location of T_κNS-1. Patterns obtained when genomic DNA from the indicated mouse strains (C3H, BALB/c), from a BALB/c sarcoma (MethA), a Chinese hamster cell line (E36) and mouse–hamster somatic cell fusion lines generated using MethA as a parent (ms5, mAE29, MA12) or BALB/c embryo fibroblasts as parent cells (MFE2/1/7, MFE2/1/1, MFE10), or A/HeJ macrophages as parent cells (MACH4AG3, MACH4B3) is analysed by Southern blotting as described in Fig. 2 legend. The origin of cell line Ecm4e has been described previously[37]. The DNA was digested with *Eco*RI which produces a 2.8-kb band that hybridizes with the *Sma*I/*Bgl*II probe used in Fig. 2. The specific murine chromosomes retained in each hybrid line are indicated with a plus sign. To simplify the figure, the complete karyotype of each cell line is not indicated. Chromosome 12 has been rearranged in the MFE10 cell lines. Hazy bands larger than 3.0 kb reflect cross-hybridization with homologous hamster-derived sequences. The positions of standard size markers are again indicated in kb at the left. Additional hybrid cell lines examined included MFE8, which contains all murine chromosomes except 6 and Y and which was positive for T_κNS-1 as was 12G/3, which lacks murine chromosomes 1, 3, 4, 7, 9, 11 and Y. Cell line 12G/2 lacking chromosomes 1, 2, 3, 4, 7, 9, 10, 11, 12, 14, 18, 19 and Y also lacked the T_κNS-1 band. Chromosomes not shown in the figure but present in the cell lines were as follows: MFE2/1/1: 1, 2, 3, 4, 7, 8, 9; 12G4: 5, 14, 16; ECm4e: 14; mAE29: 14; MACH4AG3: 2, 4, 7, 8, 16; MFE10: 1, 2, 7, 8, 9, 11, 14, 16; MFE2/1/7: 1, 2, 3, 7, 8, 9; MACH4B3: 2, 7, 16.

vening sequence has been shown to contain an enhancer element located 3′ to the *Hin*dIII site which dramatically increases transcription of C_κ-containing plasmids introduced into murine lymphoid cells[26–28]. Although translocation may occur randomly throughout the genome, as has been proposed for retroviral transduction[17], we find it provocative that two independent translocation events in murine plasmacytomas occur at highly homologous sites 180 bp apart in a sequence apparently organized to support active gene expression.

The NS-1 plasmacytoma line was developed from a mineral oil-induced tumour, MOPC-21, after adaptation to culture conditions and selection for sensitivity to hypoxanthine–aminopterin–thymidine medium[12] and we cannot be certain at which point during the genesis of NS-1 the T_κNS-1 6:10 translocation occurred. The fact that recent isolates of MOPC-21 do not contain this translocation[29] may reflect deletion of a κ allele during propagation of this tumour. In addition to the T_κNS-1 translocation, the NS-1 cell line contains a characteristic 12:15 translocation event of murine plasmacytomas, an activated oncogene common to most murine plasmacytomas[14], and a rearrangement in c-*mos* apparently analogous to that described in XRPC24 (ref. 11 and N. Takahashi, personal communication). Although the simultaneous coexistence of these four sequence abnormalities may in part reflect chromosomal instability within cultured cell lines, it is significant that three of these four abnormalities involve genes having known transforming potential. One possible explanation might be that these oncogenes act in concert, together providing growth advantages not present when only one is expressed[30] or perhaps developed sequentially, reflecting multiple stages in oncogenesis[14,31]. Thus, chromosomal translocations in neoplastic cells may provide a window for the selective identification of important genetic elements involved in differentiation and growth control.

We thank Bennett Berson for technical assistance, C. Katz for typing the manuscript, Drs M. Nahm and J. M. Davie for the gift of HGAC9 hybridoma cells, Dr M. Kronenberg for normal tissue ahd hybridoma mRNA, Dr M. Cline for cloned oncogene probes, and Drs R. Barth, M. Kronenberg and E. Kraig for critical reading of the manuscript. This work was supported by grants from the NIH; R.M.P. is the recipient of a USPHS New Investigator Award.

Received 20 July; accepted 8 November 1983.

1. Rowley, J. D. *Science* **216**, 749–751 (1982).
2. Klein, G. *Nature* **294**, 313–318 (1981).
3. Shen-ong, G. C. C., Keath, E., Piccoli, S. P. & Cole, M. D. *Cell* **31**, 443–452 (1982).
4. Crews, S., Barth, R., Hood, L., Prehn, J. & Calame, K. *Science* **218**, 1319–1321 (1982).
5. Marcu, K. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 519–523 (1983).
6. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7837–7841 (1982).
7. Dalla-Favera, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7824–7827 (1982).
8. Mushinski, J. F., Bauer, S. R., Potter, M. & Reddy, E. P. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1073–1077 (1983).
9. Stanton, L. W., Watt, R. & Marcu, K. B. *Nature* **303**, 401–406 (1983).
10. Mushinski, J. R., Potter, M., Bauer, S. R. & Reddy, E. P. *Science* **220**, 795–798 (1983).
11. Kuff, K. L. *et al. Nature* **302**, 547–548 (1983).
12. Kohler, G. & Shulman, M. *Eur. J. Immun.* **6**, 292–298 (1979).
13. Calame, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6994–6998 (1982).

14. Lane, M.-A., Sainter, A. & Cooper, G. M. *Cell* **18,** 873–880 (1982).
15. Loenen, W. A. M. & Brammar, W. J. *Gene* **20,** 249–259 (1980).
16. Ruddle, F. H. in *Genes, Chromosomes, and Neoplasia* (eds Arrighi, F. E., Rao, P. N. & Stubblefield, E.) 7–17 (Ravel, New York, 1981).
17. Bishop, J. M. *A. Rev. Biochem.* **52** (in the press).
18. Coffin, J. M. *et al. J. Virol.* **40,** 953–957 (1981).
19. Kirsh, I. R. *et al. Nature* **293,** 585–587 (1981).
20. Van Ness, B. G. *et al. Nature* **301,** 425–427 (1983).
21. Hieter, P. A., Max, E. E., Seidman, J. G., Maizel, J. V. & Leder, P. *Cell* **22,** 197–207 (1981).
22. Weintraub, H. & Groudine, M. *Science* **93,** 848–858 (1976).
23. Chung, S.-Y., Folsom, V. & Wooley, J. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2427–2431 (1983).
24. Weischet, W., Glotov, B. O., Schnell, H. & Zachau, H. *Nucleic Acids Res.* **10,** 3627–3645 (1982).
25. Storb, U., Wilson, R., Selsing, E. & Wilson, A. *Biochemistry* **20,** 990–996 (1981).
26. Rice, D. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **79,** 7862–7865 (1982).
27. Oi, V. T., Morrison, S. L., Herzenberg, L. A. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **80,** 825–829 (1983).
28. Queen, C. & Baltimore, D. *Cell* **33,** 741–748 (1983).
29. Walfield, A. M. *et al. Nucleic Acids Res.* **8,** 4689–4707 (1980).
30. Land, H., Parada, L. F. & Weinberg, R. A. *Nature* **304,** 596–602 (1983).
31. Klein, G. *Cell* **32,** 311–315 (1983).
32. Nahm, M. H., Clevinger, B. L. & Davie, J. M. *J. Immun.* **129,** 1513–1518 (1982).
33. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
34. Max, E., Maizel, J. & Leder, P. *J. biol. Chem.* **236,** 5116–5120 (1981).
35. Anderson, S., Gart, M. J., Mayol, L. & Young, I. G. *Nucleic Acids Res.* **8,** 1731–1743 (1980).
36. Mullins, J. I., Casey, J. W., Nicolson, M. O. & Davidson, N. *Nucleic Acids Res.* **8,** 3287–3305 (1980).
37. Fournier, R. E. K. & Ruddle, R. H. *Proc. natn. Acad. Sci. U.S.A.* **74,** 3937–3941 (1977).

Activation of N-*ras* gene in bone marrow cells from a patient with acute myeloblastic leukaemia

Christof Gambke*, Erich Signer† & Christoph Moroni*

* Friedrich Miescher-Institut, PO Box 2543, CH-4002 Basel, Switzerland
† Children's Hospital, University of Basel, Basel, Switzerland

Human tumour cell lines of various histological origin contain genes that can transform NIH 3T3 cells in culture[1,2]. Most frequently the gene is an activated K-*ras* gene, more rarely an activated H-*ras* gene, and sometimes the recently discovered N-*ras*[3–11]. Other transforming genes, distinct from *ras*, have been found in B- and T-cell leukaemias[9,12]. Since most of the transforming genes have been identified in cell lines, it is still unclear at what stage the genes become activated. We have therefore initiated a study to determine if the presence of a transforming gene correlates with the clinical course of a malignant disease. Here we demonstrate the presence of a transforming N-*ras* gene in bone marrow cells from a patient with acute myeloblastic leukaemia at the outbreak of the acute disease phase. Fibroblast DNA from the same patient was not transforming. In contrast to HL-60 cells[13,14], no alteration of the *myc* gene was detected.

A 9-year-old girl with anaemia and leukocytosis was admitted to the hospital where an acute myeloblastic leukaemia (AML) M2 (ref. 15) was diagnosed. Clinical and laboratory findings are summarized in Table 1. An aliquot of this patient's diagnostic bone marrow aspirate, obtained before chemotherapy administration, was examined for the possible presence of a transforming gene. AML DNA was found to contain focus-forming activity in three consecutive experiments (Table 1*a*). Four foci from three different plates were picked using cloning cylinders and grown into cell lines of clearly transformed morphology. The DNA from these lines was prepared and tested in a secondary transfection experiment to determine whether the putative transforming gene could be transferred. Table 1*b* shows that this DNA could induce foci on NIH 3T3 cells.

DNA derived from primary and secondary foci was subjected to Southern blot analysis using BLUR-8[16] as a probe for the detection of human repetitive *Alu* sequences in the transfection foci. Figure 1 shows that a representative primary focus (lane *b*) contains a large number of human repetitive DNA sequences. Three secondary foci (lanes *c–e*) share an *Alu*-containing fragment of ~8.8 kilobases (kb). A fourth secondary focus lacks this fragment, but contains two fragments of ~20 and 6 kb. We tentatively conclude that the transforming activity is associated with the 8.8-kb fragment, and perhaps with the 20- or 6-kb fragment. To characterize the transforming gene further we tested its sensitivity to seven restriction enzymes by digesting primary focus DNA before the transfection experiment. Focus formation was reduced 10-fold or more by digestion with *Eco*RI, *Hin*dIII, *Bam*HI or *Pvu*II, while *Xho*I, *Sal*I and *Cla*I did not reduce the focus-forming activity of the DNA preparation (data not shown). This restriction enzyme sensitivity pattern differs from the published patterns obtained from B- and T-cell leukaemias[9], but is identical to a pattern obtained with a transforming gene present in a fibrosarcoma and also in an embryonal rhabdomyosarcoma line[17]. In a subsequent study[8] by the same group this sarcoma-derived gene was cloned and identified as the N-*ras* gene.

Southern blot analysis was then used to determine whether the AML-derived transfection foci contained the N-*ras* gene as suggested by the restriction enzyme sensitivity experiment. The probe used was an *Eco*RI–*Sst*I subfragment from a cloned 8.8-kb *Eco*RI fragment[8] containing the 5′ region of the N-*ras* gene given by Alan Hall. With this probe, we detected an 8.8-kb *Eco*RI fragment in DNA from a primary focus (Fig. 2*b*) and from three secondary foci (Fig. 2*c–e*). In a fourth secondary focus (Fig. 2*f*), a hybridizing fragment in the 20-kb region, corresponding to the upper *Alu*-containing fragment in Fig. 1*f*, was detected. The slower mobility of this fragment is probably due to loss of one of the *Eco*RI sites flanking the 8.8-kb fragment brought about by DNA shearing required by the transfection protocol[18]. We conclude that the transforming gene present in AML bone marrow represents the N-*ras* gene, part of which can be detected in most transfection foci as an 8.8-kb *Eco*RI fragment. Consistent with this interpretation are the results of immunoprecipitation experiments by H. P. Senn using a monoclonal anti-p21 antibody which show that secondary foci contain elevated levels of a *ras* protein (data not shown).

Table 1 Primary and secondary transfection experiments

Source of DNA	Foci per no. of plates	Foci per μg DNA
a Primary transfection		
Calf thymus	1/6	0.004
AML bone marrow	42/20	0.056
b Secondary transfection		
Primary foci		
T82/73-9A1	22/6	0.100
T82/73-10/2	12/6	0.053
T82/73-11/1	37/4	0.247
T82/73-10/3	22/6	0.100

The patient under study was in good general condition; the only abnormal findings were a moderate hepato-splenomegaly, slightly enlarged lymph nodes and a small perirectal abscess. The white blood cell count was $62\times10^9\,l^{-1}$ (normal range $5–12\times10^9\,l^{-1}$); most cells were atypical myeloblasts. There was thrombocytopenia with a platelet count of $55\times10^9\,l^{-1}$ ($150–400\times10^9\,l^{-1}$) and an anaemia with 91 $g\,l^{-1}$ of haemoglobin ($120–140\,g\,l^{-1}$). The bone marrow was hypercellular with 68% atypical myeloblasts cytochemically positive for peroxidase and Sudan black, negative for Pas and nonspecific esterase. This, together with morphological criteria, established the diagnosis of acute myeloblastic leukaemia (AML), type M2 according to the FAB (French–American–British) classification[15]. No abnormal findings were detected by karyotype and chromosome banding analysis (H. J. Müller, data not shown). Bone marrow cells prepared by standard Ficoll centrifugation were lysed using 0.5% SDS in 100 mM NaCl, 10 mM EDTA, 10 mM Tris-HCl, *p*H 7.4, and digested with proteinase K. DNA was prepared by phenol and chloroform–isoamyl alcohol extractions followed by ethanol precipitation and subsequently resuspended in 10 mM Tris-HCl, *p*H 7.5, 1 mM EDTA. NIH 3T3 cells, seeded at 5×10^5 cells per 10-cm dish 1 day before the experiment, were transfected with 37.5 μg DNA as described[18]. Medium was changed 6 h later, then twice weekly. Foci of morphologically transformed cells were counted after 14 days.

0028–0836/84/050476–03$01.00

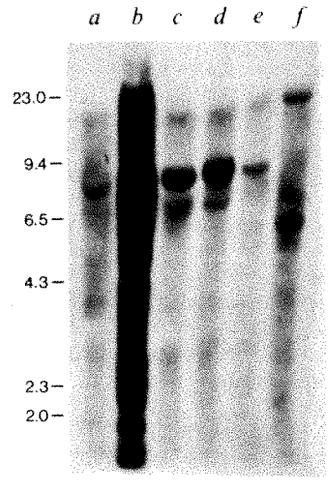


Fig. 1 Presence of human repetitive DNA in primary and secondary transfection foci. Aliquots (15 μg) of cellular DNA were digested overnight with *Eco*RI, phenol-extracted, ethanol-precipitated and subjected to agarose (0.8%) gel electrophoresis. After transfer of DNA to nitrocellulose, hybridization was done in the presence of 10% dextran sulphate at 67 °C using 2×10^7 c.p.m. of BLUR-8 probe[16] nick-translated to a specific activity of 1.4×10^8 c.p.m. per μg DNA. The final wash of the filter was in 0.5×SSC/0.1% SDS for 20 min at 65 °C. Autoradiography was at −70 °C for 16 h using Kodak X-Omat SO-282 and an intensifying screen. Cellular DNA was from: *a*, NIH 3T3 cells; *b*, primary focus T82/73-9Al; *c*, secondary focus T83/76-7b; *d*, secondary focus T83/76-9a; *e*, secondary focus T83/80-8; *f*, secondary focus T83/76-11a.

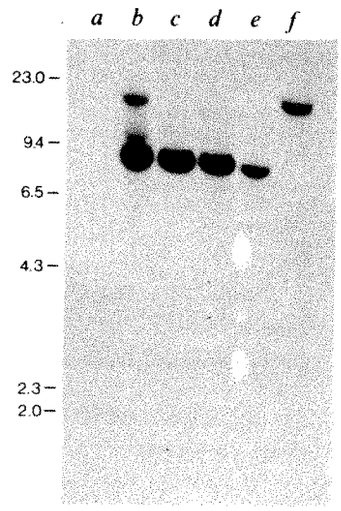


Fig. 2 Presence of the human N-*ras* sequence in primary and secondary transfection foci. Cellular DNA (15 μg) was digested overnight with *Eco*RI and a Southern blot performed as described in Fig. 1 legend except that the final wash was at 62 °C. The probe was an *Eco*RI–*Sst*I subfragment of the human N-*ras* 8.8-kb *Eco*RI fragment[9], nick-translated to a specific activity of 4×10^8 c.p.m. per μg of DNA. Cellular DNA was from: *a*, NIH 3T3 cells; *b*, primary focus; *c–f*, secondary foci as in Fig. 1.

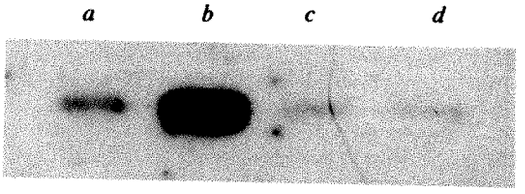


Fig. 3 Lack of *myc* gene amplification in AML DNA. Aliquots of cellular DNA (15 μg) were digested overnight with *Sst*I and a Southern blot was performed as described in Fig. 1 legend. The probe was pMC41-3RC, a 1.4-kb fragment[14] of the human *myc* gene cloned into pBR322. Specific activity was 5×10^8 c.p.m. per μg DNA. Cellular DNA was from: *a*, normal human spleen; *b*, human promyelocytic leukaemia HL-60; *c*, bone marrow from AML patient; *d*, fibroblasts derived from AML patient. The size of the fragment shown is 2.8 kb.

A transforming N-*ras* gene has been reported to be present in a neuroblastoma[7] line, two sarcoma[8,17] lines, the promyelocytic leukaemia[11] line HL-60, and, as presented here, in AML bone marrow cells at the outbreak of the acute phase of the disease. Thus, this gene is associated with tumours of different origins, but also with different maturation stages of leukaemias within the same lineage, namely the more primitive AML and the more differentiated promyelocytic leukaemia.

The HL-60 line has been shown to have an alteration of a second oncogene—an amplification of the *myc* gene[13,14] which is associated with chromosome translocations in human B-lymphomas[19–22]. While it is assumed but not known whether the N-*ras* gene was already present as a transforming variant in the original HL-60 tumour biopsy, it has been shown that the *myc* amplification had occurred *in vivo*[14]. To determine whether we could detect a *myc* gene alteration in AML DNA, we examined DNA from the original bone marrow aspirate and DNA from skin fibroblasts established in culture from the same patient. Normal human spleen DNA from an unrelated donor and DNA from HL-60 cells were included in the analysis as controls. As a probe we used a *Cla*I–*Eco*RI fragment of the human *myc* gene containing the 3′ exon given by R. C. Gallo. We detected neither amplification nor altered mobility of the *myc* gene in our AML patient, while HL-60 DNA showed the expected[13,14] amplification (Fig. 3). While we cannot exclude a minor *myc* gene alteration, it seems that N-*ras* activation in AML can occur independently of *myc* amplification/rearrangement. If indeed both the N-*ras* and *myc* genes have a role in the natural history of some myeloid leukaemias of which HL-60 may be a prototype, it would seem that in some cases, such as the more primitive AML, the activated N-*ras* gene exerts its effects independently of *myc*, and thus may require alterations of other genes.

To approach the question of whether the activation of the N-*ras* gene observed in this patient was a somatic event, we intend to compare the cloned activated gene with its alleles from the patient's fibroblasts. In three preliminary transfection experiments, this fibroblast DNA displayed a focus-forming activity of <0.005 foci per μg DNA, which corresponds to background values (compare Table 1). However, sequencing data from the cloned N-*ras* alleles will be needed to establish more firmly that N-*ras* activation in this patient was indeed a somatic event. It will be interesting to see whether this activation involves similar mutational events to those reported for the H-*ras* gene, namely a single base pair change altering either amino acid 12 (refs 23–25) or 61 (ref. 26) of the corresponding p21 protein. In addition, the present demonstration of an activated N-*ras* gene from an AML patient occurring in the absence of detectable *myc* gene alteration calls for an extensive study on the status of the N-*ras* gene and its expression in acute leukaemias.

We thank Drs R. A. Gallo and A. Hall for their gifts of DNA probes, and Dr J. A. Kop for critically reading the manuscript. We acknowledge the technical assistance of Ms Heide Luther and Mr Paul Hofer.

Received 1 August; accepted 15 November 1983.

1. Weinberg, R. A. *Adv. Cancer Res.* **36**, 149–163 (1982).
2. Cooper, G. M. *Science* **218**, 801–806 (1982).
3. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* **297**, 474–478 (1982).
4. Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S. & Barbacid, M. *Nature* **298**, 343–347 (1982).
5. Der, C. J., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3637–3640 (1982).
6. Pulciani, S. *et al. Nature* **300**, 539–541 (1982).
7. Shimizu *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 2112–2116 (1983).
8. Hall, A., Marshall, C. G., Spurr, N. K. & Weiss, R. A. *Nature* **303**, 396–400 (1983).
9. Lane, M.-A., Sainten, A. & Cooper, G. M. *Cell* **28**, 873–880 (1982).

10. McCoy, M. S. *et al. Nature* **302,** 79–81 (1983).
11. Murray, M. J. *et al. Cell* **33,** 749–757 (1983).
12. Goubin, G. *et al. Nature* **302,** 114–119 (1983).
13. Collins, S. & Groudine, M. *Nature* **298,** 679–681 (1982).
14. Dalla Favera, R., Wong-Staal, F. & Gallo, R. C. *Nature* **299,** 61–63 (1982).
15. Bennett, J. M. *et al. Br. J. Haemat.* **33,** 451–458 (1976).
16. Rubin, C. M., Houck, C. M., Deininger, P. L., Friedmann, T. & Schmid, C. W. *Nature* **284,** 372–374 (1980).
17. Marshall, C. J., Hall, A. & Weiss, R. A. *Nature* **299,** 171–173 (1982).
18. Wigler, M. *Proc. natn. Acad. Sci. U.S.A.* **76,** 1373–1376 (1979).
19. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7837–7841 (1982).
20. Dalla Favera, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7824–7827 (1982).
21. Crews, S. *et al. Science* **218,** 1319–1321 (1982).
22. Sheng-Ong, G. L. C., Keath, E. J., Piccoli, S. P. & Cole, M. D. *Cell* **31,** 443–452 (1982).
23. Tabin, C. J. *et al. Nature* **300,** 143–149 (1982).
24. Reddy, E. P., Reynold, R. K., Santos, E. & Barbacid, M. *Nature* **300,** 149–152 (1982).
25. Taparowski, E. *et al. Nature* **300,** 762–765 (1982).
26. Yuasa, Y. *et al. Nature* **303,** 775–779 (1983).

Three-dimensional structure of the light-harvesting chlorophyll *a*/*b*–protein complex

W. Kühlbrandt

Institut für Zellbiologie, ETH Hönggerberg, CH-8093 Zürich, Switzerland

Photosynthesis in the chloroplasts of green plants is carried out by a highly organized system of membranes. Chlorophyll–protein complexes in the photosynthetic membrane perform specialized functions which result in the biochemical fixation of solar energy. The light-harvesting chlorophyll *a*/*b* protein complex (LHC) has a key role in this process[1,2]. A striking property of the LHC that distinguishes it from other chlorophyll–protein complexes and makes it suitable for structural studies by Fourier methods is its tendency to form crystalline arrays *in vitro*[3]. I report here the three-dimensional structure of pea LHC determined at 16 Å resolution by electron microscopy of two-dimensional crystals and image analysis. A three-dimensional map shows that the LHC is a highly asymmetric transmembrane protein composed of three structurally equivalent monomers. The large surface area exposed on one side of the trimeric complex suggests a functional role in membrane interaction. The ability of LHC isolated from widely different plant species to form similar crystalline arrays[4–6] suggests that the structure reported here is of general significance.

Apart from its function as a molecular antenna that harve solar energy, the LHC regulates the transfer of excitation ener to the reaction centres of photosystems I and II, depending the phosphorylation of the LHC apoprotein[2]. The complex a mediates the interaction of thylakoid membranes which lea to the formation of stacks of apposed membrane vesicles, t chloroplast grana. This process is triggered by cations a resembles the formation of crystalline arrays *in vitro* whi occurs spontaneously on addition of cations to the detergei solubilized complex[7]. By controlled dialysis against moderz salt concentrations it is possible to obtain extensive, high ordered two-dimensional crystals of the LHC. These crystalli sheets have a hexagonal lattice with a 125 Å unit cell dimensio and belong to the P321 space group. They are composed interdigitating triangular units alternating in orientation to t plane of the sheet[4].

Specimens of crystalline sheets shadowed with heavy me to reveal their surface structure show a hexagonal array of ho ~90 Å in diameter (Fig. 1*a*). The holes span the tw dimensional crystal, indicating the absence of a continuous lip bilayer. The shadow cast in the holes and at the edges sho that the sheets are ~60 Å thick and therefore are single lay of molecules, an important prerequisite for this type of structu analysis. When contrasted with negative stain, the cryst resemble a honeycomb with the stain-excluding protein s rounding dark pools of contrasting agent within the ho (Fig. 1*b*).

Two-dimensional crystals contrasted with the negative sta uranyl acetate, were used to determine the three-dimensio structure of the LHC in a way similar to that described Henderson and Unwin[9]. This method takes advantage of t fact that the Fourier transform of an electron micrograph o two-dimensional crystal is a central section through the thr dimensional Fourier transform of the crystal, that is, throug lattice of lines in reciprocal space which are perpendicular the plane of the crystal. By combining the Fourier transfor of a sufficient number of different, tilted views it is thus possi to measure the amplitudes and phases of Fourier terms alo each lattice line. As in X-ray crystallography, an inverse Fou transform then yields a three-dimensional map of the structu In this analysis, amplitude and phase data were obtained computing the Fourier transforms of well-order densitometered areas of 43 electron micrographs of LHC cr tals tilted up to 82° in the electron microscope. Fourier te at the points of the reciprocal lattice (where the transform pl intersects the lattice lines) were collected and combined, us

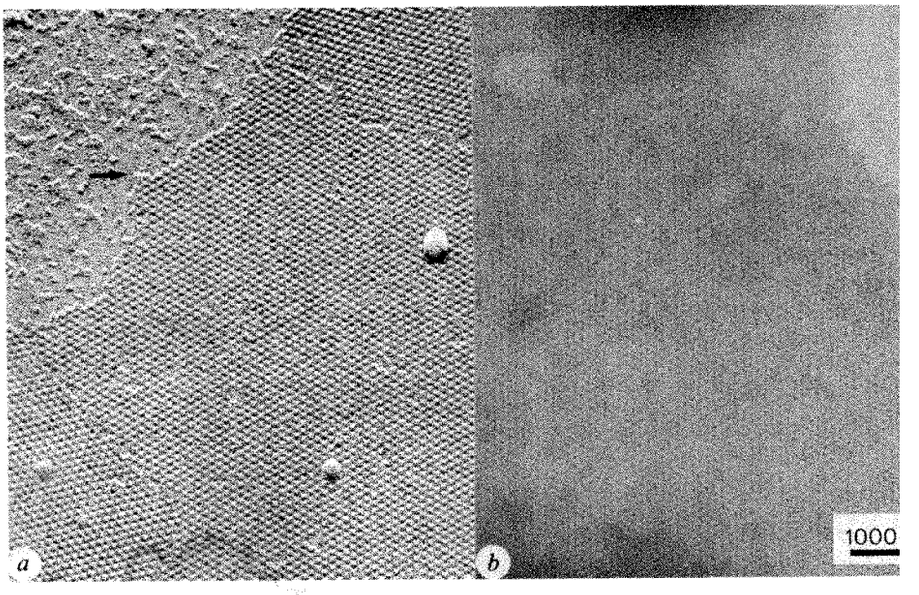


Fig. 1 Two-dimensional crystals of the LHC from pea chloroplasts: *a*, freeze-dried and shadowed with platinum at an angle of 45°; *b*, negatively stained with a 2% (w/v) uranyl acetate solution containing 0.1% (w/v) Triton X-100 (Sigma). The shadow cast at the edge of the crystalline sheet in *a* (arrow) indicates a thickness of 60 Å. The surface relief of the crystals shows a hexagonal array of holes measuring roughly 90 Å across which span the crystalline sheets. The holes appear as dark pools of stain in negatively contrasted sheets (*b*). The LHC was isolated from pea chloroplasts as described elsewhere[4]. KCl was added to the fluorescent density gradient fraction to a final concentration of 200 mM. The microcrystalline precipitate was pelleted after 3–4 min at room temperature and redissolved in 0.2% (w/v) unbuffered Triton X-100. Two-dimensional crystals were obtained by dialysis of the purified gradient fraction against 300 mM KCl, 10 mM Tris-HCl *p*H 6.9, 0.1% (w/v) Triton X-100 and 1 mM NaN_3 for ~1 h at 32–39 °C.

0028-0836/84/050478—03$01.00

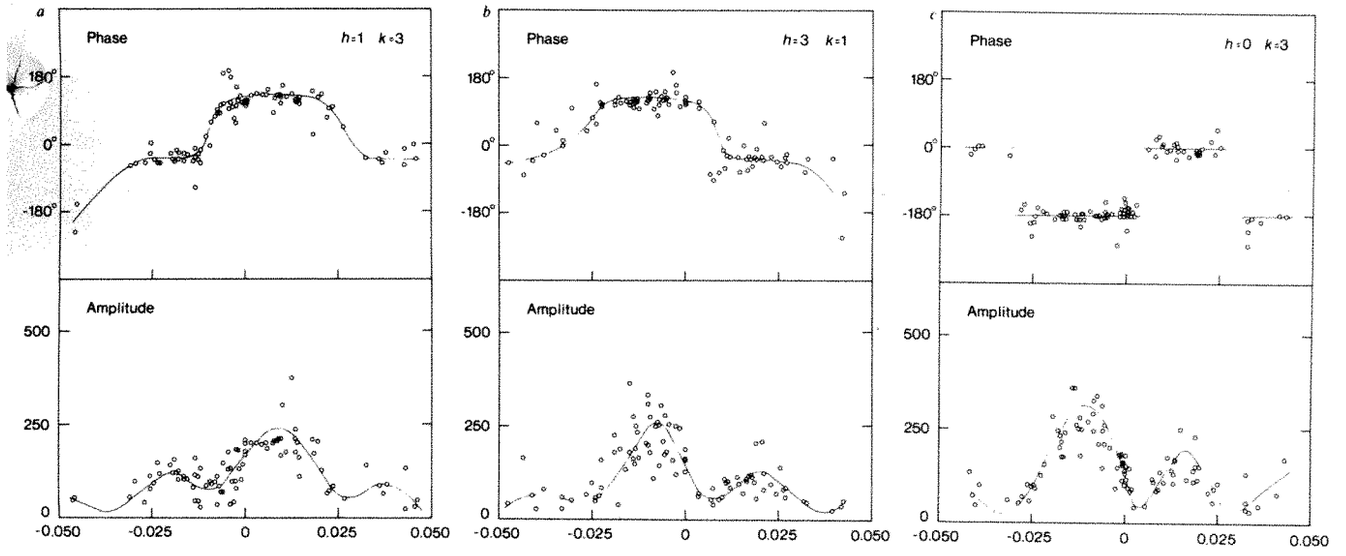
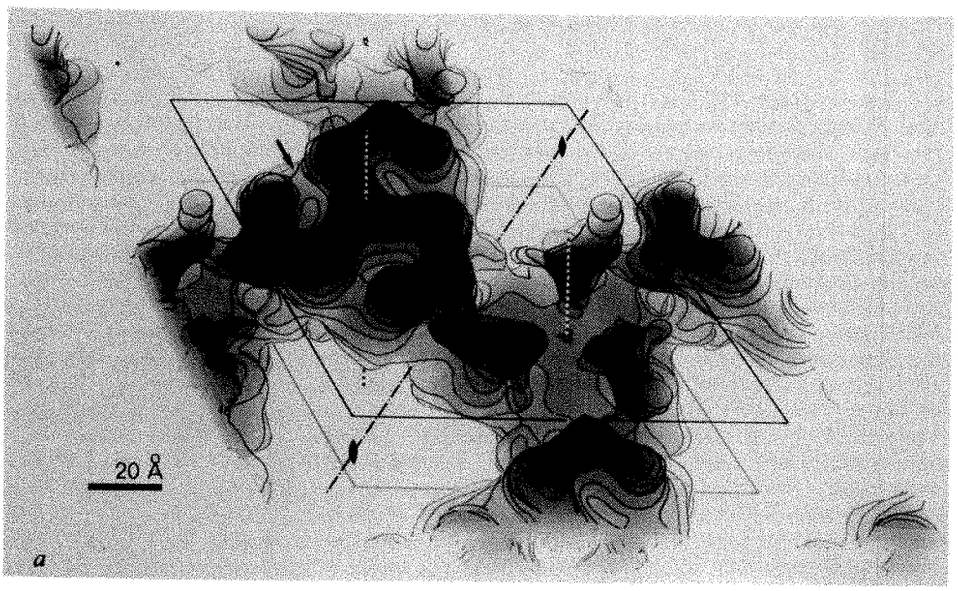


Fig. 2 Amplitude and phase of Fourier terms with Miller indices: *a*, $h = 1$, $h = 3$; *b*, $h = 3$, $k = 1$; and *c*, $h = 3$, $k = 0$ as a function of the reciprocal space coordinate, z^*. The apparent mutual mirror symmetry of pairs of lattice lines (h, k) and (k, h) (for example, *a* and *b*) and the real phase angles of 0° and 180° in the entire z^* range for axial lattice lines (for example *c*) indicate P321 symmetry. Data to a resolution of 16 Å were collected at reciprocal lattice points on computed Fourier transforms of 36 images in six tilt series of five negatively stained two-dimensional LHC crystals, with tilt angles ranging from 0° to 62°. Seven images of crystalline sheets recorded at tilt angles between 62° and 82° were incorporated in the final data set. Data were merged using the 3-fold symmetry of the two-dimensional crystals in projection. The phase residual averaged over all images with tilt angles <62° was 19.3° when no symmetry was imposed (P1 space group), 19.2° for P3, and 19.4° for P321 symmetry, confirming that the highest symmetry, P321, applied.



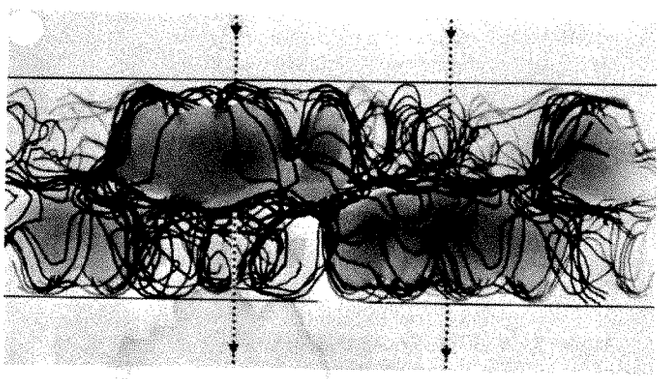


Fig. 3 Three-dimensional map of the LHC viewed from above (*a*) and edge-on (*b*) drawn to the same scale. The map shows two complexes in the unit cell. A 3-fold symmetry axis (· · · ·) runs centrally through each complex. The two trimeric LHC molecules are related by a 2-fold symmetry axis (·–·–·) so that adjacent complexes alternate in orientation perpendicular to the plane of the crystalline sheet. Different structural detail is exposed on the two external surfaces of the complex, which is a highly asymmetric, integral transmembrane protein. The edge-on view (*b*) in a direction perpendicular to the unit cell edge at the front of *a* indicates the vertical distribution of density within the complex. The main mass is found near the side of the complex which in the top view is characterized by three hook-like arms extending around three darkly staining gaps (arrow). The thickness of the LHC trimer and of the crystalline sheets is ~59 Å. The map was generated from structure factors sampled at regular intervals of 0.005 $Å^{-1}$ without imposing 2-fold symmetry. The distance between sections is 3.125 Å in the top view and 3.38 Å in the edge-on view. Contour lines were drawn at the first positive level of a linear 0 to 9 scale. Areas within the first (*a*) and third (*b*) contour level respectively are shown as opaque.

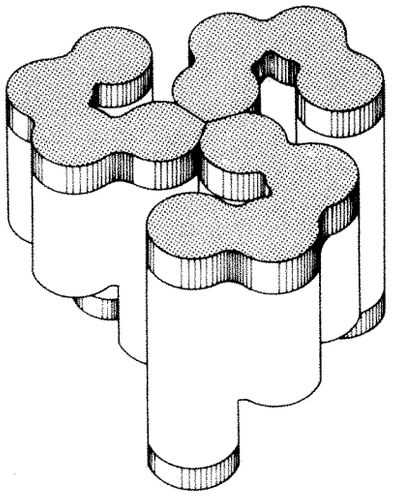


Fig. 4 Schematic drawing of one light-harvesting complex as it appears from the three-dimensional map (Fig. 3). The 3-fold symmetry of the complex indicates that it is a trimer, composed of three structurally equivalent subunits. The shaded regions show the part of the complex that would protrude from a hypothetical 45-Å lipid bilayer symmetrically positioned in the two-dimensional crystal. The platform at the top of the complex suggests a functional role in membrane interaction through van der Waals' forces between LHC molecules in stacked membranes.

the 3-fold symmetry of the crystals, to map out the variation of amplitudes and phases along the lattice lines (Fig. 2). The data extended to a resolution of 16 Å.

A three-dimensional map of the LHC (Fig. 3), generated from structure factors sampled at regular intervals along lattice lines, shows that there are two LHC molecules per unit cell (Fig. 3*a*). The two molecules are related by a 2-fold axis, a symmetry element of the crystalline sheets that form *in vitro*. In the native membrane, all LHC molecules face the same way up[10]. A 3-fold axis runs vertically through the centre of each complex, indicating that LHC is a trimer, composed of three subunits in symmetry-related positions. The trimeric nature of the complex has also been suggested by gel electrophoresis[11]. The two trimers in the unit cell represent the two main aspects of the complex as seen from above and below the plane of the crystalline sheet, respectively. Clearly, LHC is an asymmetric membrane protein, exposing different structural features on both sides. One side (termed the top side) shows three hook-shaped, somewhat angular lobes separated by stain-filled gaps that extend ~20 Å vertically into the complex (arrow in Fig. 3*a*). Each lobe appears to be composed of three vertically oriented, roughly cylindrical domains of 15–20 Å diameter, connected by narrower bridging regions as indicated in Fig. 4. The other side shows three oblong protrusions which are continuous with the hook-shaped lobes.

An edge-on view of the three-dimensional map (Fig. 3*b*) shows that the thickness of the crystalline sheet and of the LHC trimer is ~59 Å, which agrees well with measurements made on stacked sheets[4] and by heavy metal shadowing. Seen edge-on, the complex appears highly asymmetric. Roughly half way up the trimer there appears to be a change in density. The less dense region at the bottom of the complex is taken up by the three elongated protrusions. The dense region which accounts for 80% of the stain-excluding volume consists of the three hook-shaped lobes. The lobes appear to form a rigid platform at the top of the complex which presumably is exposed at the surface of the thylakoid membrane. Immunological studies[10] have shown that both sides of the complex protrude from the native membrane and expose different antigenic sites on the two membrane surfaces. Due to the absence of a continuous lipid or detergent bilayer in the two-dimensional crystals used here, the three-dimensional map does not show by how much the complex protrudes on the two sides, but, given a bilayer thickness of 45–50 Å (ref. 12), the LHC cannot protrude by more than 10–15 Å (see Fig. 4).

An effect of the pronounced asymmetry of the LHC is a large difference in surface area exposed on both sides. The area exposed on the top side is three or four times larger than that exposed on the opposite side, and roughly twice as large as that exposed by a cylindrical molecule of the same height and volume. It is suggested that the extensive platform at the top of the LHC molecules has a functional role in membrane interaction. The repeat distance of stacked sheets, 56–58 Å (ref. 4), shows that complexes in adjacent membranes can approach one another close enough for van der Waals' forces to take effect[13]. The flat top surface of the LHC molecules seems highly suited for this purpose. Another possible functional role of this part of the molecule is that of a scaffold for orienting and arranging the antenna chlorophylls to cover a large surface area. The three protrusions on the other side could serve to anchor the molecule in the membrane and to make contact with the chlorophyll–protein complexes of photosystem II which are closely associated with the LHC[14].

The purified pea LHC used in this study contains roughly equal amounts of two different, largely homologous polypeptides of 25,000 and 27,000 apparent molecular weight[4,15] which each bind chlorophyll *a* and *b*. The total number of chlorophyll molecules attached to one polypeptide is in dispute, with reported values ranging from 6 (ref. 16) to 11 (ref. 17). The polypeptide patterns of LHCs isolated from other sources are often more diverse, containing up to four different components in the same molecular weight range. The larger polypeptide of pea LHC has recently been sequenced, giving an exact molecular weight of 28,512 (ref. 18). An estimate of the stain-excluding volume of the trimer at the selected contour level gave a molecular weight of ~100,000, using a specific volume of 1.3 $Å^3$ per dalton derived from the density of bacteriorhodopsin[9]. Allowing for bound chlorophyll, this value suggests that the trimer is composed of three rather than six polypeptides. It seems, therefore, that the two different polypeptides found in pea LHC are structurally equivalent and interchangeable in the trimer, in a way similar to isoenzymes such as lactate dehydrogenase in which two different polypeptides combine to form tetramers of all possible permutations[19]. Evidence indicating that the different LHC polypeptides are products of a small multigene family[18] supports this view. LHCs isolated from widely different species such as spinach[5] and barley[6] have very similar properties and form crystalline arrays similar to those studied here. The structure reported here may therefore prove to be valid for the main antenna protein of all green plants.

I thank K. R. Leonard who made computer facilities and programs at the EMBL, Heidelberg, available to me. The data merging program was written by S. D. Fuller. I also thank O. Kübler for the use of image processing equipment and a two-dimensional Fourier transform routine, and T. Thaler for his assistance in isolating the complex. Part of this work was supported by an EMBO short-term fellowship.

Received 12 September; accepted 22 November 1983.

1. Thornber, J. P. *A. Rev. Pl. Physiol.* **26,** 127–158 (1975).
2. Bennet, J. *Biochem. J.* **212,** 1–13 (1983).
3. Mullet, J. E. & Arntzen, C. J. *Biochim. biophys. Acta* **589,** 100–117 (1980).
4. Kühlbrandt, W., Thaler, T. & Wehrli, E. *J. Cell Biol.* **96,** 1414–1424 (1983).
5. Simpson, D. J. *Carlsberg Res. Commun.* **44,** 305–336 (1979).
6. Ryrie, I. J., Anderson, J. M. & Goodchild, D. J. *Eur. J. Biochem.* **107,** 345–354 (1980).
7. Arntzen, C. J. & Burke, J. J. *Meth. Enzym.* **69,** 520–538 (1980).
8. Li, J. & Hollingshead, C. *Biophys. J.* **37,** 363–370 (1982).
9. Henderson, R. & Unwin, P. N. T. *Nature* **275,** 28–32 (1975).
10. Anderson, B., Anderson, J. M. & Ryrie, I. J. *Eur. J. Biochem.* **123,** 465–472 (1982).
11. Dunkley, P. R. & Anderson, J. M. *Biochim. biophys. Acta* **545,** 174–187 (1979).
12. Franks, N. D., Melchior, V., Kirschner, D. A. & Caspar, D. L. D. *J. molec. Biol.* **155,** 133–153 (1982).
13. Barber, J. *Biochim. biophys. Acta* **594,** 253–308 (1980).
14. Andersson, B. & Anderson, J. M. *Biochim. biophys. Acta* **593,** 427–440 (1980).
15. Bennet, J., Markwell, J. P., Skrdla, M. P. & Thornber, J. P. *FEBS Lett.* **131,** 325–330 (1981).
16. Thornber, J. P., Steward, J. C., Hatton, M. W. C. & Bailey, J. L. *Biochemistry* **6,** 2006–2014 (1967).
17. Mullet, J. E. *J. biol. Chem.* **258,** 9941–9948 (1983).
18. Coruzzi, G., Broglie, R., Cashmore, A. & Chua, N. H. *J. biol. Chem.* **258,** 1399–1402 (1983).
19. Markert, C. L. & Møller, F. *Proc. natn. Acad. Sci. U.S.A.* **45,** 753–763 (1959).

Thermodynamics of the B–Z transition in superhelical DNA

Maxim D. Frank-Kamenetskii & Alexander V. Vologodskii

Institute of Molecular Genetics, Academy of Sciences of USSR, Moscow 123182, USSR

One of the most exciting events in recent years in molecular biology was the discovery of the left-handed Z form of the DNA double helix. Originally found in linear self-complementary $d(GC)_x \cdot d(GC)_x$ polymers[1] and oligomers[2] in non-physiological conditions (a rather high salt concentration), it was recently shown to be easily enough adopted in physiological conditions when purine–pyrimidine sequences are inserted into superhelical DNA[3–9]. From such a system, superhelical DNA carrying an artificial purine–pyrimidine insert, we can obtain data allowing the determination of the energy of the junction between the B and Z stretches, F_j, and the free energy change ΔF_{BZ} per base pair (bp). We present here a simple thermodynamic consideration of the B–Z transition in such a system. By applying the results to experimental data[3–9] we have shown that the thermodynamic parameters for both sequences studied so far ($d(GC)_x \cdot d(GC)_x$ and $d(GT)_x \cdot d(AC)_x$) are similar and equal to $F_j = 4$–5 kcal per mol per junction and $\Delta F_{BZ} = 0.5 \div 0.7$ kcal per mol per bp.

We consider closed circular DNA, N bp long, carrying an insert of n bp which is potentially capable of adopting the Z conformation, that is, sequences such as $d(GC)_x \cdot d(GC)_x$, $d(GT)_x \cdot d(AC)_x$, and so on.

Note that by n we denote the total number of bp in the insert, that is, $n = 2x$.

Generally speaking, we have to allow for the B–Z transition in our statistical-mechanical treatment of superhelical DNA along with open regions and cruciform structures[10–13] because all these alterations of the B form compete for the superhelix energy. We do that elsewhere[14]. Here we present a simple thermodynamic treatment based on the assumption that DNA may experience only the B–Z transition in its artificial purine–pyrimidine insert.

As the negative superhelicity increases, the insert adopts the Z form. In general, this process consists of two stages. At the first stage some fraction of the insert, m_0 bp long, flips from the B to Z form at some superhelix density value σ_0. Then, if $m_0 < n$, the rest of the base pairs in the insert would adopt the Z conformation gradually as the negative superhelix density increases further. The flipping occurs when the free energy increase due to the formation of the m-bp Z-form fragment is balanced by the energy decrease due to the relaxation of the strain in the superhelical DNA:

$$2F_j^* + m\Delta F_{BZ} + \Delta G_m = 0 \qquad (1)$$

Here F_j^* is the effective energy of the junction between B and Z forms, ΔF_{BZ} is the free energy change per bp for the B–Z transition. ΔG_m is the change in superhelix energy when m bp are converted from the B to Z form, which is known to be[5,15,16]:

$$\Delta G_m = 10RTN\left[\left(\sigma + \frac{1.8m}{N}\right)^2 - \sigma^2\right] \qquad (2)$$

Here σ is the titratable superhelix density value in DNA, and the factor 1.8 appears because the number of bp per helical turn is assumed to be 10.5 in the B form and 12 in the Z form[2,5]. Substituting equation (2) into equation (1) we obtain:

$$32.4RTm^2 + mN(\Delta F_{BZ} + 36RT\sigma) + 2NF_j^* = 0 \qquad (3)$$

The minimal value of $-\sigma$ for which equation (3) has a real root with respect to m defines the superhelix density of flipping, σ_0. This value is determined by setting the discriminant of quadratic equation (3) at zero, leading to:

$$\sigma_0 = -\frac{1}{36RT}\left(\Delta F_{BZ} + 11.4\sqrt{\frac{2F_j^* RT}{N}}\right) \qquad (4)$$

The number of bp that flip at this superhelix density:

$$m_0 = 0.175\sqrt{\frac{2F_j^* N}{RT}} \qquad (5)$$

Equations (4) and (5) are valid only if $m_0 < n$. If they lead to an m_0 value larger than n it means that the whole insert flips to the Z form at some value, σ_n, ($|\sigma_n| > |\sigma_0|$) and this value is determined by equation (3) with m replaced by n. In the case of $m_0 < n$ the flipping is followed by a gradual growth of the Z form stretch with increasing negative superhelicity. For m values within the interval $m_0 < m \leq n$ the following equation holds:

$$\Delta F_{BZ} + 36RT\left(\sigma + 1.8\frac{m}{N}\right) = 0 \qquad (6)$$

Equation (6) reflects the fact that the energy increase due to the transition of a base pair from the B to Z form should be balanced by the energy decrease due to the decrease of the superhelix strain. Equation (6) shows that

$$\sigma + 1.8\frac{m}{N} = \text{const. for } m_0 < m \leq n \qquad (7)$$

Before comparing the results with experimental data we must emphasize that our treatment was oversimplified in one important point. We have neglected the entropic terms that originate from the fact that a number of states with the same or almost the same energy correspond to a given value of m (when $m < n$). Most probably all positions of the originally flipping stretch of m_0 bp are energetically equivalent while it is within the n-bp insert. We have performed rigorous calculations based on the partition function. The complete results will be presented at length elsewhere[14]. The general situation has proved to be rather complicated. It is simple if m_0 is small compared with n, in which case the above equations remain valid with

$$F_j^* = F_j + \Delta \qquad (8)$$

The value of Δ is a weak function of the parameters and may be considered as a constant $\Delta = -1.8$ kcal per mol. When $m_0 \geq n$, the entropic terms may be practically neglected, that is, $F_j^* = F_j$.

Turning to the experimental situation, we see that both behaviours predicted by the above theoretical analysis were really observed. In most cases the insert flipped as a whole[3–7] but Haniford and Pulleyblank[8] have recently observed the flipping of a part of the insert followed by the elongation of the Z form stretch with increasing negative superhelix density.

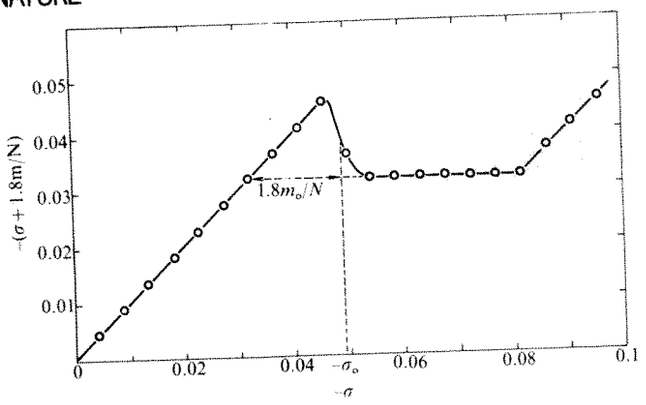


Fig. 1 The data of Haniford and Pulleyblank[8] presented in a form suitable for theoretical analysis. The value $\sigma + 1.8m/N$ is determined directly from the scaled experimental value of DNA mobility in gel. The value of σ_0 corresponds to the middle point in the flipping of the m_0-bp stretch.

© 1984 Macmillan Journals Ltd

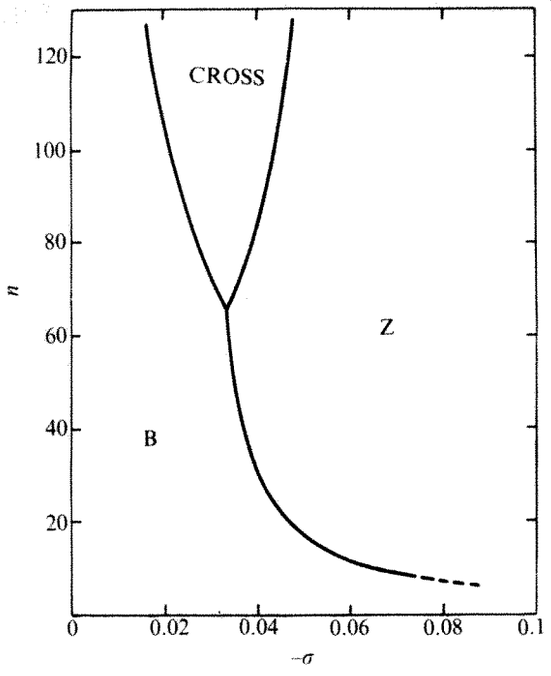


Fig. 2 Phase diagram for an n-bp $d(GC)_x \cdot d(GC)_x$ sequence inserted into long superhelical DNA with superhelix density σ. The calculations have been performed for $F_j = 4.0$, $\Delta F_{BZ}^{GC} = 0.6$, $F_s = 6$ and $\Delta F^{GC} = 2.1$ kcal per mol corresponding to 0.2 M Na^+.

In their experiments the $d(GT)_{30} \cdot d(AC)_{30}$ sequence was inserted into a plasmid with $N = 2.2 \times 10^3$ and at $\sigma_0 = -0.050$ $m_0 = 22$ bp flipped into the Z form[8]. Then the writhing number remained unchanged until the whole insert adopted the Z conformation. During this stage $\sigma + 1.8m/N = -0.032$ (Fig. 1). Using equations (5) and (6) and the above data, $F_j^* = 2.2$ kcal per mol for junction and $\Delta F_{BZ} = 0.69$ kcal per mol per bp. Substituting these values into eq. (4) we see that they give the observed value of σ_0. It follows from equation (8) that $F_j =$ 4.0 kcal per mol per junction. Since the $-\sigma_0$ value is rather low, the competition with cruciform structures can be neglected. This follows from the control two-dimensional gel electrophoresis pattern in[8] which shows no discontinuity up to the $-\sigma_0$ value. A cruciform structure would certainly have produced a clear-cut discontinuity in the two-dimensional pattern[17].

Equation (5) shows that an insert would flip as a whole if

$$n \leq 0.175 \left(\frac{2F_j N}{RT}\right)^{1/2} \tag{9}$$

For the most popular plasmid pBR322 ($N = 4.4 \times 10^3$) we conclude, substituting the above value of $F_j = 4.0$ kcal, that the insert would flip as a whole up to $n \approx 40$ bp. This estimate makes it quite clear why most people observed the flipping of whole inserts[3–7]. To interpret their data one has to use equation (3) in which m is replaced by n. This equation is similar to the one used by Nordheim *et al.*[5]

It follows from equation (3) that measuring the superhelix density values σ_1 and σ_2 of the B–Z transition for two inserts with lengths n_1 and n_2 one may determine the parameters:

$$F_j = 18RT\left[\sigma_2 - \sigma_1 + \frac{0.9}{N}(n_2 - n_1)\right]\left(\frac{1}{n_1} - \frac{1}{n_2}\right)^{-1} \tag{10}$$

$$\Delta F_{BZ} = 36RT\left[\frac{n_1\sigma_1 - n_2\sigma_2}{n_2 - n_1} - \frac{0.9(n_1 + n_2)}{N}\right] \tag{11}$$

Substituting the experimental values[5] ($n_1 = 14$, $n_2 = 32$, $\sigma_1 = -0.072$, $\sigma_2 = -0.048$) into equation (10) we obtain $F_j = 7.5$ kcal per mol in agreement with the original paper[5] but contradicting the above estimation. This may be explained by cruciform formation in the carrier pBR322 DNA between superhelix densities σ_1 and σ_2. Indeed, Singleton[18] has recently shown that in pBR322 DNA a 35 bp palindrome with the centre in position 3,065 assumes a cruciform. If in the conditions described by Nordheim *et al.*[5] it is formed within the superhelix density interval $\sigma_1 < \sigma < \sigma_2$ then we should replace in equations (10) and (11) the σ_1 value by the value $\sigma_1^* = \sigma_1 + n_+/N$ (n_+ is the number of bp in the palindrome[13]), then $\sigma_1^* = -0.064$ and equations (10) and (11) yield the values $F_j = 5.3$ kcal per mol and $\Delta F_{BZ} = 0.56$ kcal per mol. This example shows that the behaviour of comparatively short inserts that flip at high negative superhelix densities may be seriously affected by the cruciform formation in the carrier DNA[14].

The remaining difference between the values of parameters obtained for $d(GC)_x \cdot d(GC)_x$ and $d(GT)_x d(AC)_x$ inserts can hardly be regarded as significant and most probably reflects the accuracy of our present-day knowledge of the parameters. Other estimations of the same parameters have very recently led to quite similar results[19,20].

Having the parameters of the B–Z transition, we can predict the behaviour of a purine–pyrimidine insert for different n. If the sequence of the insert is not self-complementary, the behaviour is governed by the above equations. However, a self-complementary sequence (such as $d(GC)_x \cdot d(GC)_x$) can adopt a cruciform structure as well as a Z form. So in this case we have to consider the equilibrium between three possible states: the B form, the Z form, and the cruciform. For the sake of simplicity we will assume that N is very large and as a result m_0 is larger than n for all values of n under consideration. The free energies of the insert's transition from the B to Z form and from the B form to the cruciform are

$$F_z = n(\Delta F_{BZ} + 36RT\sigma) + 2F_j \tag{12}$$

$$F_+ = 20RTn\sigma + 3F_s + 4\Delta F^{GC} \tag{13}$$

Equation (12) immediately follows from equation (3) at $N \to \infty$. In equation (13) the last two terms are the energy of the cruciform formation, that is, the energy of six helix ends, and the energy of the formation of two single-stranded loops comprising four nucleotides each[10,11,13]. Using equations (12) and (13) we obtain the phase diagram plotted in Fig. 2. The diagram shows that small n values are not favourable for a cruciform structure. However, for n larger than 70 there is an interval of superhelix densities where a cruciform structure proves to be the most stable conformation. This interval grows as n increases.

Note that this behaviour may be difficult to observe because of the very large relaxation time, especially for the cruciforms[17,21].

Received 3 July; accepted 2 November 1983.

1. Pohl, F. M. & Jovin, T. M. *J. molec. Biol.* **57,** 375–396 (1972).
2. Wang, A. H. J. *et al. Nature* **282,** 680–686 (1979).
3. Peck, L. J., Nordheim, A., Rich, A. & Wang, J. C. *Proc. natn. Acad. Sci. U.S.A.* **79,** 4560–4564 (1982).
4. Singleton, C. K., Klysik, J., Stirdivant, S. M. & Wells, R. D. *Nature* **299,** 312–316 (1982).
5. Nordheim, A. *et al. Cell* **31,** 309–318 (1982).
6. Stirdivant, S. M., Klysik, J. & Wells, R. D. *J. biol. Chem.* **257,** 10159–10165 (1982).
7. Wang, J. C., Peck, L. J. & Becherer, K. *Cold Spring Harb. Symp. quant. Biol.* **47,** 85–91 (1983).
8. Haniford, D. B. & Pulleyblank, D. E. *Nature* **302,** 632–634 (1983).
9. Nordheim, A. & Rich. A. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1821–1825 (1983).
10. Anshelevich, V. V., Vologodskii, A. V., Lukashin, A. V. & Frank-Kamenetskii, M. D. *Biopolymers* **18,** 2733–2744 (1979).
11. Vologodskii, A. V., Lukashin, A. V., Anshelevich, V. V. & Frank-Kamenetskii, M. D. *Nucleic Acids Res.* **6,** 967–982 (1979).
12. Vologodskii, A. V. & Frank-Kamenetskii, M. D. *FEBS Lett.* **131,** 178–180 (1981).
13. Vologodskii, A. V. & Frank-Kamenetskii, M. D. *FEBS Lett.* **143,** 257–260 (1982).
14. Vologodskii, A. V. & Frank-Kamenetskii, M. D. *J. biomolec. struct. Dynam.* (in the press).
15. Depew, R. E. & Wang, J. C. *Proc. natn. Acad. Sci. U.S.A.* **72,** 4275–4279 (1975).
16. Pulleyblank, D. E., Shure, M., Tang, D., Vinograd, J. & Vosberg, H.-P. *Proc. natn. Acad. Sci. U.S.A.* **72,** 4280–4284 (1975).
17. Lyamichev, V. I., Panyutin, I. G. & Frank-Kamenetskii, M. D. *FEBS Lett.* **153,** 298–302 (1983).
18. Singleton, C. K. *J. biol. Chem.* **258,** 7661–7668 (1983).
19. Singleton, C. K., Klysik, J. & Wells, R. D. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2447–2451 (1983).
20. Peck, L. J. & Wang, J. C. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
21. Vologodskii, A. V. & Frank-Kamenetskii, M. D. *FEBS Lett.* **160,** 173–176 (1983).

Anatomy of higher education

Eric Ashby

The Higher Education System: Academic Organization in Cross-National Perspective. By Burton R. Clark.
University of California Press: 1983. Pp.315. £21.25, $32.50.

TO SET this book in perspective, here are three incidents from my own experience.

First, dinner at high table in the University College of the Gold Coast (now Ghana) in 1956. The students, wearing gowns, stand as we file in: the master, the senior tutor, the steward, the chaplain. A Latin grace is read. After dinner we retire to the Combination Room and are offered port. The terms, in a nation with a large Muslim and pagan population, were labelled Michaelmas, Lent and Trinity. The purchasing officer was called a manciple.

Second, a faculty discussion in Lovanium University in what was, in the 1950s, the Belgian Congo. Or, to be accurate, a briefing, not a discussion, for the rector was a Napoleonic figure unconcerned with the niceties of democracy. He was appointed from Louvain and was in direct teletype contact with Belgium from his office in Leopoldville. The number of Congolese graduates at that time was pathetically small for a country about to become independent. What curriculum (I asked) had they devised in order to prepare the Congolese to administer their affairs. Exactly as in Louvain, was the reply: a basic course in philosophy, French language and French literature before qualifying for any professional training; *Chanson de Roland* in the first year.

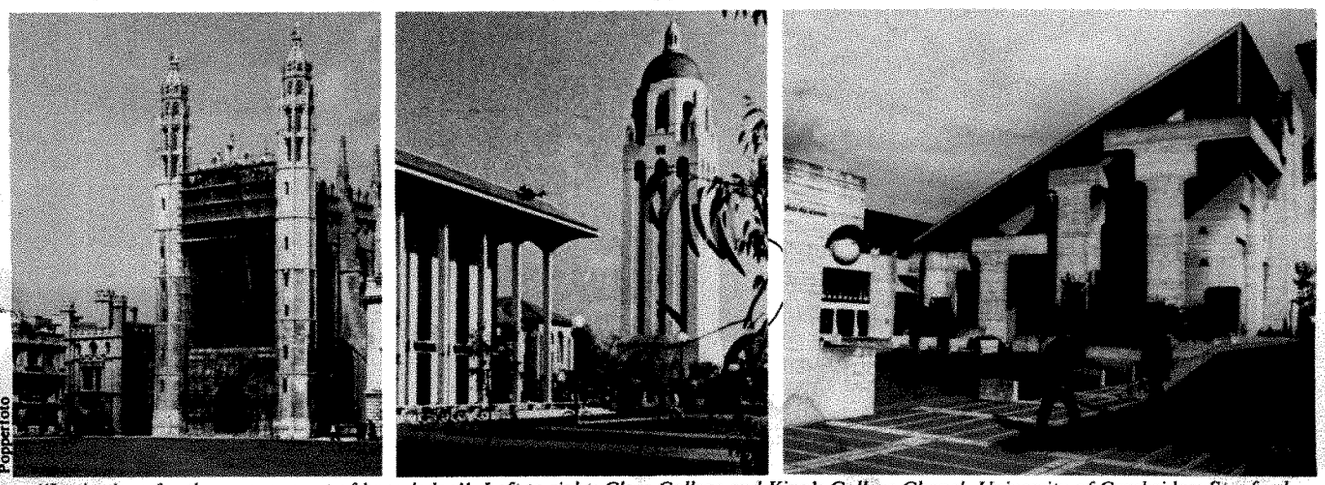
Third, a visit to the University of Nigeria in Nsukka, created in the early 1960s under the sponsorship of Michigan State University. As you come from Enugu you see the university spread below you, on a flat plain below a steep hill, with the central, predominant feature of a sports stadium. The cafeteria, I found, was crowded with somewhat subdued and perplexed farmers from the surrounding villages, attending a week-end course in poultry nutrition.

These were just the superficial signs of the genetic imprint of Cambridge on Ghana, Louvain on the Congo and Michigan on Nigeria. It permeated curriculum, style of administration, academic values. At first there was scant evidence of adaptation to the local society. This deep imprint of older universities on newer ones is not just a recent phenomenon. In 1551 the Spanish emperor Charles V founded universities in Mexico and Peru with all "the privileges, exemptions, and limitations of the University of Salamanca". Even the order of precedence in the academic procession and the exact procedure at convocations had to be prescribed from Salamanca. In Scottish universities there is a vestige of inheritance from Bologna, when the students elect the Rector. In Cambridge, candidates for the bachelor's degree are presented to the vice-chancellor with the assurance (fortunately delivered in Latin) that they have satisfied their teachers in morality as well as in learning. For centuries the university, like church and parliament, has shown extraordinary resilience and capacity for adaptation — surely a challenge to scholars to analyse how this has happened. Yet, despite shelf-loads of rhetoric about "the Idea of a University" and some good historical accounts of universities in separate countries, very few scholars have risen to it. Burton Clark is one of them. This alone makes his book an important one.

The easy way to approach the task is to describe, seriatim, the systems of higher education in different countries and then in a final chapter to apply the formula familiar to examinees — "compare and contrast". The result is rarely illuminating and invariably dull. Clark meets the challenge in a more ambitious way. What, he asks, are universities *for*? What do they believe about themselves and what does the so called "layman" (that word is historically significant too) think about them? Where does authority lie in systems of higher education? They have changed over the centuries and by implantation into alien cultures: what have been the incentives, and what have been the impediments, to change?

The raw material for universities is knowledge. They are, Clark declares, institutions for the management of knowledge; research and teaching are the technologies employed in management. But knowledge is not homogeneous. An institution for the management of knowledge brings on to one campus mediaevalists, biochemists, engineers. Why? Mediaevalists can get on perfectly well without biochemists. Are they together simply to share the central heating system and the catering facilities? In the Soviet system much professional education is in staff colleges attached to the appropriate ministries: lawyers in a college run by the ministry of justice, doctors in a college run by the ministry of health. Yet an institution embracing many diverse disciplines remains the standard pattern for higher education.

Clark's painstaking analysis of this question holds no surprises for anyone who has spent a lifetime in academe, but it includes a great deal that is not understood by the politicians and bureaucrats who now have such influence over the future of higher education. He explains how it is much more than basic facilities that bring scholars together into universities. They share certain beliefs; they have common problems of divided loyalty; they cannot work well unless they are allowed to devise for themselves a very peculiar structure of



"Institutions for the management of knowledge". Left to right: Clare College and King's College Chapel, University of Cambridge; Stanford University and the Université Catholique de Louvain

power and authority, one in which the process of decision-making does not descend in decrees from above but arises on the "shop floor" and percolates upwards, to reach (paradoxically) the desk of the individual who, *de jure*, is the boss of the institution. Prominent among the beliefs is a common purpose: to transmit, by teaching, the orthodoxy of the discipline and to generate, by research, a rigorously controlled system of dissent from orthodoxy. There is a mystique about this and there is intense loyalty to an institution that nurtures this mystique. But these communities of scholars have to come to terms with two often conflicting loyalties. On one hand their loyalty is to the discipline: what matters most to a physicist may be his status among other physicists, and this tempts him to put what he calls "my own work" before the interests of the university in which he serves. On the other hand their loyalty is to the institution and its corporate interests: what matters most may be the educational mission of the community, to produce (as the Oxford don, Mark Pattison, put it) "not a book but a man". "The culture of a discipline even includes idols", writes Clark. In the sociologist's office you see a picture of Max Weber on the wall. Yes, and in the office of a chemist who is also a tutor or a dean you see a picture of Magdalen quadrangle on the wall. It is symbolic of the split-personality that inhabits the academic mind. There are subtle differences among different countries in the emphasis given to these loyalties. An American would talk of belonging to the Yale Class of '24; a German would not state his university, but would say "I was a pupil of Heisenberg"; a graduate from Oxbridge does not even mention his university: he is called "a Clare man" or "a Wadham man".

An institution for the management of knowledge has to solve a very difficult problem in the management of its own members. The scholars are differentiated into dozens of disciplines and sub-disciplines. Those in authority over them cannot possibly tell them what to do; yet someone has to allocate space, cut the financial cake into slices, provide the common services. And someone has to defend the university's interests against pressures from what is (shortsightedly) called "the outside world". Clark describes in detail the patterns of academic authority: the dictatorial professor who has the right of patronage (as is to be found in Italy); the collective control by committees of academics (as is to be found in Britain); the administrators who manage the university as a sort of holding company for semi-autonomous oligarchies of faculties or colleges (as in California); the limited delegated control under a system managed by the state, where the professors are civil servants (as in France). He sees good and bad in all these patterns and wisely refrains from committing himself to preferences among them.

Finally, Clark discusses change in institutions of higher education. Here, it seems, is another paradox: that they are notoriously the most exasperatingly conservative bodies and yet it is from them that the most revolutionary initiatives for changes in society come. Clark finds this puzzling, but I think the explanation may be quite simple. It is that the initiatives come from the "shop floor", from the research and writing of young people. The resistance comes from the people who have become enmeshed in the administrative network of the place or whose interests would be endangered by change. The obduracy of the University of London to reform itself is a local British example of this.

Clark's book is written with authority but I must add a warning that his conclusions are at a level of generalization which I found at times hard to follow. I am not a sociologist, so it is not for me to carp about the use of abstract latinized words which to the uninitiated may seem to be jargon. In writing this "layman's" review of it, I may have oversimplified some of Clark's subtle reasoning, but I hope I have made it clear that this is a book which, even if it isn't to be read for pleasure, is certainly to be studied for profit. □

Lord Ashby is a Fellow of Clare College, Cambridge, and has been a professor in the Universities of Sydney and Manchester, and vice-chancellor in the Universities of Queen's, Belfast, and Cambridge.

Surface Physics

Dr J.E. Inglesfield asks us to point out that the title to his review of M. Prutton's *Surface Physics*, 2nd Edn (*Nature* **307**, 190; 1984) was chosen not by him but in the *Nature* office. No slight upon the book was intended by the title "Superficial science".

Editor, *Nature*.

Archaeology, A to Z

Colin Platt

The Macmillan Dictionary of Archaeology.
Edited by Ruth D. Whitehouse.
Macmillan, London: 1983. Pp.597. £25.

THE emergence of archaeology as an amalgam of several disciplines has been comparatively recent, dating back scarcely further than the 1960s. And it is this crossing of boundaries, still bewildering to many, that is the chief justification for Dr Whitehouse's new *Dictionary*. Handsomely presented in a generous layout, it has the enticing feel of a good work of reference: essential, of course, for instant instruction, but as well suited for a pleasurable browse. How many of us would have been aware, without such aid, that the earliest phase of the North Vietnamese Bronze Age is known to its students as "Go Bong"? On the very same page, we are cross-referred away from Gogo Falls, and are given a good, short account of the goat. Here is pleasure, stimulus and intelligence, all in one.

Nobody need have misgivings about the intended scope of this dictionary. Its field is the wide world; its timespan is four million years; its concern is with techniques as well as evidence. Nevertheless, what precisely were the principles, we are still bound to ask, that guided Ruth Whitehouse and her small team of subject editors in their selection of material for discussion in these pages? In the little she tells us on this fundamental matter, Dr Whitehouse is not reassuring. "I cannot claim", she confesses, "that coverage is even. . . . My own prejudices, and to a lesser extent those of my subject editors, have necessarily prevailed." One of these prejudices — perhaps a considered principle, though we are not so informed — is that the best archaeologist is necessarily a dead one. Thus David Clarke (d.1976), as one of the pioneers of the New Archaeology which itself gives purpose to this book, gets his entry. But Lewis Binford, very much more influential in the same field as Clarke precisely because he remains alive and active, does not. The editors have been spared embarrassing decisions, but only to the impoverishment of their text.

In the same way, other omissions would have been easier to accept if more trouble had been taken to explain them. Of course, this is a familiar game among critics of dictionaries, but so much the more reason to anticipate it. Why Repton and not Deerhurst? Why Krak des Chevaliers and not Sahyun? Why San Vincenzo al Volturno and not Monte Cassino? In each case, the answer seems to be that there have been excavations (or research programmes) on these sites known personally to one of the subject editors of this volume. And increasingly, as we come to follow through our individual specialisms, the *Macmillan Dictionary of Archaeology* begins to look narrowly based. Why, to go back to the general editor's opening apologia, do the compilers' prejudices, in a work of reference of this kind, "necessarily" have to prevail? They are a clannish group in which marital pairings and former scholarly cooperations are prominent. Could it be that there were just not enough of them from the start?

Fortunately, the preoccupations of Ruth Whitehouse's "team of international experts" are very likely to be those of the great mass of users of this dictionary. Nor is there any doubt that there is much of real value in these pages, clearly and professionally expressed. Mutton dressed as lamb, perhaps. But still good food. □

Colin Platt is a Professor of History at the University of Southampton.

Newton of promise

C.W. Kilmister

Certain Philosophical Questions: Newton's Trinity Notebook.
By J.E. McGuire and Martin Tamny.
Cambridge University Press: 1983. Pp.519. £50, $84.50.

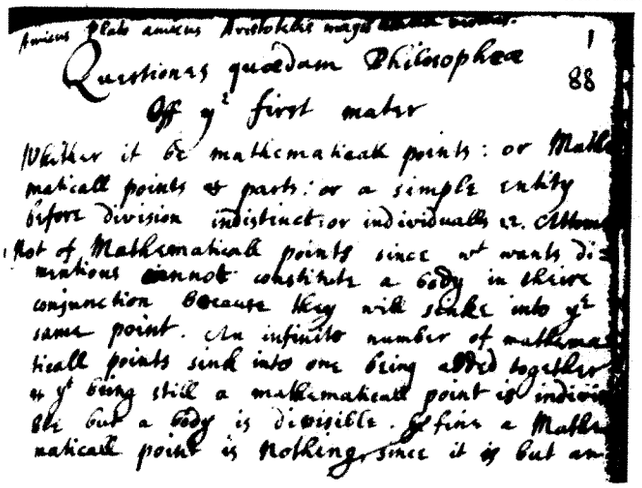
WE now know a great deal more about Newton than we did 30 years ago; this is mainly the result of Whiteside's magnificent edition of the papers, with the Halls' collection of the letters coming a close second*. As a result we can see the mistakes as well as the triumphs of a genius, and insights that, for one reason or another, never saw the light of day.

But are we any nearer to understanding this most astonishing of men? Perhaps a little, but because there is still so much left to understand one should hesitate long before relegating a work such as this to the "Newton's laundry book" category. And indeed such a judgement would be wholly unjust. In 1661, at the age of 18, Newton began to keep notes about his studies in Cambridge in a notebook which is in the University Library. But the *Questiones quaedam philosophicae* are (English) notes well on in the book and so almost certainly date from late March 1664 to late 1665. Since 1666 was to be, by his own confession, Newton's *annus mirabilis,* these notes on scientific matters could not fail to be of interest.

The book has been produced with all the care that American scholarship can provide, and the finished work is one of considerable beauty. Two-thirds of it is taken up by an essay describing the *Questiones* in the context of Newton's thought and of other thought of the time, and the remaining third presents, on facing pages, the transcription of the notebook and a modern English version (in which even Newton's sketches are carefully redrawn). So there is something for everyone. The historian and scholar have an important source presented in meticulous detail. The scientist can read the modern English and see Newton grappling with a variety of questions, some a consequence of the theoretical background with which he had been saddled by his university course, some of a much more modern kind.

He begins with a discussion of atomism and the "first matter", concluding that there must be atoms, finite in magnitude, and that as a consequence the "world" (i.e. the Universe) must be composed of a finite number of them, since "an infinite number of finite parts cannot be finite". This discussion partakes of an Aristotelian flavour in the arguments rejecting the possibilities of mathematical points serving as atoms. And Newton goes on to infer that, to discuss motion, there must be

> a least distance, a least progression in motion, and a least degree of time. Lay two globes together so close that they cannot come any nearer without touching — that is the least distance. Let them be moved together — that is the least degree of motion and it is performed in the least part of time.


Questiones quaedam Philosophicæ
Of y^e first matter
88
Whether it be mathematicall points: or Mathematicall points & parts: or a simple entity before division indistinct: or individualls &c. Not of Mathematicall points since ye wants dimensions cannot constitute a body in their conjunction because they will shake into y^e same point. An infinite number of mathematicall points sink into one being added together & y^t being still a mathematicall point is indivisible but a body is divisible. Ergo a Mathematicall point is nothing, since it is but an

In his own hand — the title page of the "Questiones", consisting of Newton's notes on Walter Charleton's consideration of the nature of first matter in "Physiologia Epicuro-Gassendo-Charltoniana".

So here was someone still far from understanding the use, let alone the nature, of fluxion or fluent, but at the same time preparing the ground for the seed. He returns to such problems later, evidently still worried, but without resolving them. This really summarizes the character of the whole book.

The next discussion is of the Sun, stars and planets: "Whether the Sun moves the vortex about (as Descartes's will) by his beams. . . . How it is that the Sun turns on his axis". Then a quotation "Hebrews, Chapter 1, Verse 2, God made the worlds by his son . . ." is followed shortly after by "On Saturday, December 10, 1664, by a subtle observation I found the distance of a comet from the centre of the Moon to be 9° 48′ . . .".

The properties of matter take Newton's attention next, including rarity, density, perspicuity, opacity, gravity and levity. He notes that "the matter causing gravity must pass through all the pores of a body". This is followed by a detailed discussion of the behaviour of this matter as it streams down to the Earth and back again. Then, quite soon, "you may observe in water that a thing moved in it does carry along with it the water behind it, as in a cone, or at least the water is moved from behind it with but a small force . . .".

There follows a varied selection of queries — on reflection of light, on atmospheric pressure, "What angle ought a windmill sail make with the wind", on water and salt, on magnetic attraction and electrical attraction, and so to much more on light, vision and colours. Even questions of memory, imagination and sympathy are touched on, succeeded almost at once by consideration of the character of oils, meteors, minerals and the tides. Here, as well as theoretical aspects of the vortex theory, there are notes about the reversing of the tides between Harris and Uist and about the alleged difference in speed of the Danube "as is perceived by the motion and noise of the clackers in mills" between six and midnight though "there is no ebb nor flow, the water keeping at a constant height".

Ever and again violent motion and motion itself return. Newton asks "Whether there can be motion in a vacuum" (since an earlier discussion interpreted the relative character of motion very strictly in terms of adjacent bodies). The argument concludes "If this going of c to d be not motion I ask what it is. But this is only to strive about terms, and if it pleases you not to call it motion call it what you will, but it is that which we aimed to prove". There are many astronomical observations and discussions, mostly of comets, before a return to motion yet again and then more optics. So it goes on, with notes on God and the soul, before various chemical queries come all together — genuine chemistry rather than alchemy, such as: "Alcalizate salts are wont to precipitate what acid salts dissolve".

So where does the notebook show us Newton standing? We see a man with at least part of one foot still in the Middle Ages, still needing to liberate himself from the weakening clutch of Aristotle; a man who has studied Descartes and is evidently not too happy about vortices. But above all we discern an enquiring mind, compulsively interested in everything around him or reported to him. In some ways a more human figure emerges. The authors tell us that "Newton's copy of Diogenes is extensively dog-eared . . . . As Newton used the technique of dog-earing, a dog-ear was used not only to mark a page but often to mark a place on a page". But the mystery is still there. The author of the notebook is the young man of promise. The Newton of 1666 still seems a different person. □

**The Mathematical Papers of Isaac Newton* (in 8 volumes). Edited by D.T. Whiteside. Cambridge University Press: 1967–1982. *The Correspondence of Isaac Newton* (in 7 volumes). Edited by A.R. and M.B. Hall. Cambridge University Press: 1959–1977.

C.W. Kilmister is Professor of Mathematics at King's College, University of London, and a past President of the British Society for the History of Mathematics.

Experiments in the gauge revolution

C.H. Llewellyn Smith

Weak Interactions of Leptons and Quarks.
By E.D. Commins and P.H. Bucksbaum.
Cambridge University Press: 1983. Pp.473. Hbk £37.50, $69.50; pbk £16.50, $27.95.

FOLLOWING the discovery of parity violation in β decay in 1957, it was soon found that a wide range of "weak" decay processes can be described by a simple "phenomenological" theory. The pejorative adjective indicates that this theory is not mathematically consistent and can only work in a limited energy domain. In 1971, 't Hooft showed that consistency could be obtained in gauge theories of the type discussed earlier by Weinberg, Salam, Glashow and others, which have the great attraction of unifying weak and electromagnetic interactions. There was then no experimental evidence for such theories but their revolutionary potential was sufficient to stem the flow of books on weak interactions which had appeared during the 1960s.

Confidence in gauge theories built up as their predictions were gradually confirmed, beginning with the discovery of "neutral current" neutrino interactions at CERN in 1973 and culminating in the discovery of the W and Z bosons — the carriers of the weak force — with the predicted masses at CERN last year. Reflecting these developments, textbooks which incorporate or describe the "gauge revolution" have begun to appear in the past few years. This one, which is suitable for graduate students or as a reference work for researchers, is the first of the new generation of books written from an experimental standpoint in which crucial experiments are described. As well as the traditional topics, there are chapters on most of the subjects which ought now to be in such books — high-energy neutrino interactions, neutrino masses and oscillations, parity non-conservation in eN and NN interactions (including in atoms — the authors' speciality) and neutrinos in astrophysics.

Turning to omissions, it is odd that there is no derivation — or even statement — of the Fierz transformation, although it is frequently used. The omission of a proper discussion of current algebra and chiral symmetry is more serious. The Adler–Weisberger and Goldberger–Treiman relations appear (although the latter is confused with pole dominance of the pseudo-scalar form factor and we are not told whether the real relation works). Discussion of the Callan–Treiman relation and predictions for slope parameters in K $\rightarrow 3\pi$ surely also deserved some space. On the other hand, the "abbreviated and superficial" discussion of path integrals will help neither the novice nor the expert and could have been left out. The authors nowhere really use or need the full Feynman rules which they derive using this technique (although some discussion of testable higher-order predictions of gauge theories might have been desirable).

A more serious criticism is that the book contains several theoretical remarks which are dubious or misleading. Two of the more subtle examples must suffice. It is not true that Lagrangians "satisfy the Euler Lagrange equations" or that current conservation is in general equivalent to invariance of the Lagrangian (the whole question of auxiliary fields in supersymmetry bears witness to this). The mass-matrix for the K^0–$\bar{K}^0$ system does not follow from the Schrödinger equation given (although the appearance of the word "effective" perhaps provides an alibi) — a derivation involves the Wigner–Weisskopf theory of decaying states. A final point is that it seems perverse in a book largely devoted to interactions of neutrinos to normalize spinors in a way which is singular for massless particles.

Despite its faults, this is not a bad book. There are better introductions to gauge theories, such as *Gauge Theories in Particle Physics* by I.J.R. Aitchison and A.J.G. Hey (Adam Hilger, 1982), and clearer discussions of the phenomenology of weak interactions, for example in L.B. Okun's *Leptons and Quarks* (North-Holland, 1982) or T.D. Lee's monumental *Particle Physics and Introduction to Field Theory* (Harwood, 1981). However, this is the only up-to-date book on weak interactions which includes a discussion of the experiments and the data, and I would therefore advise students to read it and librarians to buy it. □

C.H. Llewellyn Smith is a Reader in the Department of Theoretical Physics, University of Oxford.

Exciting stuff

F.W.J. Teale

Principles of Fluorescence Spectroscopy.
By Joseph R. Lakowicz.
Plenum: 1983. Pp.496. $32.50, £25.

EVEN the most cursory glance over the literature of biochemistry, biotechnology and clinical medicine confirms the continuing growth in the exploitation of fluorescence techniques. How then should uninitiated researchers equip themselves to join the enlightened throng? Only the most dedicated can afford the time necessary to gain experience in a recognized "fluorescence" laboratory. More ordinary mortals have recourse to reading reviews, specialized articles and older classic books and papers selected from a large and diffuse literature. This can be a hard, unrewarding slog for the newcomer, and there has long been a need for a suitable primer in this field.

Joseph Lakowicz has attempted to remedy this need by bringing within a single monograph the basic introduction, essential theory and working practice of the most important aspects of fluorescence spectroscopy, at least as encountered in the life sciences. The reader has the vicarious benefit both of what is clearly a thorough knowledge of the literature, and of the author's experience in a well-known laboratory since his treatment strongly reflects the influence of Gregorio Weber to whom the book is dedicated. This is no small recommendation in itself. Phase-modulation techniques, after two decades of undeserved and incidental eclipse by pulse-excitation, are rightly restored to pre-eminence, while the phase-dependent method developed by the author for resolution of heterogeneous emission occupies a complete short chapter. Longer, well-illustrated sections are devoted to the fundamental fluorescence parameters and to a selection of excited-state processes implied by the "spectroscopy" in the title. As the latter suggests, the main emphasis is on fundamental aspects, while examples are taken almost entirely from protein and membrane systems.

A description of the essential instrumentation, calibration methods, common malfunctions and experimental pitfalls comes immediately after a short general introduction. This introduction, however, is barely adequate in conveying the range of molecular information potentially made accessible by appropriate strategies, and an early chance to whet appetites is rather missed. That said, the level of presentation is nicely poised between the superficial and the recondite. So that the reader has some likelihood of pursuing them, references are restricted to major benchmark publications (except for several "unpublished observations", which would have been better dealt with in the text). A useful feature, stressing the didactic intention of the author, is the inclusion of simple problems at the end of most chapters; if attempted, these oblige the reader to understand fully the main thrust of the preceding material. Correct solutions are also provided for reassurance.

Lakowicz's aim in writing this book was to satisfy the requirements of those interested in understanding the principles underlying the various fluorescence techniques encountered in contemporary research. To my mind, he has succeeded. □

F.W.J. Teale is the Reader in Physical Biochemistry in the Department of Biochemistry, University of Birmingham.

NEW FROM NORTH OXFORD ACADEMIC

Two key books on Japan

Japan: Science & Technology Outlook

Based on the White Paper recently prepared by the Japanese Science & Technology Agency, this book deals extensively with Japan's current progress in the development of new and original technology in such areas as nuclear fusion, space technology, bio-engineering, electronics and communications, marine technology and new materials. Authoritative data is presented on all aspects of Japanese research and development, including details of international co-operation.

ISBN 4 938329 11 5 £44.00 net

The latest edition of the
Computer White Paper

Provides each year a comprehensive picture of Japan's DP industry. Many graphs and tables are included to give a clear and authoritative picture of the industry and its policies.

£30.00 net

Order from your bookseller, or direct (cash with order) from
North Oxford Academic
242 Banbury Road, Oxford OX2 7DR, U.K.

Circle No.18 on Reader Service Card.

NEW FROM BUTTERWORTHS

Gaseous Air Pollutants and Plant Metabolism

Edited by

M J Kozioł,
DPhil, Botany School, University of Oxford

F R Whatley,
PhD, Sherardian Professor of Botany, University of Oxford

The biochemical interactions of gaseous air pollutants with plant metabolism is a subject of increasing interest and study. This volume contains contributions from leading experts dealing with the effects of the major gaseous pollutants on aspects of plant metabolism including bioenergetic pathways, enzyme activities, metabolite pools and cell buffering capacities.

Gaseous Air Pollutants and Plant Metabolism is an essential review for research workers in botany, biochemistry, agriculture and the environmental sciences and for industrial and governmental organizations concerned with air pollution damage and air quality.

January 1984	*480 pages*		*234 × 165mm*
0 408 11152 6	*Hardcover*	*Illustrated*	*£50.00*

For further details of this and other Butterworths titles please write to Katie Bleach

Butterworths
Borough Green, Sevenoaks, Kent TN15 8PH

Circle No.16 on Reader Service Card.

COMPUTED TOMOGRAPHY OF THE BODY

NOW IN PAPERBACK

A Radiological and Clinical Approach

Janet E. Husband MRCP, FRCR Ian Kelsey Fry DM, FRCP, FRCR

'As a general text on computed tomography this book may be thoroughly recommended.' British Medical Journal

'... A much needed book that will be of use to all those involved in CT scanning and should be read by all radiologists in training – their examiners will certainly have read it.'
British Journal of Radiology

'I would highly recommend this book to anyone interested in learning about computed body tomography.'
American Journal of Roentgenology

Contents

The Contributors; Preface; Basic Principles with R. Parker; General Considerations – Technical; General Considerations–Clinical; The Thorax – Mediastinum; The Thorax – Lungs, Pleura and Chest Wall; The Abdomen – Kidney; The Abdomen – Adrenals; The Abdomen – Liver and Biliary Tract; The Abdomen – Pancreas; The Abdomen – Miscellaneous Topics; Lymph Node Disease of the Abdomen and Pelvis; The Pelvis; The Musculo-Skeletal System; The Spinal Cord by P. Pullicino; The Head and Neck with C.A. Parsons; The Orbits by G.A.S. Lloyd; Computed Tomography in Oncology; Index.

October 1983	232pp (Illustrated)	SCIENTIFIC & MEDICAL / MACMILLAN PRESS LTD	£15.00	ISBN 0 333 25585 2

Please send orders and requests for information to: Carolyn Leuw
Scientific and Medical Division, The Macmillan Press Ltd, Houndmills, Basingstoke, Hants RG21 2XS, England

Circle No.63 on Reader Service Card.

MICROLINK - THE MOST FLEXIBLE COMPUTER INTERFACE

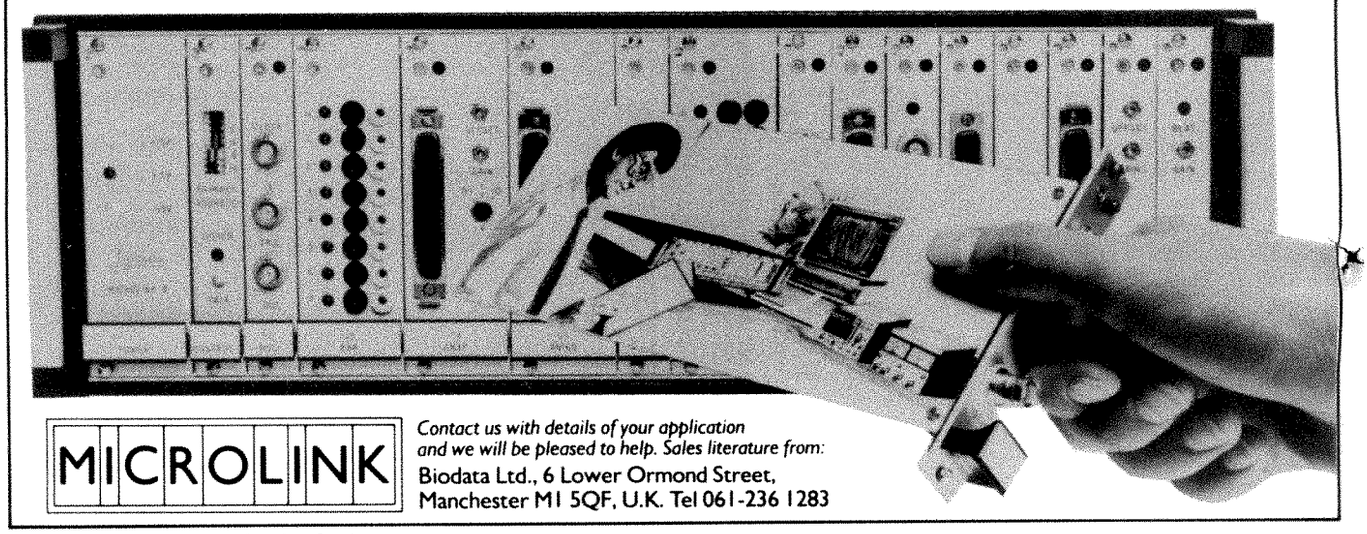
Full modularity MICROLINK is a fully modular laboratory interface designed and constructed to the highest professional standards. MICROLINK uses the IEEE-488 bus, the only real choice for instrumentation systems.

Superlative specification An unsurpassed range of 30 modules including analogue and digital inputs/outputs, timers, counters, RS-232 and thermocouple inputs, allows you to connect almost any instrument to your computer. Data transfer rates of upto 50 Kbytes per sec. are achievable and upto 256 analogue inputs can be accepted.

Full software support Programming manuals are available for most computers with IEEE-488 facilities. Sample programs are provided in BASIC so that you can get your measurement/ control system running with minimum delay.

Universal application MICROLINK has been used in environments as varied as North Sea oil production platforms and hospital neurological units, in applications such as: data logging, experimental monitoring and control, environmental monitoring, automatic test and high speed data acquisition.

USE MICROLINK WITH hp apple IBM digital SIRIUS 1 commodore

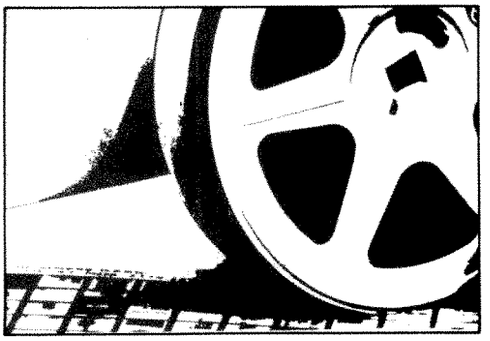


MICROLINK

Contact us with details of your application and we will be pleased to help. Sales literature from:
Biodata Ltd., 6 Lower Ormond Street, Manchester M1 5QF, U.K. Tel 061-236 1283

Circle No.12 on Reader Service Card.

nature
is available in Microform.



University Microfilms International

Please send additional information
for _____ (name of publication)
Name_____
Institution_____
Street_____
City_____
State_____ Zip_____
300 North Zeeb Road, Dept. P.R., Ann Arbor, Mi. 48106

Circle No.70 on Reader Service Card.

COLLABORATIVE RESEARCH INC

Products for Biotechnology

Recombinant D.N.A. Reagents.

Nucleosides, Nucleotides,

Templates, Primers & Probes.

Oligonucleotide Celluloses.

Cell attachment factors.

Growth factors.

Agents in the U.K.

STRATECH SCIENTIFIC

37 Mildmay Grove
London N1

Tel. 01-354 2601 **Tx 268048**

Circle No.11 on Reader Service Card.

Appointments

Professor R.L. Wain has been appointed the first Royal Society Kan Tong-Po visiting professor to Hong Kong. The visiting professor scheme has been set up to allow scientists from the Commonwealth and the United States to spend four to ten months in the universities of Hong Kong, assisting with research and lecturing whilst pursuing their own research interests.

Awards

Applications are invited from young Australians and permanent residents in Australia for the Fogarty International Centre's research fellowships. The awards are designed to allow research in health-related fields in any biological or medical laboratory in the United States. Up to six awards may be made from Australia, and each fellowship lasts for twelve months, although an extension of a year may be considered. Candidates must have achieved a doctoral degree in the health sciences, preferably within the last six years and not more than 10 years ago. A stipend of $18,000 to $22,000 is provided according to relevant postdoctoral experience. Further information and application forms are available from the Australian Academy of Science, PO Box 783, Canberra, ACT 2601, Australia.

The British Medical Research Council is looking for candidates to nominate for the NIH international research fellowships of 1985–86. A competition for nomination for these awards will be held by the MRC in June 1984. The research fellowships are awarded for 12 months at a stipend level that ranges from $16,000 to $20,000 per year depending on experience. Application forms are available from The Training Awards Group, Medical Research Council, 20 Park Crescent, London W1N 4AL, UK.

The British Institution of Production Engineers has made the following awards: the International Award to **Dr Alan Puckett** of Hughes Aircraft Company; the Mensforth Gold Medal to **Mr Peter Jefferson** of British Aerospace; the Nuffield Award to **Sir Robert Telford** of Marconi Electronics and the Institution Silver Medal to **Mr John Adler**, former chairman of the Institution's northern region.

Foulkes Foundation fellowships have been awarded to **Miss Susan Griffith** of University College, London; **Dr Mary Ninkovic** of the University College Hospital Medical School, London; **Dr Deenan Pillay** of the University of Newcastle-upon-Tyne Medical School; **Dr Richard Sheaves** of Oxford University; **Dr Kevin Tyler** of Birmingham University and **Mr David Manuel** of Cambridge University.

Meetings

1–3 March, **Symposium on the Clinical Management of the Elderly Patient,** Florida (The Health and Education Council, Franklin Square Hospital, Essex Community College, Baltimore County Health Department, 7201 Rossville Boulevard, Baltimore, Maryland 21237, USA).

6–9 March, **Oceanology International Exhibition and Conference,** Brighton, UK (Oceanology International Conference and Exhibition, Spearhead Exhibitions Ltd, Rowe House, 55–59 Fife Road, Kingston upon Thames, Surrey KT1 1TA, UK).

18–20 March, **Course on Biotechnology in Oil Production,** University of London (Professor V. Moses, School of Biological Sciences, Queen Mary College, Mile End Road, London E1 4NS, UK).

19–21 March, **Workshop on Freezing and Quality Control,** Rockville, USA (Mr David Grounds, Workshop Coordinator, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA).

21 March, **Conference on the Synthetic Applications of Ynes, Enes and Ones,** London (Dr W.B. Turner, Process Development Department, ICI Pharmaceuticals Division, Hurdsfield Industrial Estate, Macclesfield, Cheshire SK10 2NA, UK).

26 March, **International Meeting on Sensory Interactions,** London (Dr H.J.H. MacFie, MRI, Bristol BS18 7DY, UK).

26–28 March, **Workshop on the Use of Computers in Radiology and Oncology,** Geneva (Professor R.J. Berry, Organizing Secretary, Department of Oncology, The Middlesex Hospital Medical School, London W1N 7PN, UK).

26–30 March, **Residential School on Metallo-Organic Chemical Vapour Deposition: Theory and Practice,** Queen Mary College, London (Ms L.A. Hart, Royal Society of Chemistry, 30 Russell Square, London WC1B 5DT, UK).

26–30 March, **International Symposium on the Application of Computers and Mathematics in the Mineral Industries — APCOM,** London (Conference Officer, The Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR, UK).

26 March–15 June, **Course in Aflatoxin Analysis and Mycotoxins,** London (Training and Visitors Unit, Tropical Development and Research Institute, 127 Clerkenwell Road, London EC1R 5DB, UK).

27–30 March, **International Conference on Microbiological Quality Assurance in Industry,** Brighton, UK (Beverly Humphrey, Scientific Symposia Ltd, 33–35 Bowling Green Lane, London EC1R ODA, UK).

2–4 April, **Consensus Development Conference on Osteoporosis,** Bethesda, Maryland (Mr Peter Murphy, Prospect Associates, Suite 401, 2115 East Jefferson Street, Rockville, Maryland 20852, USA).

3–5 April, **Symposium on Solids/Liquids Separation Practice and the Influence of New Techniques,** Leeds, UK (Dr Paul Preece, Department of Chemical Engineering, University of Leeds, Leeds LS2 9JT, UK).

3–6 April, **International Conference on Energy Options,** London (The Institution of Electrical Engineers, Savoy Place, London WC2R OBL, UK).

9–11 April, **International Conference on Simulation for Nuclear Reactor Technology,** Cambridge, UK (The Conference Secretary, The Institution of Nuclear Engineers, 1 Penerley Road, London SE6 2LQ, UK).

9–13 April, **Residential School on Inorganic Polymers,** London (Ms L.A. Hart, Royal Society of Chemistry, 30 Russell Square, London WC1B 5DT, UK).

10–12 April, **International Conference on Flow Measurement in the Water Industry,** Glasgow, UK (Conference Section, National Engineering Laboratory, Glasgow G75 OQU, UK).

16–19 April, **Annual Chemical Congress of the Royal Society of Chemistry,** University of Exeter, UK (Dr John Gibson, The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

17–19 April, **Annual Meeting of the European Society for Clinical Investigation,** Milan, Italy (Organizing Secretariat, Fondazione Giovanni Lorenzini, Via Montenapoleone, 23-20121 Milan, Italy).

29 April–3 May, **Annual Meeting of the American Oil Chemists' Society,** Dallas, Texas (Meetings Coordinator, American Oil Chemists' Society, 508S Sixth Street, Champaign, Illinois 61820, USA).

15–16 May, **International Symposium on Cost and Benefit of Radioimmunoassay,** Milan, Italy (Organizing Secretariat, Fondazione Giovanni Lorenzini, Via Montenapoleone, 23-20121 Milan, Italy).

The **Conference on Hazardous Waste Management** due to take place on 8 December 1983 will now be held on 3 April 1984 at the Sudbury Conference Centre, Newgate Street, London EC1, UK.

The **International Conference on Advances in Surface Coating Technology** which was scheduled to take place in June 1984 has been postponed until 13–15 June 1985.

Students and industrial sponsorship

Richard Pearson*

In certain specialized areas the role of industry in supporting British undergraduates is significant. It can have unexpected consequences.

STUDENT finance has been high on the debating agenda for a number of years. The value of the means-tested grant for British undergraduates is currently £1,660 per annum plus fees although few students receive the full amount because of the means test, with many parents not making their full contribution. Although the real value of the grant was fairly stable throughout the 1970s, it has fallen by about 10 per cent in the past couple of years (Fig. 1). Even so, the British undergraduate is considered better off than his contemporary in Japan, who has to take out a loan, while students in most of Europe and North America have to rely on a combination of loans (often at low rates of interest) and grants, supplemented in some cases by scholarships. In the United Kingdom, the current pressure to reduce public expenditure means that loan

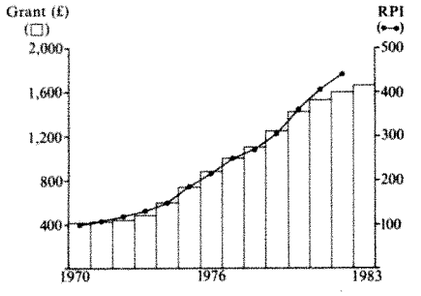


Fig.1 The "real" value of the student grant in Britain.

schemes could well be reconsidered in the next few years, although opponents argue that loans lead to only limited cost savings, while seriously affecting certain groups, such as women and those from poorer homes.

One source of finance rarely considered in this debate is industrial sponsorship. The full extent, in terms of money spent and numbers involved, is unknown. Most sponsorship is, however, concentrated in the area of engineering, with a smaller involvement in some of the pure sciences and in business and related studies. A recent report[1] from the Institute of Manpower Studies has shown that more than 2,200 (one in four) final-year engineering students were sponsored in 1983, which suggests the total number of sponsored students in higher education is probably over 10,000. Total expenditure by industry is likely to be several million pounds each year.

Sponsorship can take many forms. A student may be a salaried member of the staff of the sponsoring organization, he or she may receive a bursary worth up to £915 a year on top of the mandatory grant, or may have some mix of mandatory grant, bursary and salary when working in vacations or on periods of industrial work if on a sandwich course.

Employers sponsor students for a variety of reasons. For some it is a form of patronage, helping existing employees, often bright craftsmen and technicians, to advance their careers. For most, however, it is a means of guaranteeing a supply of well-trained recruits in the future, particularly in the "shortage" subjects of electronic and mechanical engineering. Sponsorship is also seen as providing an opportunity to make a detailed assessment of an individual's capabilities, as a means of influencing students' choice of subject and institution of study, as a way of attracting high quality school leavers, and to improve collaboration between industry and higher education. There is of course a financial cost involved, up to £4,000 per student over the duration of the course, and there is often the need to provide training and work experience.

In the past, some employers have sought legally to bind the student to the company for a period of years after graduation but this now happens only in the armed forces where the undegraduate becomes an employee. In recent years, 50–80 per cent of sponsored graduates have joined their sponsoring organizations, with each side rejecting the other in roughly similar proportions; some employers are, however, able to retain all those they want. Most students are sponsored from the start of their course, with the majority recruited directly from school, often only from local areas. More than half the employers limit their sponsorship students to particular universities, and in a few cases to particular polytechnics.

Among the 2,200 final-year engineering graduates sponsored in 1983, most were to be found in mechanical (38 per cent) and electrical/electronic (35 per cent) engineering, and relatively few in civil or chemical engineering. While two-thirds were in universities, the proportions of sponsored students, when compared on a subject basis, were similar in the universities and the polytechnics. Overall, 35 per cent of mechanical and 28 per cent of electrical/electronic engineers were sponsored (Fig. 2). It is interesting that it was the largest university/polytechnic departments (with over 50 final year students) that had the highest level of sponsorship and the middle-sized departments (25–49 final year students) that had the lowest.

In the past five years, more than 250 employers have been involved in sponsorship, some with only an occasional student, others with a regular intake of 50 to 100 a year. The number of companies sponsoring students has fallen as training budgets and recruitment levels have been cut back because of the recession. Nevertheless, the number of places on offer has grown slightly, with the number of places for electronic engineers increasing dramatically in response to expected shortages in future years.

Looking to the future, most employers expect to continue with their current sponsorship policies, while one in five expect to increase and one in seven to decrease their level of sponsorship. In the case of electronics, sponsorship has been rising rapidly. As a result, perhaps half of the graduates expecting to enter the labour

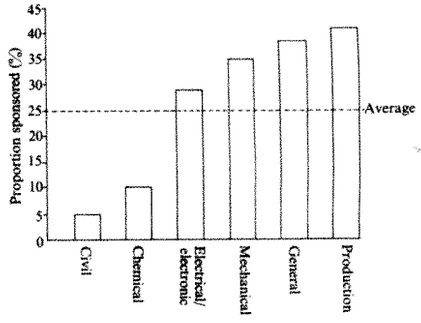


Fig.2 Proportion of final-year engineering students at UK universities and polytechnics sponsored by an employer during 1983.

market in the next few years will already be notionally committed to a particular employer. While not all will join that employer, it seems that the "free" market of electronics engineering graduates seeking employment will be even smaller than the already inadequate supply than has been forecast. This will compound the future recruitment difficulties of all types of employers, although the sustained high level of sponsorship may have an influence on subject and career choice by would-be students.

*Institute of Manpower Studies, University of Sussex, Falmer, Brighton BN1 9RF, UK.

1. Gordon, A., Hutt, R. & Pearson, R. *Undergraduate Sponsorship: Implications for the Labour Market*, Interim Report (IMS, 1983).

The items featured in this week's selection include such diverse instruments as a colloid osmometer, an infrared filter and a powder sampler

● A new line of rotary mechanical homogenizers is available from Heat Systems-Ultrasonics. The Astramixer units mix, emulsify, disperse and homogenize by high intensity or mechanical and hydraulic shearing action. Two laboratory-sized units are included in the line. The first, the micromixer, has three different rotor/stator sets; 7 mm, 12 mm and 21 mm. This allows samples of 250 μl to 1 litre in size to be processed. The standard laboratory unit is available with 35 mm and 45 mm rotor/stator sets for processing 100 ml to 20 litre batches.
Circle No. 100 on Reader Service Card.

● A new range of solid-state mini-ion selective electrodes is available from World Precision Instruments. Each electrode is 10 cm long and has a tip diameter of 3 mm. Calcium, sodium, potassium and chloride sensors can now be supplied.
Circle No. 101 on Reader Service Card.

● Wescor has released a low-cost version of its colloid osmometer. The instrument handles samples of 30 μl and can deal with whole blood flow-through sampling. It has a short response time, new wet-packed premounted membranes and a longer membrane shelf-life.
Circle No. 102 on Reader Service Card.

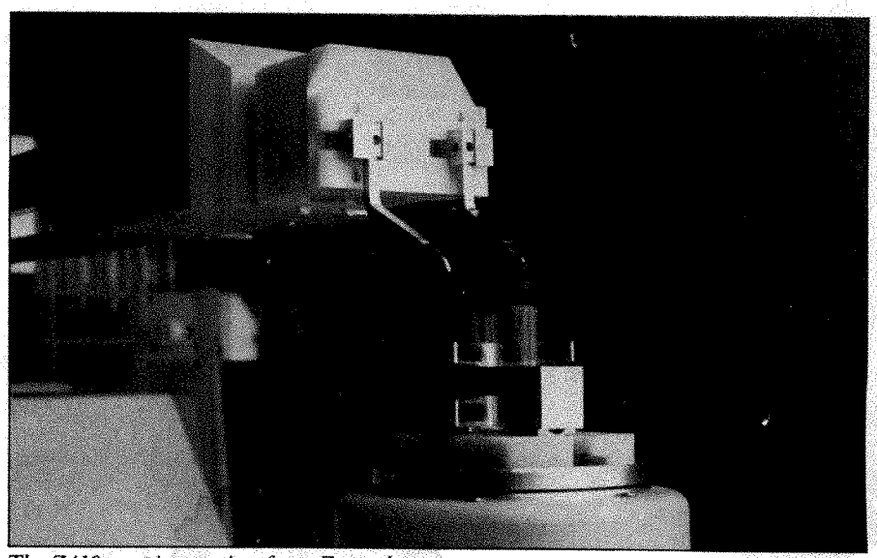
● Infrared filters designed by Infrared Engineering for inclusion in optical communications equipment are now being held as stock items. Filters ranging in size from 3 to 25 mm in diameter are available for use at wavelengths of 850, 904, 1,060 and 1,300 nm. Infrared filters of other sizes for use at other wavelengths can also be supplied to individual specifications. The filters are optimized for use with light emitting diodes (LED) which typically operate within the 800–1,400 nm waveband or with lasers used as coherent light sources operating at 1,060 nm.
Circle No. 103 on Reader Service Card.

● The new computerized model of Columbus Instruments' animal oxygen consumption monitor can simultaneously measure eight animals located in eight separate cages. The stability of the oxygen sensors is around 0.0054% oxygen which allows for 1% accuracy in measuring oxygen consumption. Results of measurements are printed at 10-min intervals in microlitres of oxygen per kilogram of body weight per hour. The system can operate for 24 h without interruptions. The oxygen consumption monitor can be used for any size of animals by changing the size of the animals' cages. A facility to measure CO_2 production can be added to the system.
Circle No. 105 on Reader Service Card.

● A new automated system for measuring red blood cell aggregation has been introduced by Myrenne Instruments. The Myrenne model MA-1 is based on the Schmid-Schönbein principle and uses a rotating cone and plate arrangement with an integrated infrared photometer. A 20-μl blood sample is subjected to a shear rate of 600 s^{-1} between a rotating transparent disposable cone and a transparent stationary plate, resulting in a dispersion of red cell aggregates. After 10 s, the cone is stopped and the optical effects of recurring aggregation are registered as an increase in optical transmission. The measured parameter, the 'mean extent of aggregation' (MEA), is the integrated area below the transmission curve during the first 10 s after the cone stopped. The MEA is computed and displayed on an LED readout. An analog signal output is provided for use with a recorder.
Circle No. 106 on Reader Service Card.

● Regency Electronics is now marketing a 30-channel programmable scanner that can monitor 15,760 frequencies (in six VHF and UHF bands). The Regency Z30 comes preprogrammed for 30 frequencies in its six covered bands: low VHF (30–50 MHz), VHF 2m amateur (144–148 MHz), high VHF (148–174 MGz), UHF 0.75m amateur (440–450 MHz), standard UHF (450–470 MHz) and extended UHF (470–512 MHz). Its telescoping antenna is electronically optimized for each band and an antenna jack can be provided for an external antenna. When searching, the Z30 covers 400 VHF frequencies (2 MHz) in about 34 s and 400 UHF frequencies (5 MHz) in about 30 s. When searching its 30 programmed channels it covers about 15 channels per second. A channel lockout feature can exclude any selected channels from being scanned. A selected priority channel is sampled every 2 s. If active, it automatically overrides any other signal.
Circle No. 107 on Reader Service Card.

● The Z410 capping station from Zymark automates the capping and uncapping of the screw cap containers used in the laboratory. It is a Zymate laboratory automation system. Sample containers can be automatically uncapped and the solution removed or reagents added. Containers can then be recapped. Vials holding samples which have been prepared for gas chromatography or HPLC can be automatically filled and capped using the Zymate system and capping station. The capping station can be added to any Zymate system and contains all the necessary software.
Circle No. 108 on Reader Service Card.



The Z410 capping station from Zymark

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

PHORBOL ESTERS
ESTERS OF 12-DEOXYPHORBOL— CALL OR WRITE FOR INFORMATION
LC SERVICES CORPORATION
165 New Boston Street
Woburn, MA 01801
(617) 938-1700
Circle No.25 on Reader Service Card.

LONDON OFFICE Keith Walesby 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024)
AMERICAN OFFICES NEW YORK Miss Gene Fein 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900
SAN FRANCISCO JJHS (415) 392 6794 ●**PASADENA** JJHS (213) 796 9200
CHICAGO Didier & Broderick (312) 498 4520 ● **BOSTON** CEL Assoc (617) 848 9306
CANADIAN OFFICE TORONTO Peter Drake 17 Pine Crescent, Toronto, Ontario M4E 1L1 (416) 690 2423

RATES UK — Rest of world — £11.00 per centimetre. Minimum 3 cm. £2 is charged for the re-direction of replies to advertisements with a box number.

CONDITIONS. The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.

OXFORDSHIRE HEALTH AUTHORITY
Churchill Hospital
MEDICAL GENETICS DEPARTMENT

A CYTOGENETICIST

is required from 1st April 1984 for two years to assist in a clinical research project on karyotyping of chorionic villi. Applicants should possess a first or upper Second Class honours degree, and have experience in human cytogenetics. Salary on Whitley Scale, commensurate with qualifications and experience scale starting at £6,520.

Enquiries to Dr Jon Jonasson, Department of Medical Genetics, Churchill Hospital, Headington, Oxford. Tel Oxford 0865/62834 or 0865/64841 ext 7059.

Job description and application form from Personnel Officer, Churchill Hospital, Headington, Oxford. (1136)A

CHAIRMAN

DEPARTMENT OF ANATOMY

Applications are invited for the position of Professor and Chairman, Department of Anatomy, Schools of Medicine and Dentistry, University of Alabama in Birmingham. We are seeking candidates with research and teaching experience, leadership qualities, and administrative skills. This department has teaching responsibilities for graduate students in anatomy, medical, dental and other students.

Please submit applications and a curriculum vitae to: Antonietta D Hyche, Administrative Officer, University of Alabama School of Medicine, Dean's Office — MEB 306, Birmingham, Alabama 35294. An Equal Opportunity/Affirmative Action Employer. (NW263)A

UNIVERSITY OF TORONTO

PHARMACOLOGIST

Assistant or Associate Professor (Tenure Stream Position). Ph.D. with several years of post-doctoral experience in pharmacology. Demonstrated excellence in teaching and research in pharmacology. Other factors being equal, Cardiovascular Pharmacologist preferred. Salary commensurate with qualifications and experience. Effective date of appointment: July 1, 1984.

Apply to: Chairman, Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, Ontario M5S 1A8. Closing date for receipt of applications: April 30, 1984.

In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents. (NW252)A

MONASH UNIVERSITY
Melbourne, Australia
DEPARTMENT OF BIOCHEMISTRY

SENIOR TUTOR

Postdoctoral graduates or those about to complete degree for position involving limited teaching to medical or science students and participation in ongoing research on connective tissue metabolism. Expertise in enzymology, particularly would be relevant to our research into regulation of turnover of connective tissue macromolecules in normal and arthritic tissue. Enquiries to Professor D A Lowther. Salary $A20,164 — $A23,100 pa.

Applications including Ref No. 30213A, curriculum vitae and referees to the Registrar, Monash University, Clayton, Vic 3168, Australia, with a copy to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, by 29/2/84. (W725)A

Head of Pharmacology

Continental Europe

This is a senior appointment within the modern and well-equipped Research Centre of a European pharmaceutical company which has a growing international reputation.

Research is centred on the company's 250-strong laboratories pleasantly located in a French speaking part of Europe, and there are strong cooperative ventures with other laboratories throughout Europe – including the UK, as well as the USA, Canada and Japan.

The company now seeks an internationally-oriented Departmental Head for its Pharmacology Group, to lead a team of 30 by coordinating the activities of the specialist section heads and, ideally, to provide a major input in cardiovascular research. An important rôle of the Head of Pharmacology will be to enhance the links with

UK equivalent: £20,000+

Medicinal Chemistry and Biochemistry, as well as the more developmentally-oriented departments.

We therefore seek an able PhD scientist who has expertise at team leader level within the pharmaceutical industry, and a broad appreciation of the major branches of pharmacology. Existing European experience would be valuable, but a clear willingness to see your career develop within continental Europe is more important.

The starting salary will be negotiable according to pharmacological and managerial experience, but is likely to provide a standard of living equivalent to at least £20,000 in the UK.

Please write in confidence for an application form, or phone if you would like to discuss the appointment further before applying:

Talentmark
Leaders in Health Care Recruitment

James A Edwards, Talentmark Limited, King House, 5-11 Westbourne Grove, London W2 4UA. Tel: 01-229 2266

(1143)A

RESEARCH HEAD

Drug Delivery Systems Research

The Upjohn Company, a worldwide leader in pharmaceutical research, is seeking applicants for the position of Research Head of the newly formed Drug Delivery Systems Research unit within the Pharmaceutical Research and Development division.

The successful candidate will lead an expanding interdisciplinary team of professionals dedicated to the elucidation of innovative drug delivery systems. The Unit will interact with other scientists responsible for development of specific human and veterinary products. Responsibilities will include the development of strategies for the discovery of future drug delivery systems, and the management of associated research activities. The successful candidate will be responsible for developing an effective program in drug delivery basic research and will assume responsibility for the evaluation, implementation, and development of collaborative research programs with academic institutions and other appropriate sources.

In addition to a Ph.D. in the biological, chemical, or pharmaceutical sciences, applicants must possess an outstanding publication record and have a minimum of three years' research management experience. Good leadership and communication skills, and the ability to relate to people at various levels, are essential.

Upjohn offers a stimulating research environment, opportunity for career growth, competitive salary and benefits, and relocation assistance.

Kalamazoo is a medium-sized Southwestern Michigan community with immediate access to a mix of excellent cultural, educational, and recreational pursuits, including numerous lakes and a four-season climate.

For confidential consideration, please send your resume to: **Lance D. Williams - 15819-J, Employment, THE UPJOHN COMPANY, 7000 Portage Rd., Kalamazoo, MI 49001, USA.**

Upjohn

A Century of Caring

An Equal Opportunity Employer M/F (NW266)A

Senior Medicinal Chemist

RESEARCH CENTRE

COTE D'AZUR

We are a well known research laboratory and we are creating a position for a Senior Medicinal Chemist in our **BIOCHEMISTRY Department.** He will take part in the creation and initiation of basic and applied research projets. Working closely with pharmacologists and biologists, he will lead a team of technicians. This position will suit a man or woman with a Ph. D. (or équivalent) in Chemistry with 3 to 6 years of research experience in the pharmaceutical industry. Good knowledge of modern methods in organic synthesis, experience of analytical techniques and familiarity with the study of structure-activity relationships are desirable. With excellent working conditions, this laboratory is at the forefront of research in its field and has excellent equipment housed in modern laboratories. This post will attract ambitious researchers of international standing who are bilingual in English and French. Please send a hand-written letter and C.V. to Mme FOURNIAT, under reference 23673/NA at Sélé-CEGOS Méditerranée, 35, rue Pastorelli, 06000 NICE (FRANCE).

Membre de SYNTEC Publival

sélé CEGOS (W729)A

CENTRE INTERNATIONAL DE RECHERCHES MEDICALES DE FRANCEVILLE — GABON

recherche

CHEF DE DEPARTEMENT ENDOCRINOLOGIE, PHYSIOLOGIE DE LA REPRODUCTION

- Docteur en Médecine, expérience de recherches en physiologie des stéroids, physiologie de la reproduction, HPLC, dosages radioimmunologiques.
- Recherches sur l'études des facteurs hormonaux de la stérilité humaine et de la puberté chez les primates.
- Recherches sur la physiologie du cycle menstruel chez le Cynomolgus.

Conditions de travail remarquables. Contrat de 2 ans renouvelable. Excellentes conditions d'expatriation.

Candidatures à adresser au Directeur Général du C.I.R.M.F., 28 boulevard de Grenelle, 75015 PARIS avec curriculum vitae. (W720)A

HINDUSTAN CIBA-GEIGY LIMITED

requires a

PHARMACOLOGIST

for its Reseach Centre in Goregaon, Bombay, India

Applications are invited from candidates of Indian nationality, aged about 35 years, and holding an MD or PhD in pharmacology, who are capable of independently planning and conducting basic and applied pharmacological research into the central nervous system, with special emphasis on electrophysiological aspects, and the cardiovascular system. The successful candidate will be expected to furnished comprehensive preclinical data on the pharmacological activity profiles of potential new drugs.

At least five years' previous experience in research is essential, and preference will be given to applicants who have received their post-doctoral training in the U.S. or in Europe.

Applications, accompanied with curriculum vitae and a full list of publications, should be sent in strict confidence to: The Personnel Department, HINDUSTAN CIBA-GEIGY LIMITED, 14, J. Tata Road, Bombay 400 020, India.

(W716)A

HINDUSTAN CIBA–GEIGY LIMITED

Specialist in biotechnology at the Science Reference Library

Applications are invited from graduates and postgraduates with a background in microbiology, biochemistry or another discipline related to biotechnology to work on the European Biotechnology Information Project (EBIP). EBIP will be based at the British Library Science Reference Library in London and will be supported by funds from the European Commission. It will be run in collaboration with other European organisations.

The successful candidate will be primarily concerned with the provision of information services in biotechnology by the exploitation of the literature and other sources and the use of computer based techniques. A degree in biochemistry, microbiology or another appropriate subject is essential with an up-to-date knowledge of the field and a keen interest in genetic engineering and other new developments.

Desirable attributes are experience of laboratory work in industry or research, a qualification in librarianship or information science, a knowledge of information provision in biotechnology, experience of using microcomputers and automated methods of information searching, and good communication skills.

The capacity to speak French or German would be a particular asset.

The duration of the post will be one year with the possibility of renewal.

Salary £7,759 pa. plus £1,250 Inner London Allowance.

Application forms (returnable by 15 February 1984) are obtainable from Marie Loftus, The British Library Personnel Section, Sheraton House, 2 Sheraton Street, London W1V 4BH.

Tel: 01-636 1544 Ext. 514. (1147)A

The British Library BL

JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

Professor of Physics

Applications are invited for the position of Professor of Physics. The appointee will also be Head of the Department of Physics. The position is available following the retirement of the Foundation Professor of Physics, Professor J.F. Ward.

The University wishes to develop its interests in physical oceanography and/or metoerology. Accordingly it seeks to appoint a person with appropriate higher degree qualifications and a proven record of research and publication in either or both of these fields.

The salary for the position of Professor is currently $A48,997 per annum, and in addition a locality allowance will be payable at the rate of $A496 per annum for a staff member with dependants (as defined) or $A248 per annum for a staff member without dependents.

Further information may be obtained from the Registrar, James Cook University, Townsville, Qld 4811, Australia, with whom applications close on March 1, 1984. In reply please quote Reference No. 83106. (W705)A

UNIVERSITY OF CAMBRIDGE

SUB-DEPARTMENT OF VETERINARY ANATOMY

POST-DOCTORAL

position available immediately to study effects of exercise on dietary metabolism and egg production in birds. The project is expected to be funded by the Agricultural and Food Research Council, for 3 years. Salary to be within the Cambridge Research Assistant scale £7,190 to £9,425 depending on age.

Send CV and names of 2 referees to: Dr J Brackenbury, Sub-Department of Veterinary Anatomy, Tennis Court Road, Cambridge CB2 1QS. (1106)A

UNIVERSITY OF ALABAMA BIRMINGHAM

DEPARTMENT OF PHARMACOLOGY

Stipends are available to support

FULL-TIME GRADUATE STUDENTS

Students accepted into the PhD program will receive a stipend of $6000 per year.

The faculty of the Department of Pharmacology are involved in a wide range of research efforts at one of the finest medical centers in the US. Students with training or interest in biology, biochemistry, chemistry, mathematics, physics, psychology or any of the physical sciences are invited to apply.

UAB is an Affirmative Action/Equal Opportunity Employer.

For further information contact: Dennis J Pillion PhD, Director of Graduate Studies, Department of Pharmacology, University of Alabama in Birmingham, Birmingham, Alabama 35294. (NW251)A

UNIVERSITY OF SYDNEY

DEPARTMENT OF MEDICINE

POSTDOCTORAL FELLOW

Appointee to carry out a study on t molecular mechanism of interacti of heparin and heparin-depende antibodies with human platelet Experience in the areas of prote chemistry, membrane biochemist and general immunological metho ology would be desirable. Appoin ment is funded by the NH&MR Grant and is available from Janua to December 1984, in the fir instance, with the possibility further renewal.

Salary range: $A20,164 — $22,5 per annum.

Applications, includi curriculum vitae and the names three referees, should be sent 24 February 1984, to the Registra University of Sydney, NSW 200 Australia, from whom furth information is available.

Equal Employment Opportunity University policy. (W729)A

UNIVERSITY OF CHICAGO

ASSISTANT OR ASSOCIAT PROFESSOR IN THE RESEARCH ASSOCIATE TRACK

to assume major responsibility in cellular electrophysiology laborato studying arrhythmogenesis and t actions of antiarrhythmic drug Experience in voltage clampin cable analysis, ion sensitive micr electrodes, and computer pr gramming preferable. Excelle scientific environment.

Renewable term appointment wi full fringe benefits. Immedia availability.

Please send curriculum vita representative reprints, and names three references to: Morton Arnsdorf, MD, Chief, Section Cardiology, University of Chicag Hospitals, Box 423, 950 E 59 Street, Chicago, Ill 60637. (NW265)A

SABBATICAL LEAVE IN SPANISH LABORATORIES

In order to promote the interaction between the Spanish Scientific Community and scientists working outside Spain, the CAICYT (Spanish National Council for Research & Development) is launching a Program encouraging scientists to spend their Sabbatical leave in Spanish Laboratories. The maximum length of stay is one year and the Program will be in operation from 1st September 1984 to 31st July 1986. The visiting scientists will receive a salary that will be awarded on the basis of qualifications and of the percentage of their current salary that is maintained by their home institution. Also some funds for expendable material in the receiving institution will be provided.

Scientists willing to take part in this Program are requested to contact the Spanish Laboratory in which they intend to stay. The CAICYT will also help, on request, to establish a connection between Scientists eventually interested in the Program and those Spanish laboratories working in their area of interest. These contacts should result in a joint proposal that must be submitted to CAICYT before 1st June 1984. Full details of the data that will be required are available either from the Spanish institutions or from CAICYT. The selected candidates will be notified not later than 1st July 1984.

All information concerning this advertisement should be requested from Dr Marisa Pacios, CAICYT, Edificio Torre Rioja, calle Rosario Pino, 14-16, Madrid 20, Spain. Tel. (34)-1-4501063.

(W704)A

THE UNIVERSITY OF SHEFFIELD

WEST RIDING CHAIR OF MICROBIOLOGY

Applications are invited for the above Chair, which fell vacant on the appointment of Professor J R Quale, FRS to the Vice-Chancellorship of the University of Bath.

The Chair will be tenable from October 1984. Salary in the range for professorial appointments (average £20,300: minimum £17,275).

Particulars from the Registrar and Secretary (Staffing), The University, Sheffield S10 2TN to whom applications (one copy), quoting the names of three referees, should be sent by 5 March 1984.

Quote ref: R32/G. (1134)A

FRESHWATER BIOLOGICAL ASSOCIATION

VACANCY FOR PHYSICAL LIMNOLOGIST AT WINDERMERE

Applications are invited for a three year post in physical limnology, to work on exchanges of energy and matter across water-atmosphere and water-sediment interfaces and transport of dissolved and particulate matter in lake ecosystems. Potential candidates should possess at least a good honours degree in physics and/or mathematics and should ideally have some experience of environmental data collection and processing.

Salary and conditions of service are analogous to those of Scientific Officer (£5,682 — £7,765) or Higher Scientific Officer (£7,149 — £9,561) in the Civil Service, gross pay depending on qualifications and experience.

Full details and application forms from the Secretary, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP (tel: 09662 2468). Closing date 14th February 1984. (1084)A

UNIVERSITY OF WARWICK

POSTDOCTORAL RESEARCH ASSOCIATES

Two positions are available in the Department of Biological Sciences on SERC funded projects:

(i) to work on an in vivo and in vitro study of microbial oxygenase-based bioconversion systems. This research is funded by a three year grant from the Biotechnology Directorate with a view to understanding the fundamental mechanisms which will enable those microbial systems to be used in the production of value added oxygenated chemicals. A working knowledge of enzyme regulation, microbial physiology/biochemistry or oxygenase enzymes would be an advantage. The successful candidate would be expected to collaborate with a co-worker already appointed on this project.

The post is available for three years (commencing as early as possible) on a starting salary up to £8,530 pa on the Research IA scale: £7,190 — £11,615 pa.

(ii) to work on the mechanism of action of the methane monooxygenase enzyme complex from bacteria. The work will cover the purification of the proteins by techniques already developed in the group, and analysis of the iron sulphur flavoproteins on the functioning complex by visible and e.s.r., spectroscopy and stopped flow techniques.

The post is available for 2½ years (commencing as early as possible) on a starting salary up to £8,080 pa on the Research IA scale: £7,190 — £11,615 pa.

Applicants should send a cv with the names and addresses of two referees to the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL by mid February. Informal enquiries and further particulars from Professor H Dalton, 0203/24011, ext 6025. (1103)A

Biotechnology Australia

Career Opportunities in Biotechnology

Biotechnology Australia Pty Ltd, Australia's leading biotechnology research company, is in the process of expanding its research and development activities. There are immediate opportunities for the research and development positions listed below.

The company provides a first class research environment for over 70 staff in modern laboratories on Sydney's North Shore. The laboratories are equipped with a full range of instruments and fermenters necessary for projects requiring the skills of molecular biologists, microbiologists and biological chemists. Topics under investigation include studies of new methods for vaccine production with recombinant DNA techniques, biological control of insect pests with genetically modified bacteria and parasitic nematodes, and the microbiological leaching of minerals. Most projects involve collaboration with leading groups in universities, CSIRO and research institutions.

The research group is supported by experts in planning and evaluation and there are extensive facilities for scale-up and process development.

Applications are now invited for the following seven positions.

Molecular Biologists

Position No. 1001 is for a candidate with Post-Doctoral experience in molecular biology or allied fields of biochemistry or microbiology, for appointment as a Research Scientist.

Position No. 1002 is for a candidate with an Honours Degree in similar subjects or in immunology, for appointment as an Experimental Officer.

Position No. 1003 is for a Post-Doctoral Fellow funded for 3 years in the first instance. Training in recombinant DNA and allied immunological techniques is required.

Position No. 1004 is for a Post-Doctoral Fellow funded for 2 years in the first instance for the development of vaccines against enteric infections using recombinant DNA technology.

Fermentation Scientist

Position No. 1005 is for a candidate with an Honours Degree in microbiology or fermentation with knowledge of chemical or biochemical engineering. Appointment will be at the Experimental Officer level to work on solid and liquid fermentations and product recovery.

Entomologist

Position No. 1006 is for a candidate with an Honours or higher degree in entomology for appointment as an Experimental Officer. The position would be for 2 years in the first instance.

Biotechnologist

Position No. 1007 is for a candidate with an Honours Degree in microbiology, biochemical engineering or allied discipline for appointment as an Experimental Officer. The position would be for 2 years in the first instance.

All positions carry salaries and conditions of employment competitive with other leading research organizations.

Applications including a c.v. and names of two referees should be sent to:

Professor N. S. Willetts
Director of Research
Biotechnology Australia Pty. Ltd.
P.O. Box 20
Roseville N.S.W. 2069 Australia

(W712)A

BIOCHEMICAL/CHEMICAL/TECHNICAL ASSISTANT

A post is available for an assistant to work on a programme concerning the biochemistry of the nervous system. Training and/or experience in biochemical and chemical techniques is essential. Salary according to BAT.

The post is limited to the end of 1984 with possibility of extension.

Application to: Max-Planck-Institut für biophysikalische Chemie, Abteilung Neurochemie, Sekretariat, Postfach 2841, D-3400 Göttingen, FR Germany. (W714)A

COMMONWEALTH AGRICULTURAL BUREAUX

Applications are invited for the post of

PLANT PATHOLOGIST

in the

COMMONWEALTH INSTITUTE OF BIOLOGICAL CONTROL

The Commonwealth Institute of Biological Control is one of the four Institutes and ten Bureaux which together comprise the Commonwealth Agricultual Bureaux, and provide a comprehensive world-wide information service in agriculture and related fields.

The C.I.B.C. undertakes research and carries out projects on a contract basis from its bases in India, Trinidad, Kenya, Pakistan, Switzerland and the U.K. It also provides an information and advisory service in all aspects of Biological Control and on the development of integrated pest management programmes.

With the increasing interest in biological control worldwide, the C.I.B.C. is expanding and diversifying its programme. A Plant Pathologist is required to join the team in the U.K. Initially he will undertake screening of rusts in the U.K. as potential control agents for weeds in other countries. At the same time, he will be expected to examine the prospects for using rusts and other plant pathogens more widely for weed control, prepare research proposals and undertake field surveys overseas in connection with C.I.B.C. projects on weeds. It would be an advantage if he had an interest in fungal parasites of insects and was able to assist and advise entomologists on their collection, isolation and screening as insect control agents.

QUALIFICATIONS Candidates should have a PhD or equivalent research qualification in plant pathology with a proven interest in, or involvement in, the use of plant pathogens as biological control agents. Overseas research experience and an interest in fungal pathogens of insects would be advantageous.

SALARY & CONDITIONS OF SERVICE The successful candidate would be based at the Commonwealth Mycological Institute initially but some field and laboratory work would be carried out at C.I.B.C., Silwood Park. At a later date, the appointee might be expected to work full-time based at Silwood Park and would be expected to travel widely and to spend periods overseas, if required.

The salary would be in the ranges of £7,149 – £9,561 or £8,970 – £11,476 depending on qualifications and experience, with London Weighting £700 while based at C.M.I.

The appointment would be made on a 3 year contract in the first place with the expectation of permanent superannuated service if performance was satisfactory.

STARTING DATE The successful candidate would be expected to take up the appointment on, or as soon as possible after, 2nd April 1984.

Further information is available from: The Assistant Director, Commonwealth Institute of Biological Control, Imperial College, Silwood Park, Ascot, Berks SL5 7PY, U.K. Tel. No. (0990) 23911 Telex: 847964 Cables Comag, Slough to whom applications, including a CV and names and addresses of two referees, should be submitted by 29th February, 1984.

(1113)A

Applications are invited for faculty appointments in

PHYSICS

to be made in the Physics Department, Memorial University of Newfoundland, effective September 1, 1984. Both tenure-track and two-year contractual positions are being considered. Research opportunities are available in a number of areas, but primary responsibility for at least two years will be for instruction at the first-year level with related activities (e.g., student interviewing and advising, school visitations, etc.). Initial appointments will normally be at the assistant professor level and subject to final budgetary approval. Applications, including curriculum vitae and the names of three referees should be forwarded to:

Dr D.H. Rendell, Head
Department of Physics
Memorial University of Newfoundland
St. John's, Nfld. A1B 3X7

In accordance with Canadian Immigration policy, this advertisement is directed, in the first instance, to Canadian citizens and permanent residents of Canada. (NW248)A

DEAKIN UNIVERSITY
Geelong, Australia

SENIOR LECTURER OCCUPATIONAL HYGIENE

Applications are invited for appointment to the above post. The appointee will be responsible for the operation and future development of the occupational hygiene program.

Applicants must be occupational hygienists and graduates, preferably in one of the physical sciences or engineering, although applicants with backgrounds in medicine, toxicology etc will also be considered.

The appointee must hold a recognised professional qualification in occupational hygiene (eg: qualifications leading to professional membership of an appropriate occupational hygiene society) and have at least eight years relevant experience. A higher degree is desirable.

Salary: $A31390 to $A36585 pa.

The position will be for an initial term of four years, during which there will be a review for continuing tenure.

Applications (quoting reference number 84/01) should include a full curriculum vitae and the names and addresses of three referees and should be sent to the Personnel Branch, Deakin University, Victoria, Australia 3217, by 30 March 1984. Applicants resident in North America and Europe should send an additional copy of their applications to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further information is available. (W707)A

THE UNIVERSITY OF WESTERN ONTARIO

BACTERIOLOGIST

Applications are invited for a tenure-track position, effective July 1, 1984, as Assistant Professor or Associate Professor, depending upon qualifications, in the Department of Microbiology and Immunology. Applicants should have a PhD and Post-Doctoral training in microbial biochemistry or biochemical aspects of microbial structure. Applicants must have demonstrated continuous ability to conduct and publish original, independent research.

The deadline for applications is March 15, 1984.

In accordance with Canadian Immigration requirements this advertisement is directed to Canadian citizens and permanent residents.

The University of Western Ontario is an equal opportunty employer.

Address correspondence to: Dr N R Sinclair, Chairman, Department of Microbiology and Immunology, University of Western Ontario, London, Ontario N6A 5C1.

(NW253)A

THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF EARTH SCIENCES

Applications are invited fr suitably qualified men and wom for appointment to the followi positions:

POSTDOCTORAL FELLOW/RESEARCH FELLOW

A number of appointments will made during 1984. The fields which these appointments are ma will be guided by the Schoo evaluation of all applications, whi are invited in the following areas Earth Physics (seismilogy and tecto ophysics), Environmental Geoche istry, Geophysical Fluid Dynami Petrochemistry, Petrophysics (rc deformation), Trace Element G chemistry, Mineralogy, and C Genesis.

Closing date: 31 March 1984.

Conditions of appointment: Sal will be in accordance with qua cations and experience within ranges: Research Fellow $A23394 $30734 pa; Postdoctoral Fell Grade 1 (at fixed point) $A20164 $23100 pa. Current exchange rate $A1 = UK 62p.

Appointment, unless otherw stated, will be: Research Fell normally up to three years with possibility of extension to maxim of five years; Postdoctoral Fell normally two years, with possibility of extension to maxim of three years.

Grants are provided towards tra and removal. Assistance towa housing is given to an appointee fr outside Canberra. Eligil appointees will be required to join Superannuation Scheme Australian Universities. T University reserves the right no make an appointment or to make appointment by invitation at time.

Prospective applicants should w to the Registrar of the Univers GPO Box 4, Canberra, ACT 26 Australia, or to the Association Commonwealth Universit (Appts), 36 Gordon Square, Lon WC1H 0PF, for further particu before submitting applications.

(W721)A

UNIVERSITY OF OXFOR
Nuffield Laboratory of Ophthalmology

RESEARCH ASSISTAN
(Or technician, subject to ad approval)

required for one year (possibl mos.) for a study of chemical cha to lens proteins and the effects drug designed to prevent catar Salary up to £7,630 depending qualifications.

Further details from Dr J Hard Nuffield Laboratory Ophthalmology, Walton St, Ox OX2 6AW to whom applicat with cv and names of two refe should be sent asap. (1099)

THE WORLD HEALTH ORGANIZATION OF THE UNITED NATIONS

invites applications for the post of

MEDICAL OFFICER

Duties of the post include: Reviewing the situation in national health systems, so as to identify the most important problems and possible solutions for their reorientation towards health for all; Promoting the exchange of experience among Member States and ensuring appropriate technical support, if required; Initiating specific tasks, such as comparative studies and analysis of technical publications, in order to develop cumulative knowledge on health systems reorientation processes in various economic and socio-political conditions; Promoting liaison with and providing support to other programmes and activities, both within and outside WHO, related to organization of health systems based on primary health care; Contributing to scientific meetings, seminars and training courses in the field considered and acting as a resource person for Organization of Medical Care and as the Secretary to the relevant Expert Advisory Panel.

Interested candidates should have graduation in medicine with postgraduate training in disciplines related to the planning and management of health systems and at least ten years of practical experience in these fields at the national level and several years' experience in health systems and at least ten years of practical experience in these fields at the national level and several years' experience in health systems research and training. Experience in international work and problems of developing countries would be an advantage. Excellent knowledge of English or French with a good working knowledge of the other is required.

Salary commensurate with present earnings but not less than US$ 54,070 per annum plus an attractive international benefits package.

Applications are invited from candidates of either sex. Qualified candidates should send their detailed curriculum vitae quoting SHS/84 to:

Personnel (MPR)
World Health Organization
CH — 1211 Geneva 27

Closing date for receipt of applications: end March 1984.

Only those candidates under serious consideration will be contacted. (W709)A

TECHNICAL CHANGE CENTRE
PRINCIPAL RESEARCH FELLOWS

The Centre is seeking to appoint full-time Principal Research Fellows to work on research projects concerned with (a) the careers of graduate scientists and engineers, and (b) the contribution which British academic research makes to the advancement of UK engineering. Applicants should be graduates in science or engineering, or have other relevant qualifications, and have experience of interdisciplinary research studies.

The appointments will be for two years in the first instance. Salaries will be not less than £15,000 pa, depending on qualifications and experience.

Applications in writing, together with a full CV, should be sent to the Secretary, The Technical Change Centre, 114 Cromwell Road, London SW7 4ES from whom further particulars may be obtained. (1098)A

NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified persons for the post of

INORGANIC/ANALYTICAL CHEMIST

Qualifications:
Post Doctoral experience in Chemical Engineering or Mineral Processing.

Salary:
Will be according to qualifications and experience on the following scales:
Senior Scientific Officer at K9444 × 372 — K10,932
Principal Scientific Officer at K10,176 × 600 — K12,576
Senior Principal Scientific Officer at K11,196 × 720 — K13,356
Chief Scientific Officer at K12,000 × 900 — K15,600

Applications giving full personal details, qualifications and experience, and names and addresses of three referees should be submitted to:
The Secretary General, National Council for Scientific Research, PO Box CH 158, CHELSTON, LUSAKA, ZAMBIA

(W717)A

Directorate of Fisheries Research, Lowestoft
Ministry of Agriculture, Fisheries and Food

Physical Oceanographer/ Dynamicist

...to work in the field of physical oceanography related to the disposal of low level radioactive wastes to the deep ocean.

You will be responsible for planning, field implementation and analysis of results of the MAFF contribution to the international SOFAR (neutrally buoyant) float experiments in the eastern North Atlantic ocean during 1984-86. Work also involves participation in projects examining the mixing processes occurring at the European Continental Slope, and in research cruises (up to 2 x 1 months duration per annum).

You should have a good honours degree or equivalent in physical sciences or mathematics and at least 2 years' relevant post-graduate experience with proven research ability in dynamical oceanography. A knowledge of ocean mixing processes would be advantageous.

Appointment as Higher Scientific Officer £7145-£9560 or Senior Scientific Officer (minimum 4 years' relevant post-graduate experience) £8970-£11475. Level of appointment and starting salary according to qualifications and experience.

RELOCATION ASSISTANCE MAY BE AVAILABLE.

For further details and an application form (to be returned by 24 February 1984) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours).

Please quote ref: SB/80/AD. (1148)A

Scientific Civil Service

THE AUSTRALIAN NATIONAL UNIVERSITY
JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

POSTDOCTORAL FELLOW/RESEARCH FELLOW/SENIOR RESEARCH FELLOW
(several positions)

Applicants for these research positions should have a PhD degree (or equivalent qualification) and expertise in any of the areas of research listed below. A number of appointments will be available from late 1984 or early 1985.

Department of Biochemistry: Head: Professor F. Gibson) Energy-transducing ATPase; membrane assembly; bacterial transport; bioenergetics; mechanism of action of classical and multifunctional enzymes; molecular biology; gene expression; differentiation; oncogenesis.

Department of Experimental Pathology: (Head: Professor P. C. Doherty) Biology and biochemistry of tumour metastasis, malignancy and host response; viral and auto-immune pathological process in the central nervous system; vascular and lymphatic pathology; inflammation; pathogenesis of malaria.

Department of Human Biology: (Head: Dr R. L. Kirk) Molecular biology of inherited disease, cytogenetics and somatic cell genetics; human population genetics, including DNA polymorphisms.

Department of Immunology: (Head: Professor B. Morris) Ontogeny of the immune response; immunology of the foetus and pregnancy; experimental surgery of the embryo and foetus; embryo transplantation; genetics of disease resistance; reproductive and developmental biology; tissue transplantation; physiology of the lymphatic system.

Department of Medicine and Clinical Science: (Head: Professor W. F. Doe) Mucosal immunity and inflammation; neutral proteases; interleukins and differentiation of bone marro wcells, macrophages and natural killer cells; pathogenesis of inflammatory bowel disease and colon cancer; neurophysiology of intestinal motility; calcium and cell regulation; makignant hyperpyrexia; asthma; platelets, atheroma and haemostasis; transplantation biology.

Department of Medicine and Clinical Science: (Head: Professor W. F. Doe) Mucosal immunity and inflammation; neutral proteases; interleukins and differentiation of bone marrow cells, macrophages and natural killer cells; pathogenesis of inflammatory bowel disease and colon cancer; neurophysiology of intestinal motility; calcium and cell regulation; malignant hyperpyrexia; asthma; platelets, atheroma and haemostasis; transplantation biology.

Department of Microbiology: (Head: Professor G. L. Ada) Histocompatability antigens; viruses; carcinogenesis; immunity to and epidemiology of viral infections.

Department of Pharmacology: (Head: Professor D. R. Curtis) Mammalian central transmitters; pain and analgesia; mammalian peripheral sympathetic synapses; biophysics of smooth muscle; nerve growth factors.

Department of Physical Biochemistry: (Head: Professor L. W. Nichol) Biological macromolecules; interacting protein systems; multienzyme complexes; enzymes of sulphur metabolism; protein conformation and hydration; secretory and blood proteins.

Department of Physiology: (Head: Professor (Elect) P. W. Gage from April 1984; Acting Head to 31 March 1984: Dr. R. I. Close)
Synaptic trasmission at peripheral and central synapses; calcium ionic and gating currents; charge movements in nerve and muscle; contractile activation in muscle; visual information processing.

Experimental Neurology Unit: (Director: Professor R. Porter) Neurophysiology; spinal cord; synaptic transmission at central synapses; respiratory neurophysiology; somatosensory pathways; control of movement; physiology of the cerebral cortex in primates.

CONDITIONS OF EMPLOYMENT: Salary will be in accordance with qualifications and experience within the ranges: Senior Research Fellow: $A32,679 — $A38,980 p.a., Research Fellow: $A23,394 — $A30,734 p.a.; Postdoctoral Fellow Grade 1 (a fixed point on scale) $A20,164 — $A23,100 p.a. Current exchange rate is $A1 = UK62p.

Appointment as Senior Research Fellow/Research Fellow will be for up to three years initially with the possibility of extension after review to maximum of five years; as Postdoctoral Fellow for one or two years initially with possibility of extension to maximum of three years.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Further information and application details are available on request from Heads of Departments, from the Registrar of the University, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Prospective applicants are encouraged to write for further information before lodging applications which close with the Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia, on *16 March 1984*. (W723)A

SALES representative qualified individual or organization for sale of state of the art chemicals for the DNA synthesis in international market. Please write in strict confidence to Chemgenes Corp, 296 Newton St, Waltham, Mass 02154. (NW259)A

Please mention
nature
when replying to these advertisements

INSTITUTE OF CANCER RESEARCH AND ROYAL MARSDEN HOSPITAL — Surrey

JOINT DEPARTMENT OF PHYSICS

MEDICAL RADIATION PHYSICIST

at Senior Lecturer level: A vacancy has arisen following the appointment of Dr R P Parker to the Chair of Medical Physics at Leeds. The person appointed will hold a senior position in this large, integrated service and research department and will be expected to undertake research, service and teaching responsibilities in the physics of radio-therapy and radiodiagnosis. The organisation is well equipped for both clinical and scientific work and there is special provision for research funding on a peer-review basis. Members of the department work in close association both with clinicians in the Hospital and with scientific colleagues in other sections of the Institute. Salary in scale £13,515 — £16,925 pa plus London Allowance of £1,186 pa.

Applications in duplicate should give full details of qualifications and experience with the names of two persons familiar with the candidate's work. These should be sent to: The Personnel Officer, The Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting Ref: 300/G/40. Closing date for applications 24th February, 1984.

Further details can be obtained by telephoning Mrs Louise Parkes, Physics Department, Royal Marsden Hospital, Sutton. Tel. 01-642 6011, ext 347. (1109)A

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CHEMISTRY

RESEARCH ASSOCIATE

Applications are invited from candidates possessing or anticipating a 1st Class or 2(i) Honours degree in Chemistry for a Research Associateship funded by the Cancer Research Campaign. Appointee will undertake research work in collaboration with Dr P L Coe on The Synthesis of Tamoxifen Analogues.

Successful candidate may be permitted to register for a higher degree on a part-time basis.

Post tenable for up to three years from 1 April 1984.

Salary on the Research Associate 1B scale £6,310-£8,530 plus superannuation.

Starting salary will depend on age, experience and qualifications.

For further particulars phone 021-472 1301 ext 2559, quoting reference F1.

No formal application form. Three copies of application, including full Curriculum Vitae and naming three referees to Assistant Registrar, (Science and Engineering), PO Box 363, Birmingham B15 2TT by 17 February 1984. (1141)A

MEDICAL RESEARCH COUNCIL

DEVELOPMENTAL NEUROBIOLOGY UNIT

Applications are invited for a

SHORT-TERM NON-CLINICAL SCIENTIFIC POST

to join a team in this Unit studying factors affecting neural development especially in culture. Background in protein separation techniques, membrane biochemistry and immunology, and experience in tissue culture are desirable. Candidates, with training in neurochemistry or cell biology, of immediately postdoctoral or equivalent status will be preferred. The appointment will be for 3-5 years. Renumeration will be at an appropriate point on the scales for University non-clinical academic staff.

Applications together with a full cv and the names of two professional referees should be sent to Robert Balazs, MRC DNU, 33 John's Mews, London WC1N 2NS (Tel: 01-242 1496) by 27 February 1984.

There is an immediate vacancy for the post of a Technician to assist in a research programme in cellular and developmental neurobiology. Applicants should have laboratory experience in biochemistry and some knowledge of tissue culture techniques and cell biology, a degree would be an advantage.

Starting salary, depending on experience, will be in the range of £5,491 — £7,747 + £932 London Weighting. Please apply with cv and the names of two professional referees to Robert Balazs at the address above. (1105)A

MRC RADIOBIOLOGY UNIT, HARWELL

TUMOUR BIOLOGIST/IMMUNOLOGIST

Applications are invited for a Short Term Non-Clinical Scientific post in this Medical Research Council Unit tenable for 3 years, to work with a group of scientists studying the effects of radiation and drugs on tumour systems. Candidates of immediately post-doctoral or equivalent status will be preferred. It is expected that the successful applicant will have some research experience in at least one of the fields of vascular physiology, experimental tumour therapy or monoclonal antibody production.

Salary and conditions of service according to MRC non-clinical scientific scales. Hostel accommodation or housing may be available.

Please write or telephone if you would like further information about this post. Applications, together with curriculum vitae and the names of two referees, should be sent to the Personnel Officer, MRC Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD, Tel 0235 834393. Closing date February 27th 1984.

(1132)A

SOUTH MANCHESTER HEALTH AUTHORITY
CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE
PATERSON LABORATORIES
LABORATORY TECHNICIANS

Required to join a new Molecular Biology team studying the role of Epstein-Barr virus in human cancer.

1. Research Technician (PL/84/4) Applicants should have a sound practical knowledge of relevant techniques including eg tissue culture, plasmid preparation, gel-electrophoresis, nick-translation and sequencing. Candidates qualified to degree or HNC standard will be preferred.

2. Junior Research Technician PL/84/5) The team also require a junior to assist, initially with routine matters such as maintenance or cells, preparation of stock solutions etc. The post would suit a school leaver with A levels in science. Day release to further qualifications will be available.

3. Part-Time Technician (PL/84/6) There are also vacancies for two part-time technicians, preferably with experience in molecular biology as indicated above. These posts will suit applicants wishing to use their experience on a part-time basis.

Salaries according to age and experience: Research Technician £5491 — £7747 pa. Junior Research Technician £3456 — £4818 pa.

Applications, enclosing a SAE, should be sent to the Laboratory Manager, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Wilmslow Road, Withington, Manchester M20 9BX. Closing date 12 February 1984.

(1127)A

Wir über uns:
BOEHRINGER MANNHEIM
gehört zu den ersten Adressen der forschenden pharmazeutischen Industrie.
Jeder 5. Mitarbeiter ist bei uns in der Forschung und Entwicklung tätig.

Unser Forschungszentrum Biochemie befindet sich in reizvoller Lage in Tutzing am Starnberger See, südlich von München.

Unsere rasch wachsende Forschungsgruppe Mikrobiologie besteht aus qualifizierten Spezialisten verschiedener Disziplinen. Für die Leitung einer weiteren Arbeitsgruppe suchen wir einen promovierten

Molekular- oder Zell-Biologen

Zielsetzung der Gruppe ist die Entwicklung von therapeutisch wirksamen Proteinen.

Von unserem neuen Mitarbeiter erwarten wir eine molekularbiologische Ausbildung mit breiter experimenteller Erfahrung in DNA-Neukombinationstechniken: Klonierung und Expression von Genen in Säugetierzellen. Am besten paßt zu uns ein Mitarbeiter mit Teamgeist und Eigeninitiative.

Wenn Sie diese anspruchsvolle Aufgabe reizt, senden Sie uns bitte zur Gesprächsvorbereitung Ihre Bewerbungsunterlagen (Zeugnisse, Lebenslauf und Lichtbild).

mannheim boehringer
diagnostica therapeutica

Boehringer Mannheim GmbH
Biochemica Werk Tutzing
– Personalabteilung –
Bahnhofstr. 9–15, 8132 Tutzing
West Germany

(W714)A

UNIVERSITY OF MANITOBA
DIVISION OF HUMAN GENETICS
MOLECULAR GENETICIST

The Division of Human Genetics of the Faculty of Medicine invites applications for a full time Assistant Professor to commence by July 1, 1984. Preference will be given to individuals with demonstrated research experience in molecular genetics using recombinant DNA technology on mammalian systems, particularly in the area of genome organization, control of gene expression or gene mapping. A PhD, postdoctoral training and potential for an independent career as an experimental scientist are prerequisites.

An interest in developing expertise and a desire to be an effective educator are desirable qualities. The position is open to both women and men. Both women and men are encourged to apply. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

For full consideration send a concise description of projected research goals and interests, names of at least 3 individuals for letters of reference, and a curriculum vitae to: Dr. P. J. McAlpine, Division of Human Genetics, Faculty of Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3. Deadline for receipt of applications: March 1, 1983. (NW254)A

ACADEMIC APPOINTMENT IN IMMUNOLOGY

The position is within the Immunology Research Center. The successful candidate will be expected to conduct an independent research program in fundamental or applied Immunology. Opportunities exist as well to participate in research programs already underway in the Center or at the Institut, to supervise and train graduate students, to become involved in production activities and/or to assume administrative duties.

QUALIFICATIONS: A candidate must have obtained a PhD or the equivalent from an accredited university in Canada or elsewere and must have a training in Immunology. Preference will be given to candidates with post-doctoral or other experience indicating that the candidate can pursue his or her research autonomously. Priority will be given to Canadian citizens and permanent residents of Canada. The candidate is expected to possess or to be willing to acquire a working knowledge of French.

LEVEL OF APPOINTMENT: Rank will be determined on the basis of the qualifications and experience and the salary will be according to the scale presently in effect at Institute Armand-Frappier (University of Quebec).

TO APPLY: Send your *curriculum vitae* including a list of publications, 3 letters of recommendation and any other pertinent information before February 29, 1984, to: Service des ressources humaines, Institut Armand-Frappier, 531 boulevard des Prairies, Case postale 100, Laval-des-Rapides, Laval, Québec H7N 4Z3.

(NW208)A

POSITIONS OPEN

The Hellenic Institute Pasteur is seeking to fill the following positions:
- An experienced Virologist PhD degree or MD having an experience in Virology, expected to assume responsibility of a research program.
- An experienced Parasitologist MD or VD having an experience in the entomological area, expected to assume responsibility of a research program on the Leishmaniasis.
- One Immunologist PhD degree or MD having an experience in the area of immunological techniques, expected to actively participate to a research program.

The candidates should be of Greek nationality.

Please write or call for additional information to the:
Secretary
Hellenic Institute Pasteur
127 Vassilissis Sofias
ATHENS 11521
GREECE
Telephone: 646 59 05
Telex: 221188 IPH GR
Deadline for submission of application: May 31, 1984.
(W719)A

BIOTECHNOLOGISTS!

Are you reading Bio/Technology, the International Journal for Industrial Biology?

For a free sample copy of this new monthly Journal from the Publishers of *Nature*, please write or phone:

Mirta Soto
Bio/Technology
15 East 26th Street
New York 10010
USA
Tel: (212) 689 5900

Felicity Parker
Bio/Technology
Houndmills Industrial Estate
Brunel Road, Basingstoke
Hampshire, UK
Tel.: (0256) 29242

MICHIGAN STATE UNIVERSITY

RESEARCH ASSOCIATE POSITIONS (2):

Veterinarian, DVM (possible PhD or MS given right experience) in biochemistry, immunology, microbiology or genetics. Immediate openings for two individuals in a University based biotechnology program dealing with domestic animal lymphokines, including interferon. Full time research positions — funded three year program.

Candidates must be experienced in cell culture, gene cloning and macromolecule purification. Candidates must be clinically innovative and have experience in dealing with domestic animals in a research setting. Expertise in basic biomedical research is essential. Salary dependent upon experience.

Applications should consist of curriculum vitae, statement of research and clinical experiences and interests and list of three references. Michigan State University is an affirmative action/equal opportunity institution.

Applications and inquiries should be directed to: Dr Robert W Bull, Department of Medicine, Michigan State University, East Lansing, Michigan 48824-1317. Telephone: (517) 355-4616. (NW255)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(UNIVERSITY OF LONDON)
Keppel Street,
London WC1E 7HT

DEPARTMENT OF MEDICAL MICROBIOLOGY

POSTDOCTORAL RESEARCH FELLOW

is required for appointment up to three years to join a group working on the development and properties of viral peptide vaccines. The research will be concerned initially with the enhancement and analysis of cellular immune responses to viral polypeptides synthesized following examination of the nucleotide sequence of the hepatitis B genome. This work will be extended at a later date to other viral systems, including hepatitis A, herpesviruses and the flaviviruses. Preference will be given to candidates with experience in cellular immunology. Salary within the range £7190 — £11,615 plus £1186 London Weighting per annum. Applications consisting of full curriculum vitae and naming two referees should be submitted to the Senior Assistant Secretary at the above address. (1120)A

INSECT NEUROBIOLOGIST

Full-time, tenure-track appointment at the rank of assistant professor. We will seek candidates having research interests in areas such as: integrative functions of the insect CNS, neuroendocrine mechanisms, neurogenetics, neurodevelopment, neuron physiology and pharmacology. Appointee will teach annual undergraduate course in general insect physiology and conduct graduate seminars. Applicants should have a Ph.D. and should demonstrate outstanding potential for research and teaching.

Applications should include the following: (1) Curriculum vitae, academic transcripts and evidence of teaching skills and experience; (2) bibliography and selected reprints or manuscripts; (3) statement of research objectives and approaches proposed; (4) names and addresses of at least three people who can provide recommendations. Submit all materials by 1 April, 1984, to E S Sylvester, Chair, Department of Entomological Sciences, 201 Wellman Hall, University of California, Berkeley Calif. 94720. The University of California is an Equal Opportunity, Affirmative Action Employer. (NW264)A

LABORATORY Scientific Officer required to assist in a 3 year research project funded by the Multiple Sclerosis Society to investigate mechanisms of immune mediated demyelination in CNS. Candidates must have HNC or equivalent qualification, with experience in light and electron microscopy. Experience in immunocytochemical techniques an advantage. Initial salary in range: £5,491 — £6,144 plus £1,042 London Weighting. Apply in writing to the Secretary, United Medical and Dental Schools, Guy's Hospital Medical School, London Bridge, London SE1 9RT, quoting Ref. AD2. (1130)A

UNIVERSITY OF SOUTHAMPTON

PROFESSORIAL MEDICAL UNIT

RESEARCH ASSISTANT

A suitably experienced graduate is required to assist in the characterisation of autoantibodies to insulin in the above department, which maintains strong connections with the adjacent Tenovus Immunology Laboratories. Broad experience in immunological methods is important, and previous involvement in monoclonal technology would be an advantage.

Salary, according to experience, up to £8,530 pa.

Applications, including cv and the names of two referees, should be sent to Dr T J Wilkin, Professorial Medical Unit, Level D, South Laboratory and Pathology Block, Southampton General Hospital, Tremona Road, Southampton SO9 4XY, telephone (0703) 777222 ext 4291, by 20 February, 1984. (1111)A

MRC RADIOBIOLOGY UNIT, HARWELL

IMMUNOLOGIST

Applications are invited for a Sh Term Non-Clinical Scientific pos this Medical Research Council U tenable for 3 years, to work wit group of scientists studying effects of radiation, and t application of monoclor antibodies in tumour systems Candidates of immediately po doctoral or equivalent status will preferred. It is expected that t successful applicant will have so research experience in the field monoclonal antibody production.

Salary and conditions of serv according to MRC non-clini scientific scales. Hostel accomn dation or housing may be availabl

Please write or telephone if y would like further information ab this post. Applications, together w curriculum vitae and the names two referees, should be sent to Personnel Officer, MRC Rad biology Unit, Harwell, Didcot, O OX11 0RD, Tel 0235 834393. Clos date February 27th 1984.
(1133)A

VETERINARY Anatomist. Assist professorship, July 1, 1984 at Io State University for PhD or DV MS. Participation in teachi preferably in gross anatomy, development of independent resea program or active collaboration current research are expected. Sal commensurate with qualificati Send résumé and three letters recommendation to: Dr Jean Carithers, Department of Veterin Anatomy, Iowa State Univers Ames, Iowa 50011, before March 1984. Iowa State University is Equal Opportunity/Affirmat Action Employer. (NW258)A

IMPERIAL COLLEGE O SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY

RESEARCH TECHNICIA (Grade 5)

A Research Technician (grade 5 required to work with Dr K O'H on the effects of gene expressio Drosophila melanogaster of chro some rearrangements induced hybrid dysgenesis. The approache be used will include Drosophila g tics and recombinant D techniques. Experience in these a is desirable but not essential.

The appointment is funded b MRC project grant to run October 1986, and the positio available immediately. Salary is the Technical Grade 5 scale (£6 — £6,689 plus £1250 pa Lon Allowance).

Suitably qualified candid should send a full curriculum v and the names of two referees to I O'Hare, Department Biochemistry, Imperial College Science and Technology, Lon SW7 2AZ. (1119)

The University of Agriculture provides agricultural education at an academic level in the Netherlands. The university has a staff of approximately 2800 and some 6000 students attending more than 70 vocational courses and conducting research. The registry comprises administrative offices, the library and a computer department.

WAGENINGEN
LANDBOUWHOGESCHOOL

Research assistant m/f

for a to be established section Plant Cell Biology for a five-year period

Duties

To stimulate plant cell biological research, the Faculty has decided to add a section Plant Cell Biology to the vocational group Molecular Biology Amongst the duties are developing plant cell biological research within the vocational group Molecular Biology. Such research shall form part of the WUB biotechnological group "Plant cell and Animal cell", in which six other University groups participate. It is expected that, after an initial period, you will conduct graduate studies and laboratory work and that graduate students can participate in research in obtaining their degree.

Requirements and benefits

You are a (molecular) (cell) biologist, experienced in plant cell biological research and an obvious interest in practical agricultural research application. You will collaborate closely with colleagues of various University vocational groups and with agricultural research institutions.
We offer a salary in accordance with Civil Service standards of Dfl. 6.561,- gross per month, depending on age, qualifications and experience.
Vacancy no. 83-066/2407.

Information regarding this position may be obtained from Prof. Dr. A. van Kammen, telephone 00 31 8370 82036/7.

The personnel policy of the University of Agriculture is aimed at achieving a reasonable balance between male and female staff members. In view of the fact that the number of women employed remains behind, women in particular are encouraged to apply. In the event of matched qualifications, preference shall be given to female applicants.

Your application should be addressed within fourteen days, to the head of the Personnel Department of the University of Agriculture, P.O. Box 9101, 6700 HB Wageningen, the Netherlands.
State the Vacancy number in the upper left hand corner of your letter and enveloppe.
A job description can also be obtained at this address. The University application code shall apply.

(W702)A

POSTDOCTORAL POSITION:

MOLECULAR BIOLOGY OF TUMOUR PROMOTION

Suitable for a PhD or MD with research experience who will join our program employing recombinant DNA technology to investigate effects of TPA on mammalian cells. Supported by the Terry Fox Training Grant Program established by the National Cancer Institute of Canada.

Send CV and references to Dr. David Denhardt, Cancer Research Laboratory, University of Western Ontario, London, Ontario, N6A 5B7 Canada. (NW257)A

SOUTH MANCHESTER HEALTH AUTHORITY
CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE
PATERSON LABORATORIES

RESEARCH TECHNICIAN

For experimental work on the biology of self-renewing systems in the Section of Epithelial Kinetics. Candidates should have a degree or HNC equivalent qualification in Biology: some experience or cell culture techniques preferred.

Salary according to age and experience £5491 — £7747.

Applications, enclosing a SAE to the Laboratory Manager, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Wilmslow Road, Withington, Manchester M20 9BX, quoting reference PL/84/3. Closing date 12 February 1984.
(1126)A

THE SCHOOL OF PHARMACY
UNIVERSITY OF LONDON
Neuropharmacology Research Group
DEPARTMENT OF PHARMACOLOGY

RESEARCH TECHNICIANS

TWO Grade 4 technical posts available for up to 5 years from date of appointment.

1. ASSISTANT ELECTRONICS TECHNICIAN

to assist in the design and construction of electronic apparatus under the direction of the electronics experimental officer. The preferred applicant should have an interest in microprocessor and/or computer applications. Ref: AET/1.

2. TISSUE CULTURE TECHNICIAN

to assist in studies on nerve cells in culture. The applicant should have an interest in tissue culture techniques and their application. Ref: TCT/2.

Practical experience for both these posts, supported by appropriate qualifications, essential. Salary £5,826 — £6,702 pa + LW £1,250 pa. 5 weeks + public holidays. Season ticket loan scheme.

Enquiries to Mr G B Withers (tel: 01-837 7651 ext 17). Application forms from: Personnel Officer, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX. (1137)A

HOUGHTON POULTRY RESEARCH STATION

GRANT-AIDED BY THE AGRICULTURAL & FOOD RESEARCH COUNCIL

IMMUNOLOGIST

The Institute is seeking an Immunologist in the Microbiology Department to provide immunological input to a multi-disciplinary project on the use of the techniques of genetic manipulation to produce novel vaccines for poultry. Specific duties will be to examine the local and systemic immune response of the chicken to viral and other antigens presented to mucosal surfaces but the appointee will also be expected to develop an interest in other immunological problems that are being investigated on the Station.

Applicants should have a first or upper second class honours degree in a biological subject and preferably a PhD, and will have had several years' post-graduate experience in immunology. At least 2 years relevant post graduate research experience is required for HSO grading; 4 years for SSO.

The appointment will be made in the Higher Scientific Officer or Senior Scientific Officer grade, according to experience. Salaries HSO: £7,149 x 6 to £9,561; SSO: £8,970 x 7 to £11,476.

For further details and application form please contact the Secretary, John Andrews, at Houghton Poultry Research Station, Houghton, Huntingdon, Cambs PE17 2DA. Tel St Ives (0480) 64101. (1088)A

MEDICAL RESEARCH COUNCIL
NEUROENDOCRINOLOGY UNIT
Newcastle upon Tyne

RESEARCH OFFICER (Electronics)

The appointee will be responsible for the design, construction and maintenance of electronic equipment to be used in biophysical investigations of the properties of neurones and neurosecretory cells. Design tasks will include: very low-level current detection circuitry; high-speed multichannel optical recording devices; computer-controlled stimulation equipment; instrumentation amplifiers; analog signal transformation circuitry. The appointee should have some experience of computer interfacing.

Minimum requirement is a university degree, HNC or equivalent qualification, with two years relevant experience after qualification. Salary scale (due for revision) Research Officer £6204 — £8126; Senior Research Officer £8204 — £10467.

For further information contact: Dr R N McBurney, MRC Neuroendocrinology Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE Telephone (0632) 735251.

(1124)A

The European Molecular Biology Laboratory, a research institute situated in Heidelberg, West Germany, invites applicants for the following vacancy

TECHNICAL ASSISTANT

The successful applicant will work in a group concerned with the problems of membrane protein traffic in animal cells. The techniques employed include cell fractionation, in vitro protein synthesis, biochemical as well as immunological isolation and characterization of membrane proteins. Some knowledge of nucleic acid methodology would be advantageous.

Appropriate training and experience in biochemical technology and analysis is essential. Preference will be given to candidates having experience in the application of immunological tools in biochemistry and cell biology (monoclonal antibody production, affinity chromatography, immuno-fluorescence microscopy etc.) A working knowledge of English is essential; a second language (French or German) is desirable.

An above average salary will be offered to the successful candidate. Certain allowances are payable in addition, depending on personal circumstances. An initial contract of 3 years' duration which can be renewed, will be offered.

Please write briefly for an application form, quoting ref. No. 84/3 to:
EMBL, Personnel Section,
Postfach 10.2209,
D-6900 HEIDELBERG
(W711)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHARMACY

TEMPORARY LECTURESHIP IN PHARMACEUTICAL AND MEDICINAL CHEMISTRY

Applications are invited for this appointment. The successful applicant will be required to contribute to undergraduate and post graduate teaching in Pharmaceutical and Medicinal Chemistry as well as to undertake research in these areas.

The appointment is for three years and preference may be given to applicants with relevant experience in medicinal chemistry and whose research interests complement those currently active in the department. These are summarised in the further particulars.

Salary in the range £7,190 to £14,125 per annum.

Further particulars and applications forms, returnable not later than 9th March 1984 may be obtained from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref No 917. (1128)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF GENETICS

Applications are invited for a

UNIVERSITY LECTURESHIP

vacant on 1 October 1984.

The scale of stipends is at present £9,875 a year, rising by annual increments to £15,085, or, if the person appointed is ordinarily resident in College, £9,776 rising to £14,986.

Well qualified applicants from any field of genetics will be considered but, all other things being equal, preference may be given to a plant geneticist.

Further particulars may be obtained from: The Secretary of the Appointments Committee of the Faculty Board of Biology 'A', Department of Zoology, Downing Street, Cambridge CB2 3EJ, to whom applications should be sent as soon as possible and in any case to reach him not later than 1 May 1984. (1115)A

REGIONAL CYTOGENETICS LABORATORY EAST BIRMINGHAM HOSPITAL

BASIC GRADE SCIENTIFIC OFFICER

Required in the above laboratory. Experience in cytogenetics or cell culture desirable.

Salary range, depending on experience and qualifications £5,681 — £8,349 per annum.

For application form and job description please contact Regional Cytogenetics Laboratory, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST. Tel: 021-772 4311 ext 4354. (1129)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

DEPARTMENT OF IMMUNOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

required to work with a team actively studying rheumatoid arthritis and autoimmune thyroiditis. Initially the project will involve production, characterization and functional activity of human monoclonal autoantibodies.

The candidate must have considerable experience of tissue culture techniques. The appointment is for 3 years in the first instance, and salary is according to age, qualifications and experience within the range £8,376-£12,801 pa inclusive of London Allowance.

Applications to include the names of two referees, should be sent to Professor I M Roitt, Department of Immunology, The Middlesex Hospital Medical School, Arthur Stanley House, 40-50 Tottenham Street, London W1P 9PG. (1101)A

THE UNIVERSITY OF BRITISH COLUMBIA

GRADUATE STUDIES IN CHEMISTRY

Applications are invited from candidates with high academic records who wish to pursue further studies towards the MSc and PhD Degrees in Chemistry. Excellent research facilities are available in all areas of modern Chemical Science, ranging from Biological Chemistry to Chemical Physics. Teaching and research assistantships are available for all students in the programme. Stipends up to $20,800 are available to highly qualified students who hold scholarships; the minimum stipend for 1983/84 is $11,000.

Application forms and further particulars may be obtained from: Dr L Weiler, Professor and Head, Department of Chemistry, The University of British Columbia, 2036 Main Mall, Vancouver, BC, Canada V6T 1Y6. (NW773)A

IMPERIAL CANCER RESEARCH FUND RESEARCH OFFICER/SENIOR RESEARCH OFFICER

required to run a cytogenetics service at the ICRF Medical Oncology Unit, St Bartholomew's Hospital London, and to work in close collaboration with the Human Cytogenetics Laboratory at the main laboratories in Lincoln's Inn Fields.

Degree or equivalent plus experience in human cytogenetics essential.

Salary range including London Weighting, £7454 to £11,717.

For further details and application form write or telephone Ms S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 on 01 242 0200 ext 305, quoting reference 45/84. (1125)A

SCIENTIFIC DIRECTOR

Large regional blood center searching for a Scientific Director for the direction of an expanding research and development program in collaboration with academic departments of the University of Connecticut Health Center. Significant internal research support and faculty appointment at University of Connecticut Health Center and/or Yale University School of Medicine available to qualified applicants.

Applicant should possess an MD or PhD degree and have at least five years of research experience at the post-doctorate level with demonstrated ability to perform independent research and direct the research of others. Salary is competitive and a complete benefit package is available.

Submit Curriculum Vitae and research plans to Dr Ritchard Cable, Director, American Red Cross Blood Services, Connecticut Region, 209 Farmington Avenue, Farmington, Ct 06032. (NW267)A

University College London

POSTDOCTORAL POSITION IN RECEPTOR RECONSTITUTION

Applicants are invited for work on reconstitution of neurotransmitter receptors into lipid bilayers. It is expected that the primary approach will involve single ion channel recording from such preparations. The laboratory has long term MRC support (to D Colquhoun and S G Cull-Candy) and is well-equipped for this work, which will involve collaboration with Professor E A Barnard of Imperial College London.

A salary in the range of £8,376 — £10,611 will be available from October 1984.

Applications, together with cv and names and addresses of the referees should be sent to Prof D Colquhoun Dept of Pharmacology, University College London, Gower Street London WC1E 6BT, UK. (1100)A

COMMITTEE FOR THE FACULTY OF BIOLOGY 'A'

DEPARTMENT OF ZOOLOGY

UNIVERSITY LECTURER

Neurobiologist required experienced in the analysis of neurons in the vertebrate brain or spinal cord using modern cellular techniques. Interest in local circuit neurons is desirable but not essential. ("New Blood" post with a normal age limit of 35.)

Appointment will be for three years with the possibility of re-appointment to the retiring age. The pensionable scale of stipends if not ordinarily resident in College is £9,875 a year rising by eleven annual increments to £15,085.

Further information from the Secretary of the Appointments Committee for the Faculty of Biology 'A', Department of Zoology, Downing Street, Cambridge CB2 3EJ to whom applications (5 copies) curriculum vitae and names of three referees should be sent by 21 February 1984. (1117)A

UNIVERSITY OF OXFORD

READERSHIP IN ATMOSPHERIC PHYSICS

In association with JESUS COLLEGE

Applications are invited for the above post, to be filled from October 1st 1984. The post carries the headship of the Department of Atmospheric Physics and the successful candidate may be offered a fellowship at Jesus College.

Details of the University and College appointments may be obtained from the Chairman of the Interdepartmental Committee for Physics, Professor K W Allen Department of Nuclear Physics Keble Road, Oxford, to whom completed applications (six typed copies one for overseas candidates) should be sent by February 28th. (1116)A

UNIVERSITY OF EAST ANGLIA
Norwich

POSTDOCTORAL RESEARCH ASSOCIATE

in Physical Organic Chemistry, required in the School of Chemical Sciences. The post is funded jointly by SERC and ICI (Pharmaceuticals Division), to investigate potential pro-drugs based on masked lactones. The appointment is for one year, and will commence April 1984, or as soon as possible thereafter. The starting salary will be up to £8,530 per annum. Applicants should have experience in techniques of physical organic chemistry, particularly kinetic procedures, and in organic synthesis, and be prepared to develop interests in the biochemical and medicinal chemical fields. The work will be carried out at Norwich.

Applications with curriculum vitae and names and addresses of two referees to be sent to:- Dr C.D. Johnson, School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ as soon as possible. (1108)A

ASTRONOMY FACULTY position. The Department of Physics & Astronomy invites applications for one or two tenure-track faculty positions starting September 1984 at a professorial rank commensurate with the qualifications of the candidate. The position is open to any astronomer with expertise in millimeter wavelength observations, who will be expected to conduct an active research program at the Five College Radio Astronomy Observatory. Candidates with exceptionally strong credentials in related observational programs, eg infrared, will also be given consideration and are urged to apply. A résumé and the names of three references should be sent to Dr David Van Blerkom, Astronomy Program Chairman, GRC Tower B, University of Massachusetts, Amherst, MA 01003. Deadline for submission of application is March 15, 1984. The University of Massachusetts is an Affirmative Action/Equal Opportunity Employer.
(NW262)A

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

Applications are invited for the post of
LECTURER in PHARMACOLOGY.

The duties of the post are to teach in the BSc Honours Pharmacology course and to engage in research in Pharmacology.

Initial salary within the range £7,190 — £9,425 per annum on a scale rising to £14,125 per annum.

Applications, together with the names of three referees, should be received not later than 20 February, 1984 by the Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/216/N (1118)A

THE INSTITUTE OF ZOOLOGY, THE ZOOLOGICAL SOCIETY OF LONDON

POSTDOCTORAL RESEARCH ASSISTANT

required to study surface antigens of gametes and early embryos in mammals. Experience of appropriate biochemical and immunological techniques is essential.

POSTDOCTORAL RESEARCH ASSSISTANT

required to investigate ultrastructural aspects of sperm maturation and embryo development. Experience of electron microscopy and immunocytochemistry is essential.

LABORATORY TECHNICIAN

required to work on the production of monoclonal antibodies raised to mammalian gametes. Experience of tissue culture is essential and of monoclonal development is desirable.

The appointments are within the MRC/ARC Comparative Physiology Research Group and are for two years initially, commencing from April 1984, with possible extension for a further two years. Salary will be appropriate to age, qualifications and experience in relation to the scales for research assistants or research technicians in the University of London.

Please apply in writing by providing c.v. and names and addresses of two referees to the Establishment Officer, Zoological Society of London, Regent's Park, London NW1 4RY, to arrive before 15 February 1984. (1104)A

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF GENETICS

POSTDOCTORAL POSITIONS

Applications are invited for a postdoctoral post funded by SERC from 1 April 1984 to study the genetics and evolution in the rDNA multigene family in Drosophila.

Applications, including curriculum vitae and names of two academic referees, to Dr G A Dover, Department of Genetics, Downing Street, Cambridge CB2 3EH.
(1114)A

NEUROSCIENCES AT BAYLOR COLLEGE OF MEDICINE

Baylor College of Medicine offers an interdepartmental Neuroscience Program for students interested in advanced study leading to the PhD in neuroscience. Applications must be completed by 1 April 1984, and applicants will be notified regarding admission and financial support by 15 May 1984.

Information regarding the program curriculum, faculty, research opportunities, and application procedures may be obtained from:

Scott F Basinger, PhD, Director, Predoctoral Program in Neuroscience, Cullen Eye Institute, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030.
(NW256)A

THE UNIVERSITY OF MANCHESTER

Research Assistants in Radio Astronomy

Applications are invited for appointments as Post-Doctoral Research Assistants in the Nuffield Radio Astronomy Laboratories, Jodrell Bank. Research Assistants are expected both to carry out their own research and assist with existing research activities. Current areas of research include quasars and active galaxies; radio spectral line studies of galaxies and stars; pulsars; and the isotropy of the microwave background radiation. The appointments are tenable from August 1st, 1984 initially for a period of one year with a good possibility of extension up to a maximum of 3 years. A number of appointments are available immediately.

Salary range p.a.: £7,190 — £11,615 (Superannuation) depending upon age and experience.

Enquiries should be addressed to: The Director, Nuffield Radio Astronomy Laboratories, Jodrell Bank, Macclesfield, Cheshire SK11 9DL. (1138)A

THE UNIVERSITY OF WAIKATO
New Zealand

OFFICE OF VICE-CHANCELLOR

(Re-advertisement)

The position of Vice-Chancellor of the University of Waikato will become vacant on 1 February 1985, following the retirement of Dr D R Llewellyn, who has held the office since 1964 as the first Vice-Chancellor of the institution.

The Vice-Chancellor is the academic and administrative head of the University responsible to the Council.

The Council invites enquiries from or suggestions concerning suitably qualified persons. Correspondence should be marked *Personal and Confidential* and should be addressed to the Chancellor at the following address: University of Waikato, Private Bag, Hamilton, New Zealand.

This position is being readvertised and applications will now close on *30 April 1984.* (W706)A

ETH

INSTITUTE OF ASTRONOMY
Zurich, Switzerland

RESEARCH ASTRONOMER

Applications are invited for a position on the regular scientific staff of the Institute of Astronomy, ETH Zurich (Swiss Federal Institute of Technology). Candidates should have earned a doctorate in astronomy or a closely related physical science. Their research record should include experience in plasma astrophysics or solar radio astronomy.

The successful applicant should perform independent research work within the framework of the current research programs of the Institute of Astronomy, preferably with an emphasis on research related to solar radio astronomy.

Initial appointment will be for a period of three years, but can be renewed. The salary will depend on the qualifications of the applicant, according to the regulations of the ETH.

Applicants should submit curriculum vitae, list of publications, the name of two referees, and date of availability, to:

Prof. J.O. Stenflo, Director
ETH-Zentrum
CH-8092 Zurich, Switzerland.

Closing date for the applications is April 1, 1984. (W703)A

THE UNIVERSITY OF LEEDS

ASTBURY DEPARTMENT OF BIOPHYSICS

POSTDOCTORAL RESEARCH FELLOW in the Deisgn of Enzyme Inhibitors using Computer Graphics in collaboration with G D SEARLE & CO

Applications are invited for the above post for research on computer model-building and molecular mechanics studies leading to the development of enzyme inhibitors for clinical use as drugs. The project will make use of an Evans & Sutherland PS300 colour display system. Applicants should have, or be about to obtain, a PhD degree in protein crystallography, molecular graphics or related structural studies. The appointment is funded by an SERC Co-operative Research Grant and is for a fixed period of up to two years.

Salary on the IA Range for Research and Analogous Staff (£7,190-£11,615), according to age, qualifications and experience.

Informal enquiries may be made to Dr A J Geddes or Professor A C T North of the University of Leeds, telephone (0532) 431751 ext 7088 or 6130.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference no 42/5/D. Completed applications should be returned to the Registrar as soon as possible.

(1145)A

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post which is funded by the Medical Research Council. The post is for a period of three years and is available immediately.

The work, with Dr D C S White and in collaboration with Dr D R Trentham FRS and Dr M R Webb both of NIMR, Mill Hill, will involve a study of the kinetics of the reactions following the hydrolysis of ATP in intact muscle fibres, using phosphate-water oxygen-exchange as the probe. Experience in kinetics and/or enzyme mechanisms is desirable.

Salary range: £7,190 — £8,530 pa.

Four copies of applications, including a full curriculum vitae and the names of two referees should be sent as soon as possible to the Registrar, University of York, Heslington, York YO1 5DD. Please quote reference number 11/6189.

(1144)A

THE UNIVERSITY OF ADELAIDE

invites applications from both men and women for the following position:

POSTDOCTORAL FELLOW IN APPLIED GEOPHYSICS (S8805) (FOR MATHEMATICIAN, PHYSICIST OR ENGINEER)

in the field of airbone magnetic surveys. The position will be in the Department of Economic Geology which will be amalgamated with the Department of Geology and Mineralogy in 1985, to form a single Department of Geology.

The appointee will participate with Professor D Boyd and a small research group in the development of a practical system of computer aided interpretation of digital aeromagnetic data which is being applied to problems in mineral exploration and geological studies.

Applicants should hold a PhD degree in one of the three fields of mathematics, physics or engineering, and should have used computing in postgraduate studies and be interested in solving complex real problems. Experience in geophysics is an advantage but is not essential.

The person appointed should take up the post before the end of June 1984. The appointment will be for one year in the first instance and, subject to the satisfactory performance of duties, will be extended for a further two years.

Further information on the duties of the position may be obtained from Professor D Boyd (telephone: (08) 228 5843) and Dr P Brooker (telephone (08) 228 5842) or telex: UNIVAD AA89141.

Further information about the general conditions of all appointments may be obtained from the Senior Assistant Registrar (Personnel) of the University.

Salary per annum: $A20164 × 5 — $23100.

Applications, in duplicate, quoting reference numbers and giving full personal particulars (including citizenship), details of academic qualifications and names and addresses of three referees, should reach the Senior Assistant Registrar (Personnel) at the University of Adelaide, GPO Box 498, Adelaide, South Australia 5001 (telex: UNIVAD AA89141), not later than 1 April 1984.

The University reserves the right not to make an appointment or to appoint by invitation. (W731)A

WANTED AND FOR SALE

Scientific and Technical Reviews.

Please inquire for runs or single volumes.

SANTO VANASIA,
58 Via M. Macchi,
20124 MILANO, Italy.

(W547)L

CONGRESS

THE III INTERNATIONAL CONGRESS OF INFLAMMATION will be held in Paris, FRANCE September 3-8, 1984

Scientific Committee: Jacques BENVENISTE (F), Robert M. FAUVE (F), Rodolfo PAOLETTI (I), Bengt SAMUELSSON (S), John R. VANE (GB), B. Boris VARGAFTIG (F), Gerald WEISSMANN (USA)

Abstracts are invited. For further information please contact

SOCFI
14 rue Mandar
75002 Paris, FRANCE

(W708)Q

FELLOWSHIPS

ONE YEAR FELLOWSHIP
(from 75,000 to 180,000 FF)
AVAILABLE IN PARIS
for a Biochemist to work on growth factors. Send application to Dr BARRITAULT for Jan.'85 before March 1st at: INSERM U. 118— 29 rue Wilhem 75016 PARIS — France.

(W727)E

THE UNIVERSITY OF AUCKLAND New Zealand

RESEARCH FELLOWSHIP IN GEOTHERMAL RESERVOIR ENGINEERING

Applicants should have a PhD in engineering or applied mathematics and some experience in reservoir modelling is desirable. The Fellowship will be for one year in the first instance with the probability of renewal for at least 2 further years.

Commencing salary up to NZ$22,487 per annum. Conditions of Appointment and Method of Application are available from the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1 0PF.

Applications in accordance with Method of Application should be forwarded to the University as soon as possible but not later than 31 March 1984. (W730)E

THE FLINDERS UNIVERSITY OF SOUTH AUSTRALIA

SCHOOL OF MEDICINE

RESEARCH FELLOWSHIP

Applications are invited fron suitably qualified persons for Research Fellowship tenable withi any of the fields represented in th School of Medicine of the University The Research Fellowship, which i available immediately, is tenabl initially for one year, bu applications may be entertained fro persons who wish to take up th position for a shorter perioc Applicants should have completed PhD degree prior to taking up th appointment, or have had equivaler research experience.

The appointment will be mad according to qualifications an experience within the salary rang $A20,164 — $A23,100. Persons wit a PhD will normally be appointed o the third step ($A21,338). A contribution will be made towarc the travel costs of the Fellow an his/her dependents in taking up th Fellowship.

Further details concerning the stal of the School and their fields c interest may be obtained from th Registrar.

Applications, including fu personal details, details of academi record and publications, outline c research interests and names an addresses of at least two referee should be lodged, in duplicate, wit the Registrar, The Flinder University of South Australia Bedford Park, South Australia 5042 by 29 February 1984. Applicant should ask their referees to forwar their reports directly to the Registra before the closing date. (W724)E

UCG Coláiste na hOllscoile Gaillimh

UNIVERSITY COLLEGE GALWAY

POSTDOCTORAL FELLOWSHIP

Applications are invited for a postdoctoral position that will soon be available in the laboratory of Dr. Frank Gannon. The successful candidate will work on the molecular cloning and manipulation of eucaryotic genes as part of a recently established active research group.

Previous experience with recombinant DNA techniques, while not essential, will be an advantage to the applicant.

Please send Curriculum Vitae to Dr. Frank Gannon, Department of Microbiology, University College, Galway, Ireland. (1135)E

UNIVERSITY OF GÖTEBORG, Göteborg, Sweden

A POST-DOCTORAL FELLOWSHIP in arterial cell and molecular biology arterial.

A post-doctoral fellowship is available for 12 months in the arterial biology group of the University of Göteborg, Göteborg, Sweden. The arterial biology group is involved in research on the cellular and molecular biology of atherosclerosis. Graduate or post-graduate training in either cell or molecular biology is required.

Post-graduates with the appropriate back-ground should write to Dr G. Bondjers, Arterial Biology Group, Medical Faculty, University of Göteborg, S-413 45 Göteborg, Sweden, enclosing a curriculum vitae, reprints and manuscripts, and the names of two referees. (W710)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENTS OF ICROBIOLOGY AND GENETICS

POSTDOCTORAL ESEARCH FELLOWSHIP

plications are invited for the ve post which is funded by an C grant to Dr C W Penn (Micro- logy) and Dr C M Thomas netics). The work will involve iing and expression of antigens es of the syphilis agent ponema pallidum in Escherichia . Candidates should have a back- und in microbiology and/or bio- nistry and should preferably have e experience of recombinant A techniques, although this is not ntial.

ost tenable for up to three years n 1.4.84 or as soon as possible eafter.

alary on the Research Fellow 1A e £7,190-£11,615 plus super- uation.

or further particulars phone -472 1301 ext 2559, quoting rence M1.

o formal application form. Three ies of application, including full riculum Vitae and naming three rees to Assistant Registrar, ence and Engineering), PO Box Birmingham B15 2TT by ebruary 1984. (1142)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF ZOOLOGY & COMPARATIVE PHYSIOLOGY

POSTDOCTORAL RESEARCH FELLOWSHIP/ RESEARCH ASSOCIATESHIP

Applications are invited for a SERC-funded Research Assistantship to study the physiological aspects of feeding behaviour in birds. Preference may be given to candidates with a working knowledge of data handling and electronics.

Post tenable for up to three years from 1.3.84 or as soon as possible thereafter.

Salary on the Research Associate 1B scale £6,310-£8,530/Research Fellow 1A scale £7,190-£11,615 plus superannuation.

For further particulars phone 021-472 1301 ext 2558 quoting reference J1.

No formal application form. Three copies of application, including full Curriculum Vitae and naming three referees to Assistant Registrar, (Science and Engineering), PO Box 363, Birmingham B15 2TT, by 17 February 1984. (1140)E

POST DOCTORAL FELLOWSHIP

BIOCHEMIST

Post Doctoral Fellowship available for biochemist with interest in one or more of the following: Immuno-chemistry, tissue culture or protein purification. Available immediately.

Send resume to: Dr Jeffrey A Nisker, Victoria Hospital, Colborne 3, London, Ontario, Canada N6A 4G5. (NW249)E

PLANT DISEASE BIOTECHNOLOGY

POST DOCTORAL FELLOWSHIPS

in plant disease biotechnology (monoclonal antibodies, recom-binant DNA): $25,488/yr, possible renewal for second year; preference to Canadian citizens.

Contact: Dr R I Hamilton, Agriculture Canada Research Station, 6660 NW Marine Drive, Vancouver, Canada V6T 1X2. (NW250)E

SEMINARS and SYMPOSIA

TENTH EMBO ANNUAL SYMPOSIUM 1984

Control of Transcription in Eukaryotes

24—27 September 1984

EMBL Heidelberg (F.R.G.)

Invited speakers include:

M. E. Akam (Cambridge; V. G. Allfrey (New York); M. Beato (Marburg); P. Borst (Amsterdam); F. Cuzin (Nice); E. M. De Robertis (Basel); A. van der Eb (Leiden); H. Eisen (Paris); D. Gallwitz (Marburg); W. J. Gehring (Basel); F. G. Grosveld (London); I. Grummt (Würzburg); P. Gruss (Heidelberg); L. Hereford (Boston); A. Johnson (San Francisco); C. Kédinger (Strasbourg); W. Keller (Heidelberg); U. Laemmli (Geneva); T. Maniatis (Cambridge, USA); K. Nasmyth (Cambridge); M. S. Neuberger (Cambridge); C. S. Parker (Pasadena); M. Revel (Rehovot); R. G. Roeder (New York); W. Schaffner (Zürich); U. Schibler (Lausanne); W. T. Schrader (Houston); G. Schütz (Heidelberg); A. Sentenac (Gif-sur-Yvette); P. A. Sharp (Cambridge, USA); T. Shenk (Stony Brook); K. Struhl (Boston); G. Tocchini-Valentini (Rome); C. Weissmann (Zürich); N. M. Wilkie (Glasgow); K. R. Yamamoto (San Francisco); M. Yaniv (Paris)

Although the main theme of the Symposium is the control of transcription in eukaryotes, some aspects of RNA processing will also be discussed.

The Symposium will be at the European Molecular Biology Laboratory, Heidelberg, with registration on Sunday, 23 September 1984. Plenary sessions will be held in the mornings and evenings of 24 — 26 September, and the morning and afternoon of 27 September. There will be poster sessions in the afternoons of 24 — 26 September inclusive, to allow participants to present their work. It is also intended to organize informal workshops during the Symposium, the themes of which, depending on the posters received, may include inducible genes, cell-type specific gene expression, promoter organization and the structure of active chromatin.

Applications for participation, including a curriculum vitae, a brief description of current research interests, and a 1-page (DIN A 4) abstract of your poster, if you wish to present one, should be sent to: Dr. J. Tooze, Executive Secretary, EMBO, Postfach 1022.40, D - 69 Heidelberg, F.R.G.

The registration fee, which includes daily transport to the EMBL from Heidelberg during the Symposium, lunches, and the Symposium reception is DM 180, for graduate students DM 90, and for participants from industry DM 360. Participants will be accommodated in hotels in Heidelberg, and a small number of inexpensive rooms will be available in the EMBL Guest House. The registration fee does NOT cover the cost of accommodation.

Applications should reach Heidelberg by 1 June 1984. The facilities available make it necessary to restrict the number of participants to about 220 and, therefore, the Organizing Committee may be obliged to make a selection from the applications received. Those accepted will be notified by 31 July 1984 at the latest. The registration fee should NOT be sent until you have been notified of your acceptance for the Symposium.

Organizing Committee: L. Philipson (EMBL Heidelberg) — Chairman, M. Birnstiel (Zürich), P. Chambon (Strasbourg), R. Cortese (EMBL Heidelberg), H. Pelham (Cambridge), J. Tooze (EMBO). (W715)M

FOURTH INTERNATIONAL SYMPOSIUM ON RAPID METHODS AND AUTOMATION IN MICROBIOLOGY AND IMMUNOLOGY

Sponsored by: German Society of Hygiene and Microbiology (DGHM)
International Union of the Microbiological Societies (IUMS)
(1981 Washington, 1976 Cambridge, 1973 Stockholm)

BERLIN, JUNE 7-10, 1984

18 seminars with 80 invited speakers will cover:

Time realities in the evaluation of vaccines for safety and efficacy; hybridisation technology in microbiology; hybridisation technology in virology; rapid diagnosis by IgM-detection; application of monoclonal antibodies; problems of hybridoma technology; data processing in microbiology and serology; fluoroimmunoassays; bioluminescence; physical detection of microorganisms; chromatography; antigen detection; isolation of microorganisms; identification of microorganisms; antimicrobial susceptibility tests; assays of antimicrobial agents in fluids and tissues; food microbiology; environmental microbiology.

Chairmen:

A. Balows (Atlanta); T. Bergan (Oslo); J. Chidlow (London); L. Corey (Seattle); T.O. Diener (Beltsville); S. Falkow (Standord); A.A. Gershon (New York); D. Gröschel (Charlottesville); N. Guttman-Bass (Jerusalem); K. -O. Habermehl (Berlin); P. Halonen (Turku); W.J. Hausler, Jr. (Iowa City); W. Heeschen (Kiel); L. Hertzenberg (Stanford); M.R. Hilleman (West Point); H.D. Isenberg (New York); B. Jarvis (South Ruislip); L. Kaufman (Atlanta); U, Krech (St. Gallen); S. Krugman (New York); M. Lovett (Los Angeles); J.M. Matsen (Salt Lake City); C.W. Moss (Atlanta); S.W.B. Newsom (Cambridge); C.E. Nord (Stockholm); S.H.W. Notermans (Bilthoven); I. Phillips (London); K. Ryan (Tucson); A.N. Sharpe (Ottawa); T. Staehelin (Basel); A. Thore (Bromma); R.C. Tilton (Farmington); L.S. Tompkins (Palo Alto); A. Turano (Brescia); S. Ulitzur (Haifa); I. Yano (Osaka); R.H. Yolken (Baltimore); H. zur Hausen (Heidelberg).

In addition: 16 seminars of 130 contributors and additional poster sessions. The Symposium will be held at the International Congress Center Berlin in combination with an Industrial Exhibition.

Requests for the preliminary program, registration forms, and applications for scientific contributions should be sent to: **Institute of Clinical and Experimental Virology, Free University Berlin, Hindenburgdamm 27, D-1000 Berlin 45, Tel. (030) 7983696.**

Registration fee: Participants before March 1st, 1984 DM 350.-, after March 1st, 1984 DM 450.-. Students (with certification of the University) DM 120.-. Accompanying persons DM 60.-. The registration fee includes a volume of abstracts and a book of proceedings, published by Springer Verlag Berlin, Heidelberg, New York, Tokyo.

(W722)M

European Society for Animal Cell Technology
International Association of Biological Standardization

The joint ESACT/ IABS meeting on the PRODUCTION AND EXPLOITATION OF EXISTING AND NEW ANIMAL CELL SUBSTRATES

will be held on MAY 21st-25th 1984
in GARDONE RIVIERA, Italy

The Meeting is organized by:
"Menarini" International Foundation, Florence, Italy
Zooprophylactic Foundation, Brescia, Italy

SUBJECT AREAS: Genetic Engineering or Modification of Animal cells, Characterization of Cell Substrates.

Safety Aspects Relating to the Exploitation of Animal Cells, Oncogenes, Regulatory Proteins, Regulatory Authorities.

Production of Antibody from Hybridomas and the Scale-Up of such Systems.

Developments in Large-Scale Cell Production Equipment.

Medium Developments for Cell and Product Generation.

Serium-Free Media (Pains and Pleasures).
Downstream Processing of Materials Produced from Cultured Animal Cells.

New Product Areas (Lymphokines, Enzymes, Hormones).

Organizing Secretariat: G. F. Panina c/ o FONDAZIONE INIZIATIVE ZOOPROFILATTICHE, Via Bianchi 1, 25125 Brescia — Italy. (W649)M

INTERNATIONAL SYMPOSIUM on "RETROVIRUSES AND HUMAN PATHOLOGY"

September 25-26, 1984 — S. Terenzo di Lerici, La Spezia, Italy

INVITED SPEAKERS

M. BARBACID (USA)
A. BURNY (Belgium)
J.C. CHERMANN (France)
R. DULBECCO (USA)
R.C. GALLO (USA)
J. HILGERS (The Netherlands)
J.A. LEVY (USA)
B.A. NEXO (Denmark)
D. STEHELIN (France)
J. SVOBODA (Czechoslovakia)
R.A. WEISS (England)
J. WYKE (England)
O. JARRETT (Scotland)

PRELIMINARY PROGRAMME

W.P. Rowe Memorial Lecture
Lecture sessions on:
retroviruses and the murine model system
retroviruses and the vertebrate model systems
retroviruses and human pathology
round table discussion on:
the role of retroviruses in nature: an update
non-stop poster session,
limited to 100 participants

Deadline for abstract submission:
March 30, 1984

ORGANIZING AND SCIENTIFIC SECRETARIAT:
OLIVIERO E. VARNIER
Institute of Microbiology, School of Medicine
Viale Benedetto XV, 10
16132 GENOVA, Italy — Phone: (010) 501337 (W718)M

INTERNATIONAL SYMPOSIUM ON MONOCLONAL ANTIBODIES: BIOLOGICAL AND CLINICAL APPLICATIONS
Florence, Palazzo dei Congressi, October 16-19, 1984

Scientific Secretariat: A. Pinchera — G. Doria — F. Dammacco — A. Bargellesi

The meeting is intended to cover three broad areas of biological and clinical applications of monoclonal antibodies: Markers of the Immune System; Hormonal Receptors and Related Autoimmune Disorders; Experimental and Clinical Oncology.

The Scientific Program will consist of Invited Lecturers and Poster Sessions. A limited number of Posters will be selected for oral presentation.

Invited Speakers: L. Adorini (Italy), R. C. Bast (USA), C. A. Bona (USA), M. I. Colnaghi (Italy), P. Comoglio (Italy), G. Corte (Italy), S. Dower (USA), P. A. W. Edwards (UK), A. Falaschi (Italy), I. D. Goldfine (USA), M. F. Greaves (UK), G. L. Greene (USA), C. Grossi (Italy), S. Hakomori (USA), L. D. Kohn (USA), J. Lindstrom (USA), R. G. Lynch (USA), J. P. Mach (Switzerland), S. L. Morrison (USA), K. Rajewski (FRG), M. D. Scharff (USA), R. H. Schwartz (USA), J. Strominger (USA), J. Schlessinger (Israel), T. Tada (Japan), I. S. Trowbridge (USA), P. E. Thorpe (UK), J. C. Venter (USA), T. A. Waldmann (USA), L. N. Warner (USA).

The deadline for submission of Abstracts is June 15, 1984. For further information write to the *Organizing Secretariat:* Organizzazione Internazionale Congressi (O.I.C.), Via Gustavo Modena 19, 50121 Florence (Italy). Tel: (055) 53962-571082 — Telex 570243 OICCONGRE.

Preliminary Program — International Symposium on MONOCLONAL ANTIBODIES '84: BIOLOGICAL AND CLINICAL APPLICATIONS
Florence, October 16-19, 1984

Tuesday, October 16 — 17:00 — 19:00 OPENING CEREMONY

R. Masi (Italy): Presidential Address

A. Falaschi (Italy): New Frontiers of Biotechnology

Wednesday, October 17 MARKERS OF THE IMMUNE SYSTEM

Morning 8:30 — 12:20

Chairmen: G. DORIA and A. BARGELLESI

M. D. SCHARFF (USA): Advances in Monoclonal Antibody Technology

S. L. MORRISON (USA): Ig Gene Expression in Transformed Lymphoid Cells

C. A. BONA (USA): Regulatory Idiotopes

K. RAJEWSKI (FRG): Monoclonal Antibodies in the Analysis of the Idiotypic Network and the Selection of the Antibody Repertoire

J. STROMINGER (USA): Human Histocompability Antigens: Genes and Proteins

12:20 — 14:00 — LUNCH AND POSTERS

Afternoon 14:00 — 18:20

Chairmen: M. RICCI and F. DAMMACCO

L. ADORINI (Italy): Antigen-Specific Monoclonal T Cell Products

R. H. SCHWARTZ (USA): Cellular Co-Recognition of MHC and Antigen

T. TADA (Japan): The Immunoregulatory Molecules Studied with Monoclonal Antibodies

C. GROSSI (Italy): Multiple Marker Analysis of Human Lymphocyte Subpopulations

N. L. WARNER (USA): New Developments in the Definition of Human Leucocyte Subsets by Monoclonal Antibodies

M. F. GREAVES (UK): Human Lymphoid Malignancies

18:20-19:20

Chairmen: G. TRIDENTE and S. ROMAGNANI

POSTER DISCUSSION Thursday, October 18

HORMONAL RECEPTORS AND RELATED AUTOIMMUNE DISORDERS

Morning 8:30 — 12:20 Chairmen: A. PINCHERA and A. ALBERTINI

L. D. KOHN (USA): Thyrotropin Receptor

I. D. GOLDFINE (USA): Insulin Receptor

J. LINDSTROM (USA): Acetylcholine Receptor

G. L. GREENE (USA): Immunochemical Studies of Estrogen Receptor in Normal and Neoplastic Tissues

B. MONCHARMONT (Italy): Application of Monoclonal Antibodies to the Study of the Estrogen Receptor Structure

12:20 — 14:00 — LUNCH AND POSTERS

Afternoon 14:00 — 17:00

Chairmen: M. SERIO and L. MARTINI

J. C. VENTER (USA): Monoclonal and Autoantibodies to Beta-Adrenergic Receptors

I. S. TROWBRIDGE (USA): The Biology of the Transferrin Receptor

J. SCHLESSINGER (Israel): Monoclonal Antibodies Against the Epidermal Growth Factor Receptor: A Versatile Tool for Structural and Mechanistic Studies.

T. A. WALDMANN (USA): Interleukin-2 Receptors on T Cells

17:00 — 18:00

Chairmen: M. MUGGEO and V. MACCHIA

POSTER DISCUSSION Friday, October 19

EXPERIMENTAL AND CLINICAL ONCOLOGY

Morning — 8:30 — 12:20 Chairmen: G. SALVATORE and G. L. BURAGGI

P. COMOGLIO (Italy): Oncogene Products as Targets for Monoclonal Antibodies

S. HAKOMORI (USA): Glycolipid Tumor Cell Markers

R. G. LYNCH (USA): Myeloma Proteins as Targets and Inducers of Immunoregulatory Signals

M. I. COLNAGHI (Italy): Tumor Detection by Immunocytochemical and Radioimmunometric Methods

J. P. MACH (Switzerland): Detection of Tumors by Immunoscintigraphy Using Monoclonal Antibodies against Tumor Markers

12:20 — 14:00 — LUNCH AND POSTERS

Afternoon 14:00 — 17:40 Chairmen: U. MELDOLESI and G. DELLA PORTA

P. A. W. EDWARDS (UK): Human Monoclonal Antibodies and the Prospects for Therapy

R. C. BAST (USA): Treatment of Bone Marrow with Monoclonal Antibodies *in Vitro*

G. CORTE (Italy: Monoclonal Antibodies for the Control of GVH Reaction after Bone Marrow Transplantation

S. DOWER (USA): A Rational Approach to the Development of Monoclonal Antibody Reagents for *in Vivo* Tumor Diagnosis and Treatment

P. E. THORPE (UK): Monoclonal Antibody-Toxin Conjugates

17:40 — 18:40 Chairmen: A. NICOLIN and L. FRATI

POSTER DISCUSSION

(W726)M

CONFERENCES and COURSES

Royal Postgraduate Medical School

(University of London)

Advances in the Applications of Monoclonal Antibodies in Clinical Oncology 6—8 June 1984

This course will cover advances in the understanding of antibody — antigen interactions, and uses in diagnosis and therapy of the new and expanding field of antibody guided localisation of cancer.
The course is suitable for both laboratory scientists and clinicians.

TOPICS INCLUDE:

Heterogeneity of tumours

Blood group antigens on tumours

Differentiation of human germs cells

Growth factors and their receptors on normal and neoplastic cells

Tumour markers

Antibody guided scanning by external body scintigraphy using
a) Radioiodines
b) Other isotopes eg Indium III

Potential uses of antibodies in
a) NMR Imaging
b) Positron emission tomography

Clinical Case Presentations

Ways to improve localisation
a) Use of antibody fragments
b) Use of second antibodies
c) Emission tomography

Therapeutic applications
a) *In vitro*
Bone marrow transplantation
b) *In vivo*
Cytotoxic antibodies
Chemotherapy—antibody conjugates
Radiolabelled antibodies
Antibody-boron complexes for neutron capture
Immunotoxins

SPEAKERS INCLUDE:

Professor K Bagshawe, London, UK,
Professor R W Baldwin, Nottingham, UK,
Dr R Beany, London UK
Dr R Begent, London, UK
Dr W F Bodmer, London, UK
Dr A R Bradwell, Birmingham, UK
Dr K E Britton, London, UK
Dr D Carr, London, UK
Dr D Catovksy, London, UK
Dr K A Chester, London, UK
Dr A A Epenetos, London, UK
Dr R Fairchild, Brookhaven, USA
Dr S Ferrone, New York, USA
Professor D Galton, London, UK
Dr J Goldman, London, UK
Dr P Goodfellow, London, UK
Dr W Gullick, London, UK
Dr J Habeshaw, London, UK
Dr K E Halnan, London, UK
Dr P Lavender, London, UK
Dr E Lennox, Cambridge, UK
Dr J P Mach, Lausanne, Switz.
Dr M Myers, London, UK
Dr M Neville, Sutton, UK
Professor S Orr, London, UK
Professor D K Peters, London, UK
Professor R Steiner, London, UK
Dr P Thorpe, London, UK

COURSE ORGANISERS:
Dr A A EPENETOS, Senior Research Fellow in Clinical Oncology, RPMS, Hammersmith Hospital
Dr K E HALNAN, Director of Radiotherapy and Oncology, RPMS, Hammersmith Hospital

COURSE FEE: **£100 including catering**
Application forms, abstract forms and further details may be obtained from: School Office (SSC), Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London, W12 0HS. (Telephone: 01-743 2030 ext 351).

Course enquiries:— **Dr A A Epenetos (Telephone: 01-743 2030 ext 353).**

One half day will be open for paper presentations.
Abstracts will be considered for oral and/or poster presentation and, if accepted, will also be considered for publication by the British Journal of Cancer.

CLOSING DATE: 1 April 1984 (1102)C

Industrial Liaison Program of the Massachusetts Institute of Technology presents a short course entitled:

"CONTROLLED RELEASE TECHNOLOGY: POLYMERIC DELIVERY SYSTEMS FOR DRUGS, PESTICIDES, AND FOODS"

Dates: June 4-7, 1984
Chairman: Professor Robert Langer, MIT
Other Speakers: Dr Frank Harris, University of Akron
Dr Jorge Heller, SRI International
Dr Marcus Karel, MIT
Dr Nicholas Peppas, Purdue University
Dr Frank Szoka, University of California

Location: Vienna, Austria

Contact: Maria Clara V. Suva Martin
Industrial Liaison Program
Tel: (617) 253-2691; TELEX: 921473

Attendence Fee: Industrial Liaison Program (ILP) Members — $700.00, Non ILP Members — $900.00.

Topics: Fundamental Principles of Controlled Drug Release; Pharmaceutical Applications; Agricultural Applications; Pesticides; Food Applications; Fabrication Technology of Controlled Release Systems; Liposomes; Microencapsulation; Polymer Technology for Release Systems and Diffusion of Drugs in Polymers.

Abstract:
Controlled release systems improve the ways in which chemical or drugs are administered. Normally, a chemical is administered in a high dose only to have to repeat that dose several hours or days later. This is not economical and sometimes results in damaging side-effects. Thus, increasing attention has been focused on methods of giving drugs continuously for prolonged time periods and in a controlled fashion by incorporating the drugs within polymers.
The program will explore recent advances in controlled release technology and cover polymers systems as they relate to achieving appropriate design of controlled release formulations. (NW260)C

UNIVERSITY OF NOTTINGHAM
MSc COURSE IN MEAT SCIENCE

Applications are invited from GRADUATES in appropriate subjects (normally in Agricultural, Biological, Chemical or Veterinary Sciences) for admission to an advanced course of study leading to the degree of MSc (by examination) in Meat Science. The course, which is of one year's duration, includes Production of Meat Animals, Physiology of Meat Animals, Animal Health & Meat Inspection, Commodity Science of Meat and the Economics and Marketing of Meat. A short dissertation based on research of a topic selected from one of these 5 subjects constitutes part of the overall assessment for the degree.
The course is designed to provide those having appropriate scientific training with specialist knowledge of meat whereby they would be suitably qualified to accept responsibility for the scientific control of ...odity in relation to developments within E.E.C. and in ...

...nts, scholarships may be available.
...the 1984-85 session should write before ...plication form, from the following:
...**Of Agriculture, University of ...hborough Leics LE12 5RD**
(1110)C

KING'S COLLEGE LONDON
MSC in HUMAN AND APPLIED PHYSIOLOGY

A one year course to train graduates in measuring and studying the physiological functioning of man in normal and abnormal environments. The course will equip students to specialise in the applications of physiology to man in industrial, Service and clinical situations and the teaching of human physiology. Topics studied include:— basic measurements; life support systems; exercise; locomotor; postural; climatic; high altitude and hyperbaric physiology.

September 1984 — September 1985

Applicants should normally have a good degree in Physiology or be medically qualified.

Further details and application form from: Dr S Rowlands, King's College London, Strand, London WC2R 2LS, quoting reference N921.

Closing date for applications: March 31 1984. (1121)C

LOUGHBOROUGH UNIVERSITY OF TECHNOLOGY

SEPARATIONS FOR BIOTECHNOLOGY AND BIOCHEMISTRY

SHORT COURSE
26th—30th March 1984

This residential course (forme entitled "Gel Filtration and Electrophoresis") provides thorough and up-to-date coverage of the separation methods used in preparative and analytical biochemistry. Lectures will be supplemented by extensive practical sessions involving the most modern techniques. The course fee including accommodation and all meals, is £275. For reservations and further details contac Miss C D Newton, Departmen of Chemistry, tel: 0509 26317 ext. 351. (1097)C
Loughborough Leicestershir

Please mention
nature
when replying to these advertisements

1984 Cold Spring Harbor Laboratory

Meetings Schedule

Molecular Biology of the Cytoskeleton, April 25-29
Organizers: C. Borisy, *University of Wisconsin*, D. Cleveland & D. Murphy, *Johns Hopkins Medical School*

First International Meeting on Ciliate Molecular Genetics, May 2-6
Organizers: P. Bruns, *Cornell University*, M. Gorovsky, *University of Rochester*

Molecular Biology of the Photosynthetic Apparatus, May 9-13
Organizers: L. Bogorad, *Harvard University*, C. Arntzen, *Michigan State University*, S. Bonitz, *CSH Lab*

RNA Processing, May 16-20
Organizers: H. Robertson, *Rockefeller University*, A. Shatkin, *Roche Institute*, M. Mathews, *CSH Lab*

RNA Tumor Viruses Ninth CSH Meeting, May 22-27
Organizers: T. Hunter, *Salk Institute*, S. Martin, *University of California, Berkeley*

Recombination at the DNA Level, 49th CSH Symposium on Quantitative Biology, May 30-June 6
Organizers: A. Klar & J. Strathern, *CSH Lab*

Molecular Biology of SV40, Polyoma and Adenoviruses, August 15-19
Organizers: T. Grodzicker, *CSH Lab*, M. Botchan, *University of California, Berkeley*

Phage and Bacterial Regulatory Mechanisms, August 21-26
Organizers: S. Adhya, *NIH*, H. Echols, *University of California, Berkeley*

Polypeptide Hormones, August 28-September 1
Organizers: O. Rosen, *Albert Einstein College of Medicine*, R. Ross, *University of Washington*, J. Feramisco, *CSH Lab*

Modern Approach to Vaccines, September 12-16
Organizers: R. Chanock, *NIH*, R.Lerner, *Scripps Clinic*

For information and applications write to:
Meetings Coordinator *or* Course Registrar

Cold Spring Harbor Laboratory
Box 100, Cold Spring Harbor,
New York 11724

Courses in Neurobiology

Neurobiology of Human Disease, June 8-17
Instructors: X. Breakfield, *Yale University*, I. Black, *Cornell University*

Immunoglobulins: Molecular Probes of the Nervous System, June 8-28
Instructors: S. Hockfield, *CSH Lab*, C. Kintner, *Medical Research Council*, L. Swanson, *Salk Institute*, E. Weber, *Stanford University*

Neurobiology of *Drosophila*, July 1-21
Instructors: L. Jan, Y. Jan, & P. O'Farrell, *University of California, San Francisco*, R. Greenspan, *Princeton University*

Molecular Biology of the Nervous System, July 5- 18
Instructors: R. Kelly, *University of California, San Francisco*, R. McKay, *CSH Lab*

Developmental Neurobiology, July 20-August 2
Instructors: D. Purves, *Washington University*, P. Patterson, *California Institute of Technology*

Advanced Electrophysiological Methods, July 24-August 13
Instructors: P. Ascher, *Ecole Normale Supérieure*, S. Schuetze & S. Siegelbaum, *Columbia University*

Courses in Genetics

Molecular Biology of Plants, June 8-28
Instructors: I. Sussex, *Yale University*, R. Malmberg, *CSH Lab*, J. Messing, *University of Minnesota*

Molecular Cloning of Eukaryotic Genes, June 8-28
Instructors: A. Bothwell, *Yale University*, F. Alt, *Columbia University*, J. Mullins, *Harvard School of Public Health*

Advanced Bacterial Genetics, July 1-21
Instructors: T. Silhavy & M. Berman, *Frederick Cancer Research Facility*, L. Enquist, *Molecular Genetics, Inc.*, S. Bear, *NIH*

Molecular Embryology of the Mouse, July 8-21
Instructors: B. Hogan, *Imperial Cancer Research Fund*, F. Costantini, *Columbia University*, L. Lacy, *Sloan-Kettering Cancer Center*

Advanced Techniques in Molecular Cloning, July 24-August 13
Instructors: T. Atkinson & M. Smith, *University of British Columbia*, J. Fiddes, *California Biotechnology, Inc.*, D. Shortle, *State University of New York, Stony Brook*

Yeast Genetics, July 24-August 13
Instructors: F. Sherman, *University of Rochester*, G. Fink, *Massachusetts Institute of Technology*, J. Hicks, *CSH Lab*

(NW261)C

STUDENTSHIPS

UNIVERSITY OF EAST ANGLIA
NORWICH

POSTGRADUATE STUDENTSHIPS
SCHOOL OF BIOLOGICAL SCIENCES

Applications are invited for SERC Studentships from candidates with interests in any of the following areas: —

Biophysics, Biochemistry, Ecology, Genetics, Microbiology, Plant Biology, Physiology, Zoology

Candidates should have, or expect to obtain in 1984, a First or good Upper Second Class degree. It is expected that at least five Studentships will be available. Applicants should write to the Director of Graduate Studies (C), School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, enclosing the names of two academic referees. Details of the research interests of members of faculty are available on request. (1112)F

SOUTH MANCHESTER HEALTH AUTHORITY

CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE

STUDENTSHIPS FOR TRAINING IN RESEARCH

Five Cancer Research Campaign financed studentships will be awarded in these multidisciplinary laboratories for full time studies leading to higher degrees in research fields relevant to cancer. Successful applicants will work in one of the following divisions; biophysical chemistry, radiobiology, experimental chemotherapy, carcinogenesis, cell biology, experiment haematology, cell and tissue kinetics, ultrastructure, immunology and molecular biology.

Awards will be tenable for three years from 1 October 1984, with a tax-free grant of £2,880. Applications are invited from candidates who have or expect to obtain a first or upper second class honours degree.

Applications, which should include a detailed curriculum vitae and the names of three academic referees, should be sent to the Director, The Paterson Laboratories, Christie Hospital and Holt Radium Institue, Wilmslow Road, Withington, Manchester M20 9BX (Tel. 061-445 8123 ext 400) as soon as possible and in any case not later than 1 March 1984. (1087)F

GRANTS and SCHOLARSHIPS

GUNNAR NILSSON CANCER RESEARCH TRUST FUND

Grant applications up to £15,000 sterling will be considered to assist cancer research projects where money is not available from the normal grant giving bodies. An application form is available, and applications should be received by 30 June 1984.

Applications to be sent to Dr Norman Howard, Chairman, Gunnar Nilsson Cancer Research Trust Fund, Department of Radiotherapy and Oncology, Charing Cross Hospital, Fulham Palace Road, London W6 8RF. (1096)H

THE ROYAL SOCIETY GOVERNMENT GRANT FOR SCIENTIFIC INVESTIGATIONS

Applications for grants from the first allotment of the Government Grant for Scientific Investigations for the year 1984 (for which the amount available will be substantially less than in recent years) should be made not later than 15 March 1984 on forms of application to be obtained from the Executive Secretary of the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

Applicants must be British subjects domiciled in the United Kingdom. Grants may be made to promote and support research in science and to assist scientific expeditions and for personal stipends or [illegible]ions.

ASSISTANTSHIPS

AFRC UNIT OF NITROGEN FIXATION

RESEARCH ASSISTANTSHIP

Applications are invited for a 3 year research assistantship in the Unit of Nitrogen Fixation, leading to a D.Phil. degree at the University of Sussex. The project is under the supervision of Drs J R Dilworth and B E Smith and will involve chemical and spectroscopic studies of the iron-molybdenum co-factor of nitrogenase and the synthesis of relevant iron and molybdenum sulphur complexes.

Applicants should hold or be likely to hold a 2.1 (minimum) honours degree in Biochemistry or Chemistry and should send a curriculum vitae to the Secretary, AFRC Unit of Nitrogen Fixation, University of Sussex, Brighton, BN1 9RQ. (1122)P

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF BIOCHEMISTRY

POST DOCTORAL RESEARCH ASSISTANTSHIP

Available immediately for approximately two years to work on "Control of Testis Leydig Cells by Intra- and Extra-Testicular Factors". Appointment supported by a grant to Professor B A Cooke from the Wellcome Trust. Candidates should have experience in one or more of the following:- Cell purification and culture, radioimmunoassay, receptor assays and steroid biochemistry.

Full curriculum vitae, including the names and addresses of two referees, should be submitted by 17 February 1984 to the School Office, RFHSM, Rowland Hill Street, London NW3 2PF (telephone 01-794 0500 ext 4262) from where further particulars are available. Please quote reference BAC/RA. (1139)P

LADY TATA MEMORIAL TRUST
INTERNATIONAL AWARDS FOR RESEARCH ON LEUKAEMIA AND ALLIED CONDITIONS

ACADEMIC YEAR 1984 — 198

The Trustees of the Lady Ta Memorial Trust invited applicatio for Awards for personal support f research on leukaemia, in th Academic Year beginning 1 October, 1984. In view of the affini between leukaemia and other form of neoplastic disease, candidate with programmes of research on an aspect of malignant disease whic may throw light on problems leukaemia will be eligible f consideration. The Trustee specially wish to encourage studie of the leukaemogenic viruses animals, the epidemiology pathogenesis, and immunology leukaemia.

Awards are open to suitab qualified investigators of a nationality, working either in th own institutions or in other cent abroad.

Stipends will be adjusted accordi to the place and institution at whi the work will be done, with provisio for annual increments an superannuation if required. Awar in recent years have usually bee between £5,000 and £10,000.

Awards are tenable for 1 year in t first instance, renewable up to maximum of 3 years.

Further particulars and applicatio forms may be obtained by writing the Secretary of the (Europea Scientific Advisory Committe Lady Tata Memorial Trust, MR Leukaemia Unit, Royal Pos graduate Medical School, DuCa Road, London W12 0H Applications must be submitt before 1st March, 1984 and awar will be announced by the Trustees June. (1131)N

ASSOCIATESHIPS

THE UNIVERSITY OF SYDNEY RESEARCH ASSOCIATESHIP — PLASMA PHYSICS

Applications are invited for Research Associateship in plas physics. The appointment would for two years from a date in period July-December 1984. T appointee would collaborate in theoretical aspects of t Department's Alfven wave heat program.

Salary range: $A20,164 $A22,513 pa.

Further information can obtained from Professor M Brennan, Wills Plasma Phys Department.

Applications close with Registrar, University of Sydn NSW 2006, Australia, on 31 ma 1984.

Equal employment opportunity University policy. (W728)C

AWARDS

The Wellcome Trust

Major Award for Psychological Research into Mental Disorders

The Wellcome Trustees invite proposals from suitable departments in the United Kingdom for an award totalling £250,000 over five years, for the pursuit of experimental and development psychological research into the genesis of mental disorders.

Please write for an application form to: The Grants Section, The Wellcome Trust, 1 Park Square West, London NW1 4LJ.

Completed applications must be returned not later than 6th April 1984.

(1123)N

Ltd, London EC4Y 0HP. Printing: Kingsdale Press Ltd, Reading, Berks, in association with Godfrey Lang Ltd, London WC1R 4EB
A Published by MACMILLAN JOURNALS LTD, 4 Little Essex Street, London WC2R 3LF. — 2 February 1984.

The living proof



The 1,000 delegates to Biotech '83 were proof positive that the business of biotechnology is booming. There is tremendous enthusiasm and need to know.

Biotech '84 Europe builds on that success to present a totally fresh forum. The solid information you want and need to know will be presented by key figures in the fast-growing fields of biotechnology and their commercial applications.

Topics featured strongly in the programme include *Novel Tools and Technologies* such as protein modelling using computer graphics and site-directed mutagenesis, new approaches in *Healthcare and Diagnostics, Agricultural Biotechnology* and the vital, but frequently neglected intricacies of *Regulations* and *Finance*.

The informal programme includes a Government-hosted reception at the Banqueting House in Whitehall, visits to the concurrent Biotech '84 exhibition and, naturally, the opportunity to glean news from colleagues from every corner of world biotechnology.

Biotech 84
europe

Wembley Conference Centre
London 15 - 17 May 1984

BE84 220.3

Name Mr/Ms/Dr

Position

Organisation

Address

Country Zip/Post Code

Phone Telex

☐ My organisation may wish to exhibit. Please send details.

☐ I may wish to participate as a delegate. Please send details.

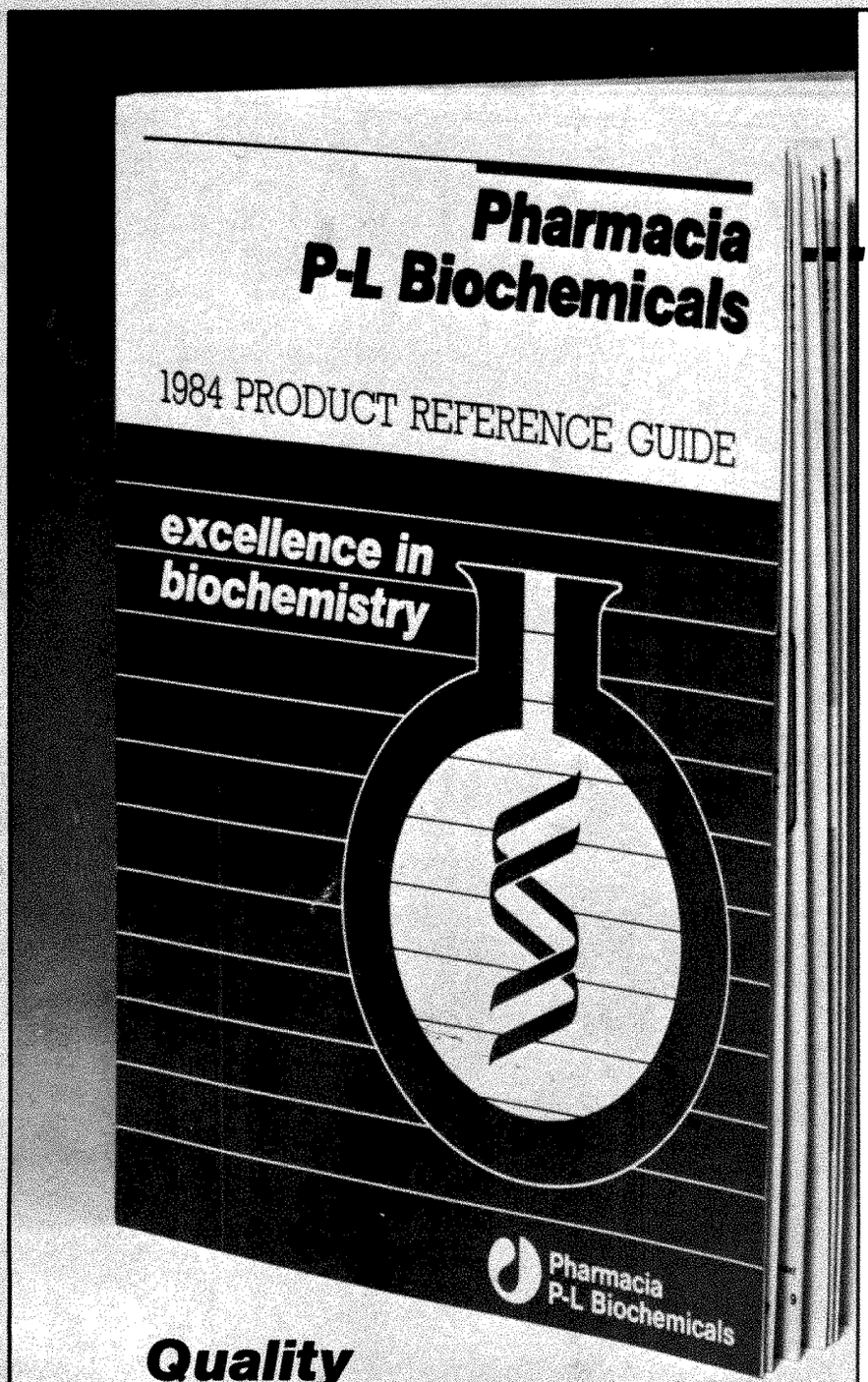
online

The world's most important events on the business impact of high technology.

Online Conferences Ltd,
Pinner Green House,
Pinner HA5 2AE, Middx. UK
Phone: 01-868 4466

Circle No. 13 on Reader Service Card.

There is only one product in this catalog...



...Quality

Actually, the Pharmacia P-L Biochemicals Product Reference Guide lists over 2000 products for biochemistry and molecular biology. The products range from restriction nucleases to coenzymes, from lipids to polynucleotides – but our most important product is the quality that goes into each biochemical that we make.

At Pharmacia P-L Biochemicals, we take our commitment to "excellence in biochemistry" seriously. We make our biochemicals with quality – we know no other way.

The 1984 Product Reference Guide from Pharmacia P-L Biochemicals

Quality biochemicals for quality research.

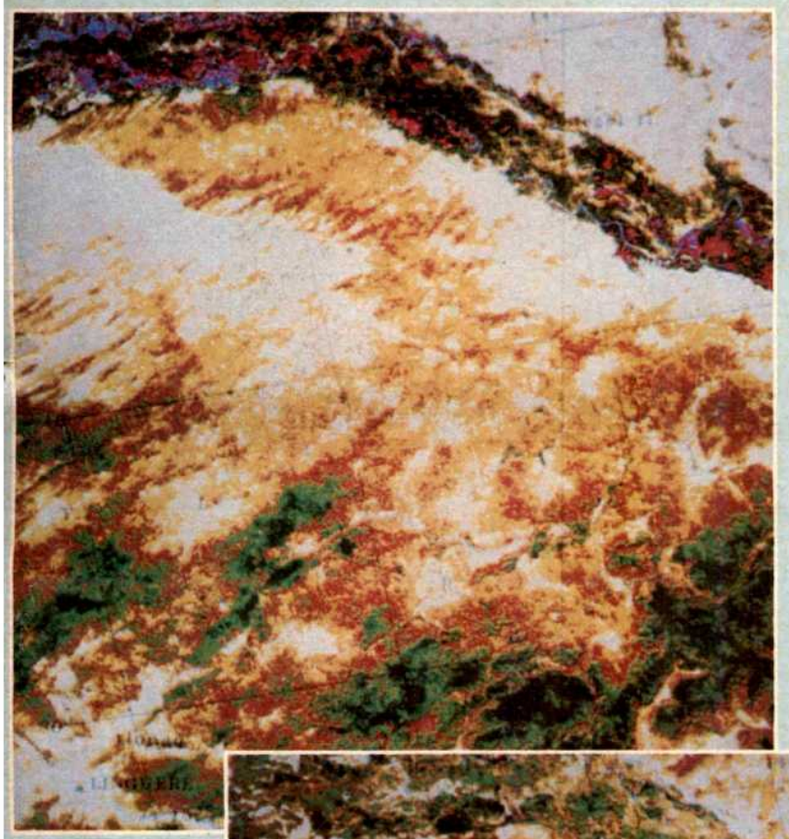
For your copy, contact your local Pharmacia Fine Chemicals/Pharmacia P-L Biochemicals representative or write to one of the addresses below.

Pharmacia P-L Biochemicals Inc.
...macia Inc.

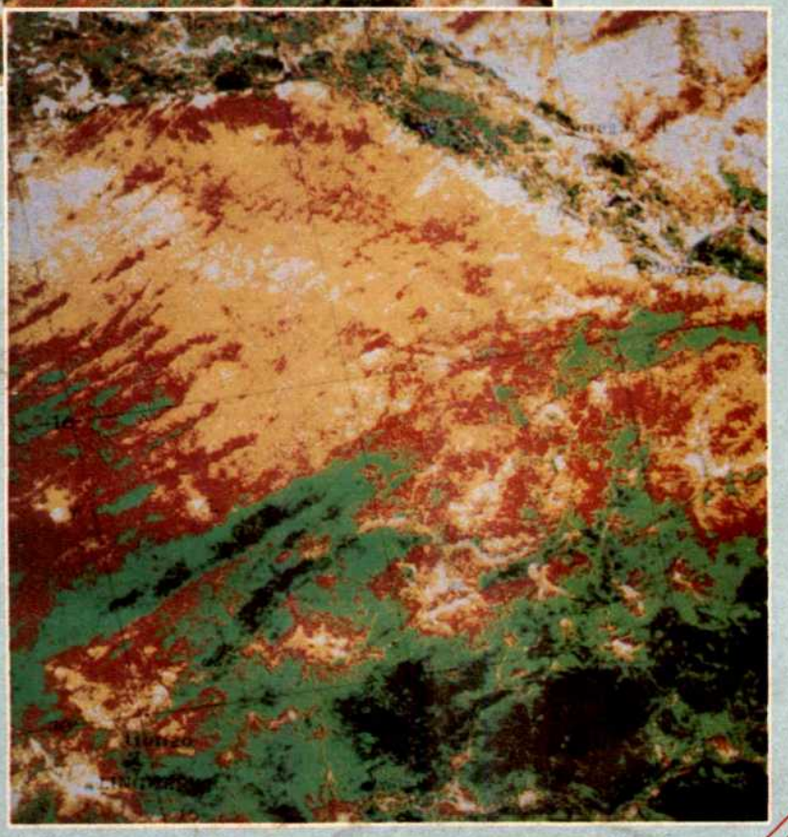
Pharmacia P-L Biochemicals Inc.
Subsidiary of Pharmacia Inc.
Box 175
S-751 04 Uppsala 1, Sweden

Pharmacia P-L Biochemicals

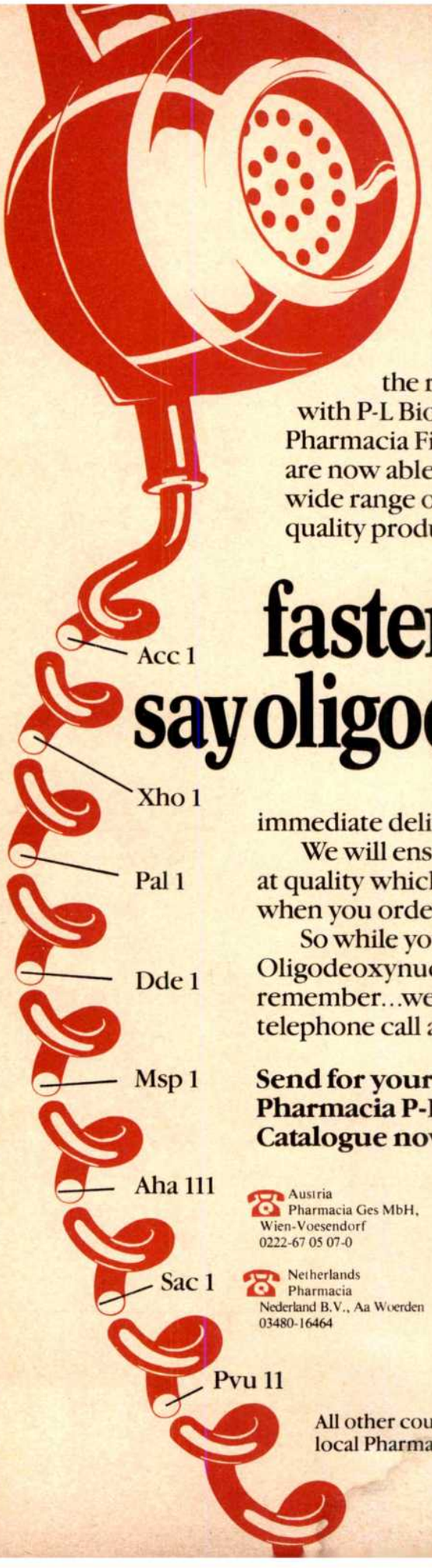
nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Vol 307 No 5951 9-15 February 1984 £1.80 $4.50



SURFACE ALBEDO AND THE SAHEL DROUGHT



CHROMATOGRAPHY AND ELECTROPHORESIS
product review



Now order your research biochemicals...

As a result of the recent merger with P-L Biochemicals, Pharmacia Fine Chemicals are now able to bring you a wide range of over 2,200 high quality products for molecular biology and biochemical research.

And all you have to do to order any of our speciality biochemicals is to contact your local Pharmacia office who are holding large stocks ready for

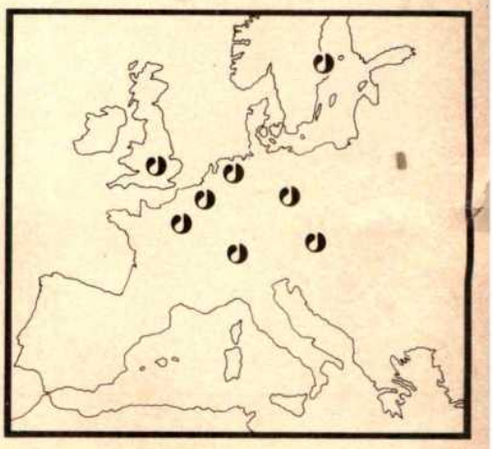
faster than you can say oligodeoxynucleotides

immediate delivery.

We will ensure a fast delivery at quality which you expect when you order from Pharmacia.

So while you are trying to say Oligodeoxynucleotides, remember...we are only a telephone call away.

Send for your free Pharmacia P-L Biochemicals Catalogue now.



Austria
Pharmacia Ges MbH,
Wien-Voesendorf
0222-67 05 07-0

Belgium
Pharmacia Belga,
Brussels
02/7369990

France
Pharmacia France SA,
Bois D'Arcy
043-9919

German Federal Republic
Deutsche Pharmacia GMB
Freiburg (0761) 49 03-239

Netherlands
Pharmacia
Nederland B.V., Aa Woerden
03480-16464

Sweden
Pharmacia Fine
Chemicals AB, Uppsala
018/165450

Switzerland
Pharmacia-Rolf-
Ag, Dübendorf
01/821 18 16

United Kingdom
Pharmacia Ltd.
Milton Keynes
0908-661101

All other countries contact your local Pharmacia representative.

Pharmacia
P-L Biochemicals

Circle No.17 on Reader Service Card.

Why researchers pay a little more for our acrylamide.

They have discovered the unsurpassed performance of Bio-Rad acrylamide:

- Reproducible separations. Even small impurities can make a big difference in results!
- Higher resolution from techniques such as Laemmli SDS-PAGE, 2-D electrophoresis, and electrofocusing.
- Gels that polymerize reproducibly and quantitatively.
- Separations free of artifacts which interfere with clear interpretation of results.
- High purity also means longer shelf life.

If your bottle says "store at 4°C," it's not pure. Bio-Rad acrylamide can be stored dry at ambient temperatures for long periods.

The acknowledged quality of our acrylamide is typical of all Bio-Rad electrophoresis chemicals, including glycine, Bis, Tris and SDS.

Furthermore, Bio-Rad supports you with a wealth of technical data, applications assistance, and tips on proper technique. That alone is worth the price.

Contact us for our latest Technical Bulletin on step-by-step polymerization protocols.



BIO-RAD

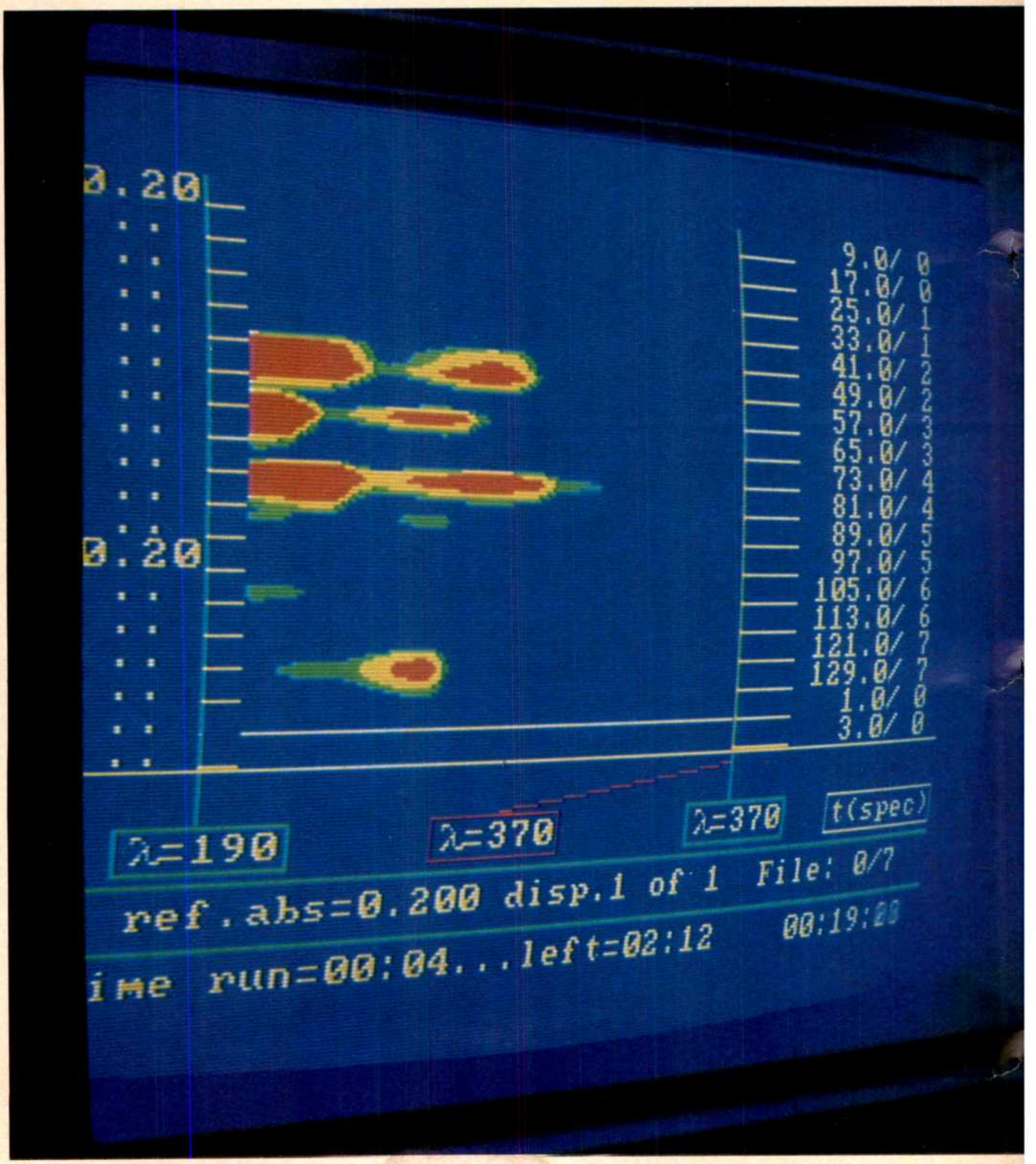
Bio-Rad Laboratories
2200 Wright Ave., Richmond, CA 94804
(415) 234-4130

Also in Rockville Centre, NY, Australia, Austria, Canada, England, Germany, Italy, Japan, The Netherlands and Switzerland.

Circle No. 10 on Reader Service Card.

YOU JUST CAN

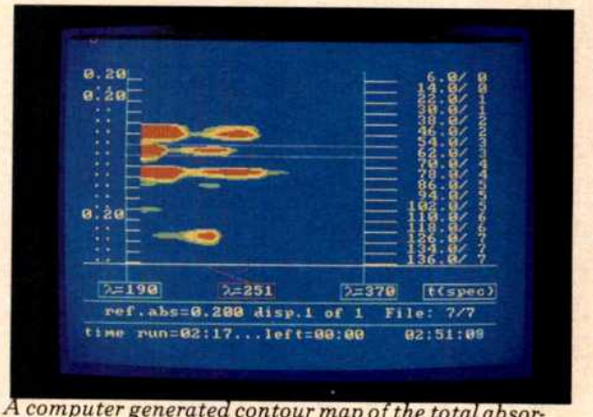
WITH LKB's NEW DI



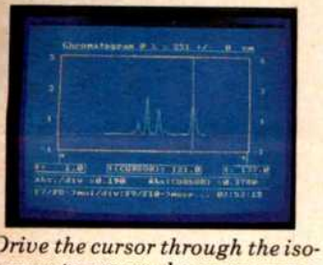
T MISS PEAKS

E ARRAY DETECTOR

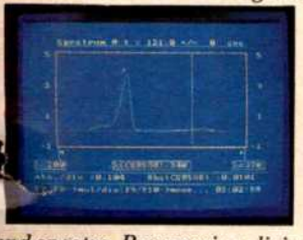
ush-button access to chromatograms and spectra, combined with an innovative icing technique, automatically surveys and explores the whole chromatographic ndscape. Equips you with colour-graphic ed back for an analytical follow-through no her system offers. Power to track, expose id pinpoint all your peaks. Only LKB's Rapid pectral Detector lets you rapidly pursue, sily discover and clearly reveal every single eak—and then deliver it blown-up in perfect detail. So however small, or wherever it's hidden, you'll just never miss a peak—you just can't! The Rapid Spectral Detector is a computer based diode array detection system that offers dramatic breakthroughs in HPLC. With real time display and raw data storage Rapid Spectral provides the true visual command you need for complete analytical insight. Lets you probe new perspectives to give you more information and better results. So if you want to find those missing peaks, get in touch with LKB. We'll send you a broschure that describes the very best in diode array technology. You'll find it infinitely revealing, just like our Rapid Spectral Detector.



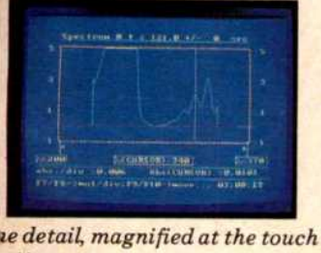
A computer generated contour map of the total absorbance pattern after separation. The vertical and horizontal axes are time and wavelength respectively. Colour coding is used to represent different levels of absorbance for each isogram.



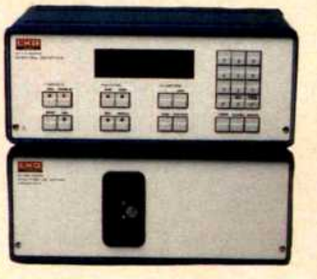
Drive the cursor through the isograms to access chromatograms...



and spectra. Progressive slicing rapidly reveals every peak, hidden or not.



ue detail, magnified at the touch of utton.



LKB BROMMA

Circle No.42 on Reader Service Card.

Head office: LKB-Produkter AB, Box 305, S-161 26 Bromma, Sweden. Tel. 08-98 00 40, telex 10492
in US sales office: LKB instruments, Inc. 9319 Gaither Road, Gaithersburg, Maryland 20877. Tel. 301-963-3200, telex 909870 (dom.), 64634 (intern.)
UK sales office: LKB Instruments Ltd., 232 Addington Road, S. Croydon, Surrey CR2 8YD, England. Tel. 01-657 8822, telex 264414
Other sales offices in: Athens (for Middle East), Copenhagen, Ghent, Zoetermeer, Hong Kong, Luzern, Munich, Paris, Rome, Turku, Vienna

Specifically labelled ^{125}I-peptides

These peptides are purified by HPLC to provide

- increased sensitivity in RIA and receptor studies
- high specific activity
- more stable tracers
- increased consistency and reliability

Compatible antisera are also available

● **CCK-8 (SO_4), ^{125}I-labelled with Bolton and Hunter reagent**	IM.159
Antiserum	N.1591
● (3-[^{125}I]*iodotyrosyl*^{27})**ß-Endorphin**	IM.162
Antiserum	N.1621
● (3-[^{125}I]*iodotyrosyl*^{10})**Glucagon**	IM.160
Antiserum	N.1601
● (3-[^{125}I]*iodotyrosyl*^{3})**Neurotensin**	IM.163
Antiserum	N.1631
● (3-[^{125}I]*iodotyrosyl*^{11})**Somatostatin, Tyr^{11}**	IM.161
Antiserum	N.1611
● **Substance P, ^{125}I-labelled with Bolton and Hunter reagent**	IM.157
Antiserum	N.1571
● (3-[^{125}I]*iodotyrosyl*^{10})**VIP**	IM.158
Antiserum	N.1581

Please contact Amersham or your local representative for more details

Amersham International plc
Amersham England HP7 9LL
telephone Little Chalfont (024 04) 4444
telex 83141 ACTIVA G

Amersham

Amersham Australia PTY Limited Sydney **Amersham Belgium SA/NV** Brussels **Amersham Buchler GmbH & Co. KG** Braunschweig W Germany
Amersham Corporation Arlington Heights USA **Amersham Denmark ApS** Birkerød **Amersham France SA** Paris **Amersham Japan** Tokyo **Amersham Nederland BV** Utrecht

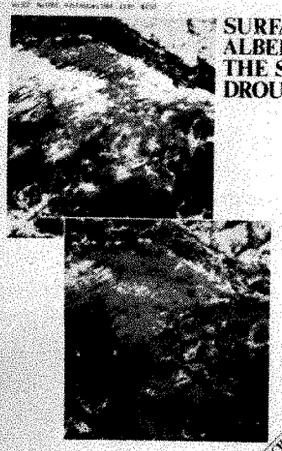
Circle No.50 on Reader Service Card.

nature

NATURE VOL 307 9 FEBRUARY 1984

COVER

nature



SURFACE ALBEDO AND THE SAHEL DROUGHT

CHROMATOGRAPHY AND ELECTROPHORESIS product review

Bio-unit changes in the Sahel desert (Ferlo Senegal region) between 1973 (left) and 1979 (right) determined from Landsat data and field studies (see page 528). Colour code: blue, normal Senegal River bed; red, flood plain; dark green, trees and bushes; olive to light green, continuous grass cover with decreasing trees and bushes; brown, continuous grass cover with no trees; yellow, patchy grass, denuded soil; white, bare soil. The problems caused by the Sahel drought are discussed on page 497.

OPINION

Reagan votes for basic research 489
Time to move Greenwich 490
How not to test drugs

NEWS

US budget 491
Agricultural research 492
Space science
US military research 493
US foundations
European information technology 494
Soviet computing
UK data protection
Fast reactors 495
Remote sensing
UK drug trials 496
France seeks rules
Sahel drought 497
***Nature* index of biotechnology stocks**

CORRESPONDENCE

Post-nuclear survival/Dating the Crucifixion/Animal rights/Indian science city 498

NEWS AND VIEWS

Cell and cancer biology meld
Peter Newmark 499
More nerve growth factors?
Joshua R Sanes 500
Non-aqueous electrodes make wet cells work
S Dennison 501
Selfish DNAs with self-restraint
W F Doolittle, T B L Kirkwood & M A H Dempster 501
Waves and particles: the diffraction of electrons
Jon Darius 502
DNA methylation — how important in gene control?
Adrian P Bird 503
Time for plant cell culture?
M W Fowler 504

BOOK REVIEWS

Government and Research: The Rothschild Experiment in a Government Department *by M Kogan and M Henkel*
Philip Gummett 571
Excitons
E I Rashba and M D Sturge eds
R J Elliott 572
Lead and Lead Poisoning in Antiquity *by J O Nriagu*
Josef Eisinger 574
Eddington: The Most Distinguished Astrophysicist of his Time *by S Chandrasekhar*
Fred Hoyle 575
Culture of Animal Cells: A Manual of Basic Technique *by R Ian Freshney*
G D Clarke

ARTICLES

Deep seismicity and modes of deformation in Tonga subduction zone
D Giardini & J H Woodhouse 505

Nd–Sr isotopic composition of granulites and constraints on the evolution of the lower continental crust
D B Othman, M Polvé & C J Allègre 510

Effect of chromosomal position on amplification of transfected genes in animal cells
G M Wahl, B R de St Vincent & M L DeRose 516

Close similarity of epidermal growth factor receptor and *v-erb-B* oncogene protein sequences
J Downward, Y Yarden, E Mayes, G Scrace, N Totty, P Stockwell, A Ullrich, J Schlessinger & M D Waterfield 521

LETTERS TO NATURE

Interstellar scintillation and ultra-low-frequency gravitational wave observations
J W Armstrong 527

Surface albedo and the Sahel drought
M F Courel, R S Kandel & S I Rasool 528

Predictability of north-east Brazil droughts
S Hastenrath 531

Evidence against surface state limitations on efficiency of p-Si/CH_3CN junctions
C M Lieber, C M Gronet & N S Lewis 533

Photochemical dehydrogenation of ethanol in dilute aqueous solution
J Handman, A Harriman & G Porter 534

Large mantle heterogeneity beneath French Polynesia
Ph Vidal, C Chauvel & R Brousse 536

Metabolic activity and bioluminescence of oceanic faecal pellets and sediment trap particles
C C Andrews, D M Karl, L F Small & S W Fowler 539

Positional determination of supernumerary blast cell death in the leech embryo
M Shankland 541

Inverse relationship between neurotensin receptors and neurotensin-like immunoreactivity in cat striatum
M Goedert, P W Mantyh, P C Emson & S P Hunt 543

Suppression of sprouting at the neuromuscular junction by immune sera
M E Gurney 546

Contents continued overleaf

Nature (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Annual Index (mailed in February). Annual subscription for USA and Canada US $230 (for subscription prices elsewhere, see next page). USA and Canadian orders only to: *Nature*, Subscription Dept PO Box 1018, Manasquan, New Jersey 08736, USA. All other orders to *Nature*, Brunel Road Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY10010 and additional mailing offices. US Postmaster send form for 3579 to: *Nature*, 15 East 26 Street, New York, NY 10010. Published in Japan by Macmillan Shuppan K.K., 5th Floor Eikow Building, 1-10-9 Hongo, Bunkyo-ku, Tokyo, Japan. 1984 Macmillan Journals Ltd.

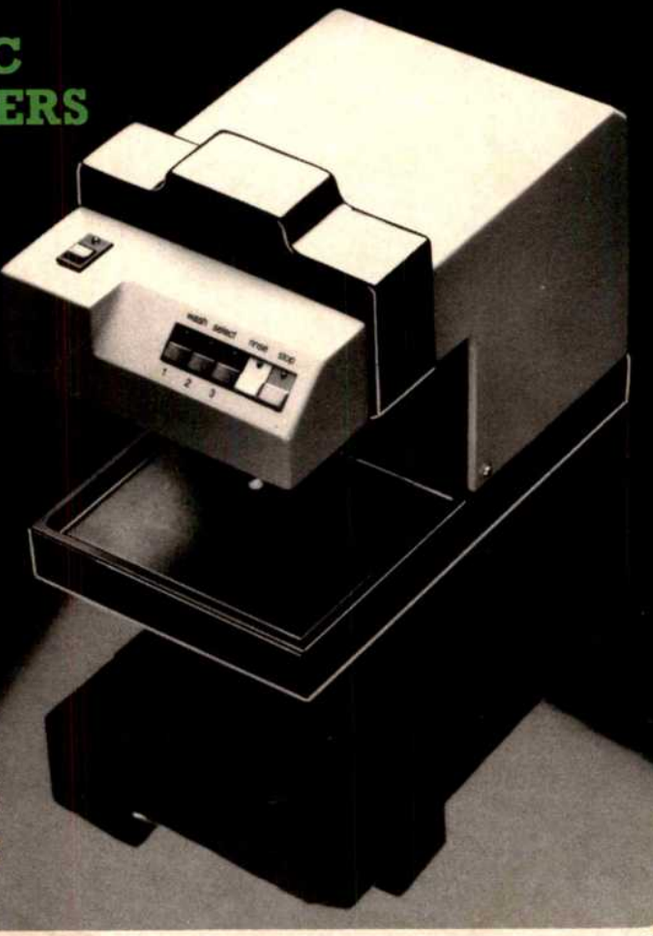
SKATRON MICROFRACTION COLLECTOR



The Skatron microfraction collector was designed to be used with 96 well microplates or macrowells in microplate pattern and very short tube change. This gives also the following features:

- Fractions are automatically numbered
- Easy handling and storage in minimal space
- Improved economy
- Direct measurements by ELISA or RIA
- No sample loss
- Collection of equal sized fractions

SKATRON MICROWASH 96 WELL AUTOMATIC MICROPLATE WASHERS



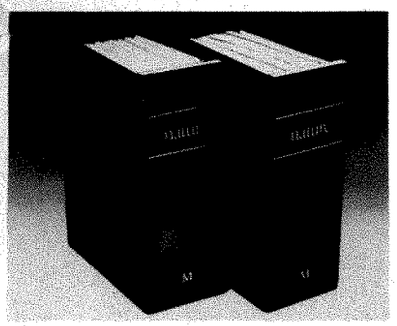
2 models of 96 well washers available. The Microwash will wash both rigid and flexible microplates.
An off centre inlet tube combines turbulence with dilution to maximize washing efficiency, a semi-beveled outlet tube permits thorough aspiration regardless of well shape, and an overflow prevents crosscontamination of wells by wash fluid.

SKATRON
– YOUR RELIABLE PARTNER
WORLDWIDE –

SKATRON AS
DØLASLETTA 1 – TRANBY – P.O.BOX 8 – 3401 LIER
NORWAY – PHONE 473/85 07 70 – TLX: 18625 SKA N

Circle No.22 on Reader Service Card.

Keep your copies of *Nature* protected in these tough black PVC binders



- Each binder is supplied with a set of wires for the easiest possible loading
- *Nature* logo blocked in gold on spine
- Transparent plastic pocket provided for your own reference
- Each binder takes two volumes of *Nature*; three binders will serve for a year
- Prices and address for orders are set out below

Annual Subscription Prices

UK & Irish Republic		£98
USA & Canada		US$230
Australia & NZ	Airspeed	£150
Continental Europe	Airspeed	£120
India	Airspeed	£115
Japan	Airspeed	Y85000
Rest of World*	Surface	£120
Rest of World*	Airmail	£180

**(not USA, Canada, Europe & Japan)*

Orders (with remittance) to:

USA & Canada
Nature
Subscription Dept
PO Box 1018
Manasquan
NJ 08736
USA

UK & Rest of World
Nature
Circulation Dept
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to:
Japan Publications Trading Company Ltd
2-1 Sarugaku-cho 1-chome
Chiyoda-ku, Tokyo, Japan
Tel: 03-292-3755

Personal subscription rates
These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
15 East 26 Street
New York,
NY 10010, USA
Tel: (212) 689-5900

UK & Europe
Felicity Parker, Nature
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

Credit card orders only (in USA):
Call toll-free: (800) 824-7888 (Operator 246)
In California: (800) 852-7777 (Operator 246)

Back issues
UK, £2.50; USA & Canada, US$6.00 (surface), US$9.00 (air); Rest of World, £3.00 (surface), £4.00 (air)

Binders
Single Binders: UK, £4.50; Rest of World, $10.00
Set(s) of 3 Binders; UK, £12.00; Rest of World, $25.00

Annual indexes (1971–1982)
UK, £5.00 each; Rest of World, $10.00

Nature First Issue Facsimile
UK, 75p; Rest of World (surface), $1.50; (air) $2.00

Nature in microform
For Information:
University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

Myelin-associated glycoprotein in human retina
K Stefansson, M L Molnar, L S Martin, G K Molnar, M Mihovilovic, R C Trivedi & D P Richman **548**

Modulation of the response of a photosensitive muscle by β-adrenergic regulation of cyclic AMP levels
L J Rubin & J F Nolte **550**

Complementation in monocyte hybrids revealing genetic heterogeneity in chronic granulomatous disease
M N Hamers, M de Boer, L J Meerhof, R S Weening & D Roos **553**

Amino acid sequence of rat submaxillary tonin reveals similarities to serine proteases
C Lazure, R Leduc, N G Seidah, G Thibault, J Genest & M Chrétien **555**

Computer-based characterization of epidermal growth factor precursor
R F Doolittle, D F Feng & M S Johnson **558**

Erythrocyte receptors for *Mycoplasma pneumoniae* are sialylated oligosaccharides of Ii antigen type
L M Loomes, K-i Uemura, R A Childs, J C Paulson, G N Rogers, P R Scudder, J-C Michalski, E F Hounsell, D Taylor-Robinson & T Feizi **560**

Stability of expression-linked surface antigen gene in *Trypanosoma equiperdum*
G A Buck, S Longacre, A Raibaud, U Hibner, C Giroud, T Baltz, D Baltz & H Eisen **563**

Three-dimensional reconstruction from tilted sections of fish muscle M-band
P K Luther & R A Crowther **566**

Three-dimensional reconstruction from a single oblique section of fish muscle M-band
R A Crowther & P K Luther **569**

MISCELLANY

Books received **575**

Product review: Computers in chromatography
R Patience **577**

NATURE CLASSIFIED

Professional appointments — Research posts — Studentships — Fellowships — Conferences — Courses — Seminars — Symposia: **Back pages**

Next week in *Nature*:

- Sheep-goats
- Oncogene activity in benign tumours
- Acetylcholine receptor cloned
- Connective tissue monoclonal antibodies
- Gap junction switching
- Glycophorin and red blood cytoskeleton
- Seasonal signals in deep oceans
- Acid rain budgets
- Adirondack acid rain
- Pliocene–Pleistocene boundary
- Pulsar in Cygnus X-3?
- I. J. Good reviews Andrew Hodges's biography *Alan Turing: The Enigma*

GUIDE TO AUTHORS

Authors should be aware of the diversity of *Nature*'s readership and should strive to be as widely understood as possible.

Review articles should be accessible to the whole readership. Most are commissioned, but unsolicited reviews are welcome (in which case prior consultation with the office is desirable).

Scientific articles are research reports whose conclusions are of general interest or which represent substantial advances of understanding. The text should not exceed 3,000 words and six displayed items (figures plus tables). The article should include an abstract of about 50 words.

Letters to *Nature* are ordinarily 1,000 words long with no more than four displayed items. The first paragraph (not exceeding 150 words) should say what the letter is about, why the study it reports was undertaken and what the conclusions are.

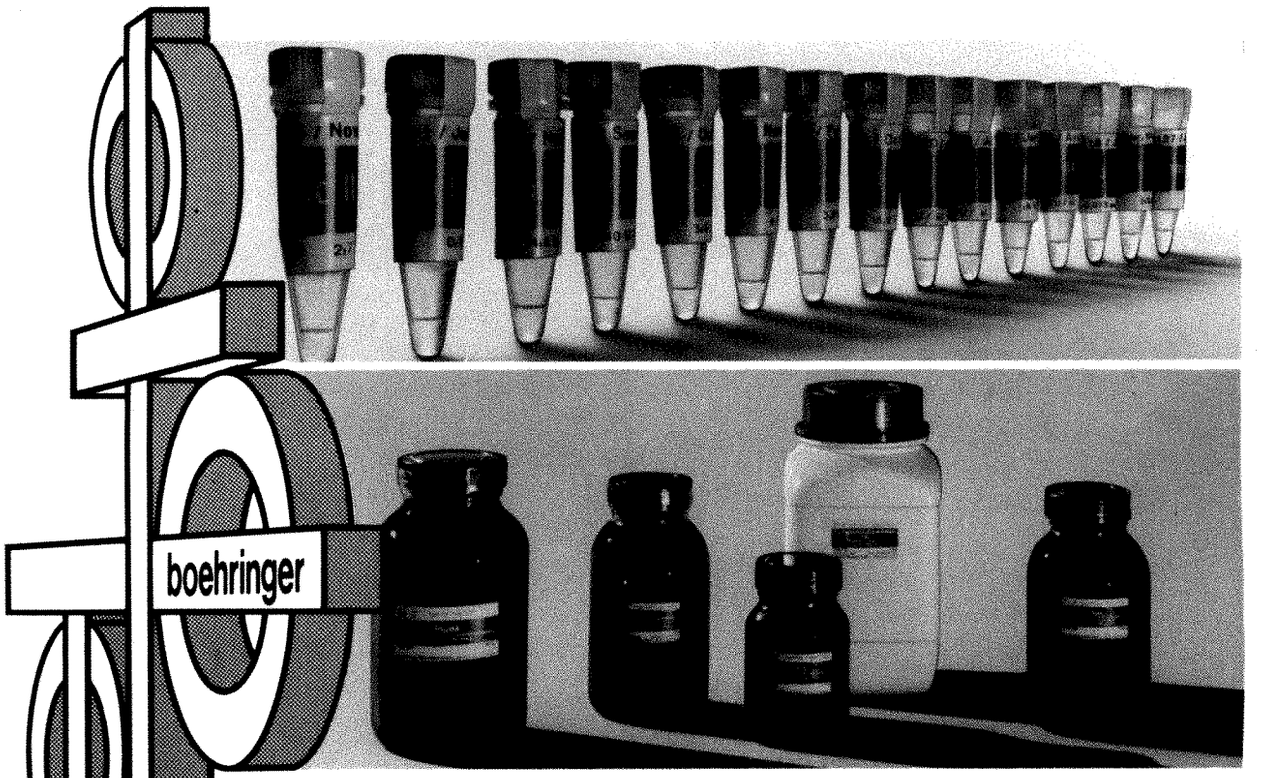
Matters arising are brief comments (up to 500 words) on articles and letters recently published in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted.

Manuscripts may be submitted either to London or Washington (decisions being made only in London). Manuscripts should be typed (double spacing) on one side of the paper only. Three copies are required, each accompanied by copies of lettered artwork. No title should exceed 80 characters in length. Reference lists, figure legends, etc. should be on separate sheets, all of which should be numbered. Abbreviations, symbols, units, etc. should be identified on one copy of the manuscript at their first appearance.

References should appear sequentially indicated by superscripts in the text and should be abbreviated according to the *World List of Scientific Periodicals*, fourth edition (Butterworth 1963–65). The first and last page numbers of each reference should be cited. References to books should clearly indicate the publisher and the date and place of publication. Unpublished articles should not be formally referred to unless accepted or submitted for publication, but may be mentioned in the text.

Each piece of artwork should be clearly marked with the author's name and the figure number. Original artwork should be unlettered. Suggestions for cover illustrations are welcome. Original artwork (and one copy of the manuscript) will be returned when manuscripts cannot be published.

Requests for permission to reproduce material from Nature *should be accompanied by a self-addressed (and, in the case of the United Kingdom and United States, stamped) envelope.*

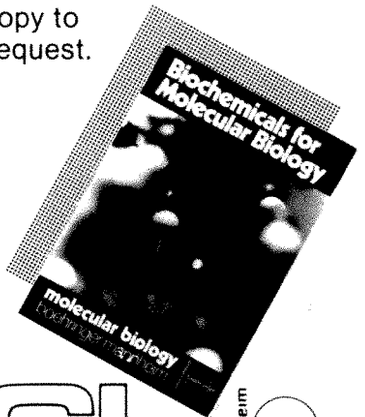


Boehringer Mannheim are extending their range of restriction enzymes and buffers

Aha III, Sau I (MstII) and Stu I are part of the rapidly expanding programme of restriction enzymes from Boehringer Mannheim. Not only is our range *wider* than ever, but each new enzyme is supplied with a comprehensive batch-specific data sheet for optimal control of experimental conditions.

Boehringer Mannheim's range of buffers is expanding too. Each one undergoes the highest standard of purification and quality control to provide you with a reliable yet economical preparation. *Tris*, for example, is notable for its low level of heavy metal contamination, whilst *Hepes* is suitable as a cell culture buffer since it is function tested for cell growth.

A new book *"Biochemicals for Molecular Biology"* presents information on our range of products for the Molecular Biologist. We will send a copy to you on request.



BCL
The Boehringer Corporation (London) Ltd.

mannheim boehringer

Boehringer Mannheim House, Bell Lane, Lewes, East Sussex BN7 1LG. Telephone: Lewes (07916) 71611

177/83/311

Circle No.12 on Reader Service Card.

POCKET SIZE FOR POCKET MONEY



For use in the laboratory, industrial plant or in the field, the Gallenkamp pH stick features:
0 to 14pH, with resolution to 0.01pH • Automatic temperature compensation from 0 to 45°C • Battery powered–typically 2 years life per set • Quick response digital LCD display • Epoxy-bodied gel-filled electrode • Impact resistant splash-proof case with full length electrode sheath • Supplied ready to use with batteries, buffer powders, lanyard and carrying case • £139 excluding VAT • Add one to your next Gallenkamp order–reference number PHK-120-B

Orders accompanied by cheque are welcome; add £23.15 to cover p&p plus VAT (total £162.15)

Gallenkamp

PO Box 290 Technico House Christopher Street London EC2P 2ER
Telephone 01-247 3211 Telex 886041

A member of Fisons plc Scientific Equipment Division

No.13 on Reader Service Card.

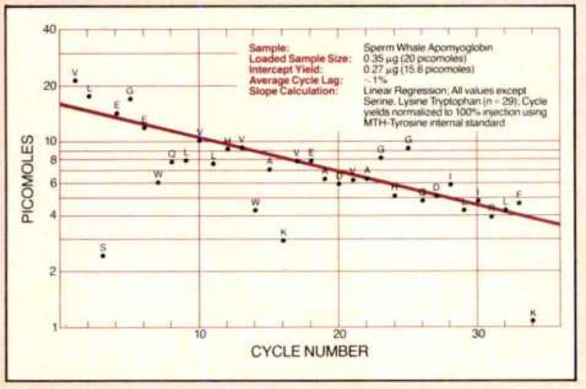
BIOSYSTEMS UPDATE

Gas-Phase Protein Sequencing

A Progress Report

Less than two years ago, Applied Biosystems introduced new gas-phase protein sequencing technology which, though in its infancy, set new standards of analytical performance and sensitivity. We are now pleased to announce the first in a series of improvements in chemistry, programming and hardware which begin to further realize the still untapped potential of the Model 470A Gas-Phase Protein Sequencer.

Changes in the coupling, cleavage and solvent extraction times, and use of an optional miniature sample cartridge, substantially increase repetitive yields and decrease lag on picomole level samples. Sequencer artifacts which can interfere with high sensitivity PTH-AA chromatography are also reduced by a factor of three to five.

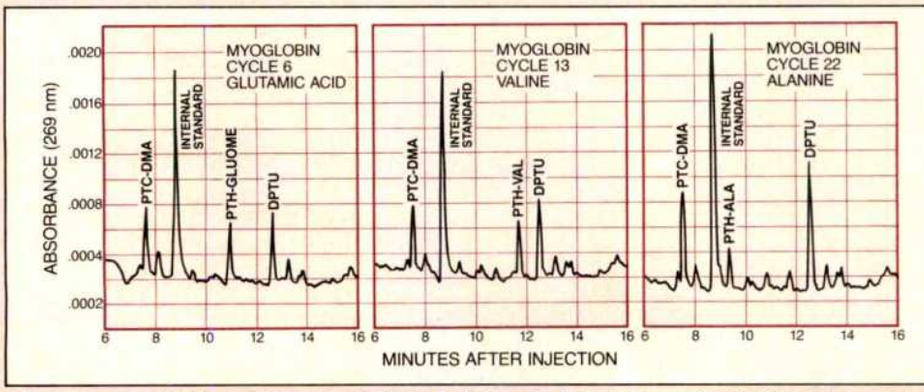


34 residues of 20 picomoles of sperm whale apomyoglobin. Repetitive yield is 96% calculated from the least squares linear regression plot of individual amino acids quantitatively recovered at each sequenced cycle. Lag averages less than 1%/cycle.

The improvements are particularly dramatic with peptides. A user evaluating our new chemistry has reported sequencing the octapeptide angiotensin II to completion with only 25 picomoles of sample.

A new miniature PTH conversion flask increases typical PTH-AA recovery to greater than 95%. The improvement is most significant on serine, threonine and tryptophan since drying times are reduced threefold.

Best of all, these improvements, and others still under development, are designed to be compatible with every instrument we've built so our current users will continue to be at the leading edge of protein sequencing technology. The 470A Gas-Phase Protein Sequencer is capable of setting even higher performance standards and Applied Biosystems, in collaboration with many of our users, is continuing to investigate further improvements to this remarkable instrument. If you'd like more information, please circle number 8.



Actual HPLC chromatograms, without background subtraction or data enhancement, of residues 6, 13 and 22 from sequencing analysis of 20 picomoles of sperm whale apomyoglobin. The data illustrate the low background and high sensitivity of the Model 470A Gas-Phase Protein Sequencer.*

**Chromatography: IBM Cyano Column. Internal Standard: MTH tyrosine. PTH yields normalized to 100% injection: Cycle 6 (glutamic acid)—11.7 pmol, cycle 13 (valine)—9.1 pmol, cycle 22 (alanine) 6.2 pmol. PTC-DMA, phenylthiocarbamyldimethylamine; DPTU, diphenylthiourea.*

Applied Biosystems

APPLIED BIOSYSTEMS, INC., 850 Lincoln Centre Drive, Foster City, CA 94404 • (415) 570-6667 • (800) 874-9868
IN EUROPE: APPLIED BIOSYSTEMS GMBH, Bergstrasse 104, D6102 Pfungstadt, West Germany • 06157-6036



synonym for
high-standard economic
production design

Eupergit®

the carrier for advanced biotechnology

Eupergit®

Research & Development
Industrial large scale processing

the carrier for a wide range of application in bioengineering:

- covalent immobilization
- bio-catalysis
- all other bio-affinity techniques

Advantages

- high quality and quantity product yield
- excellent stability of matrix and bonded ligands
- suitable for most reactors
- easy to handle
- ecologically safe
- non-toxic

Go with Eupergit into production of biopharmaceuticals, e.g.:

- 6-amino-penicillanic acid
- amino acids for parenteral infusions
- optically pure substances

Pharmaceutical grade:

- deoxy nucleotides and nucleosides
- enzymes, enzyme inhibitors, hormones, blood proteins, immuno globulins, antibodies and their fragments
- immune modulators, such as interferons or interleukins

Röhm Pharma
GMBH WEITERSTADT

Postfach 4347
D-6100 Darmstadt
West-Germany

Eupergit®

symbol for advanced biotechnology

Circle No.44 on Reader Service Card.

Please send more detailed information about Eupergit to

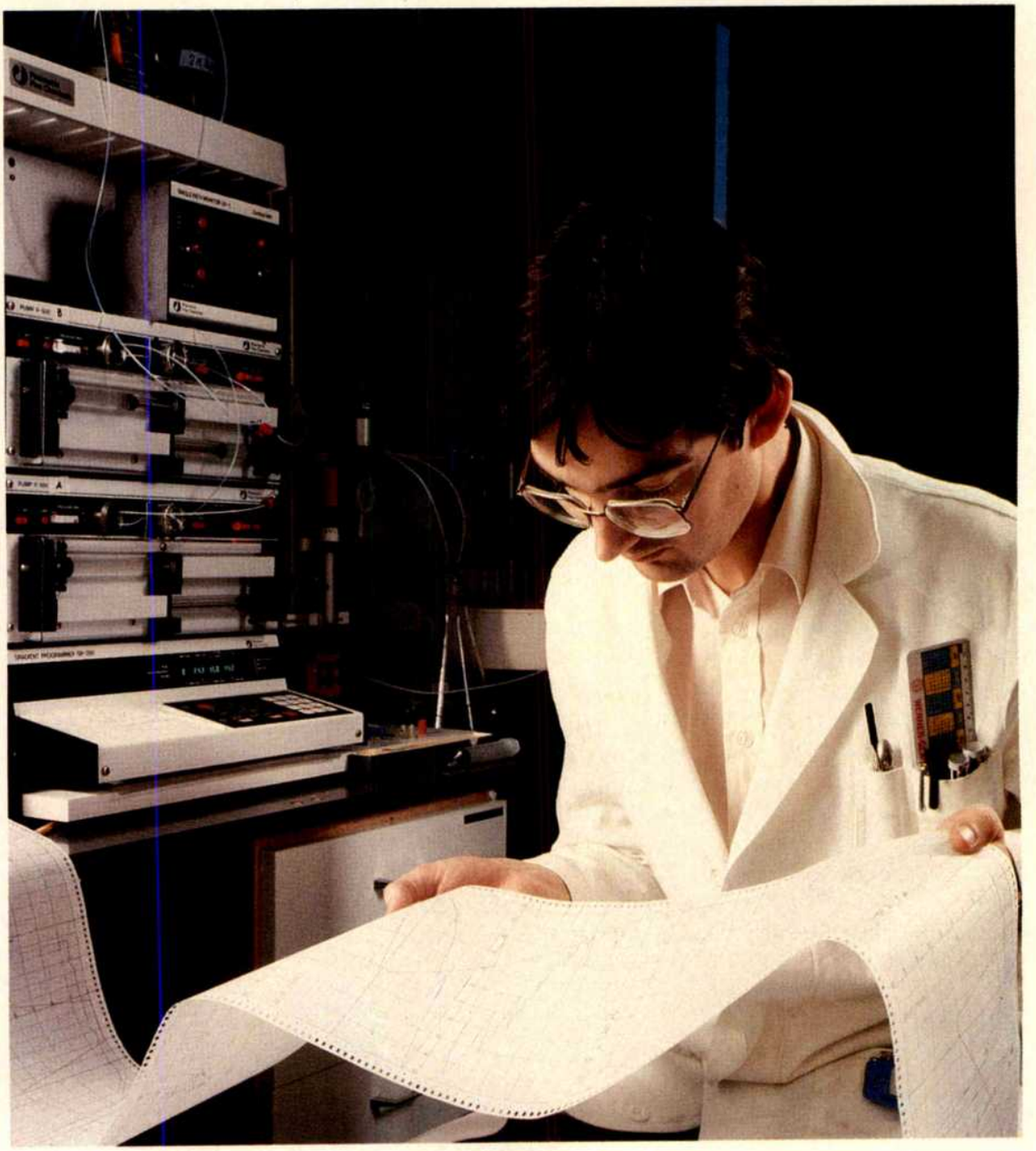
name

street

city

country

FPLC means ...

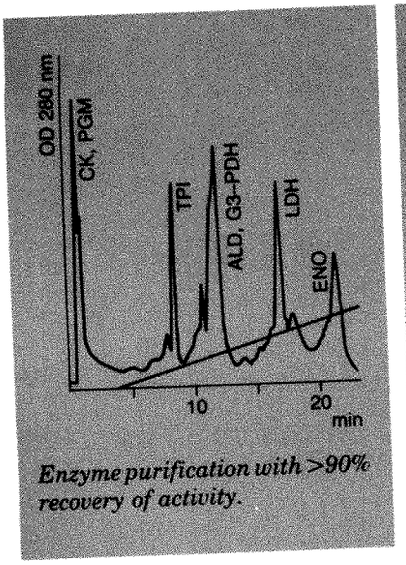


Separation power

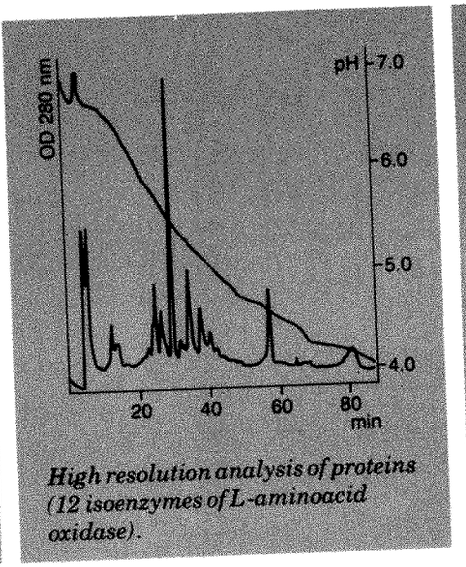
Fast Protein/Peptide/Polynucleotide Liquid Chromatography – but speed is not its only virtue.

FPLC means new unique, hydrophilic, monodisperse media providing high performance resolution and high recovery of biological activity: and new silica based media providing high performance resolution, superior yields of proteins, peptides and polynucleotides – with sample sizes up to 100 mg.

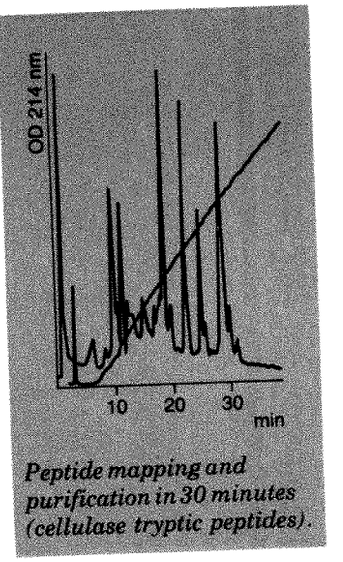
FPLC means new instruments, new media and methods – the combination of which is a high performance liquid chromatography system providing a degree of resolution, capacity and speed previously unattainable for purifying proteins, peptides and polynucleotides.



Enzyme purification with >90% recovery of activity.



High resolution analysis of proteins (12 isoenzymes of L-aminoacid oxidase).



Peptide mapping and purification in 30 minutes (cellulase tryptic peptides).

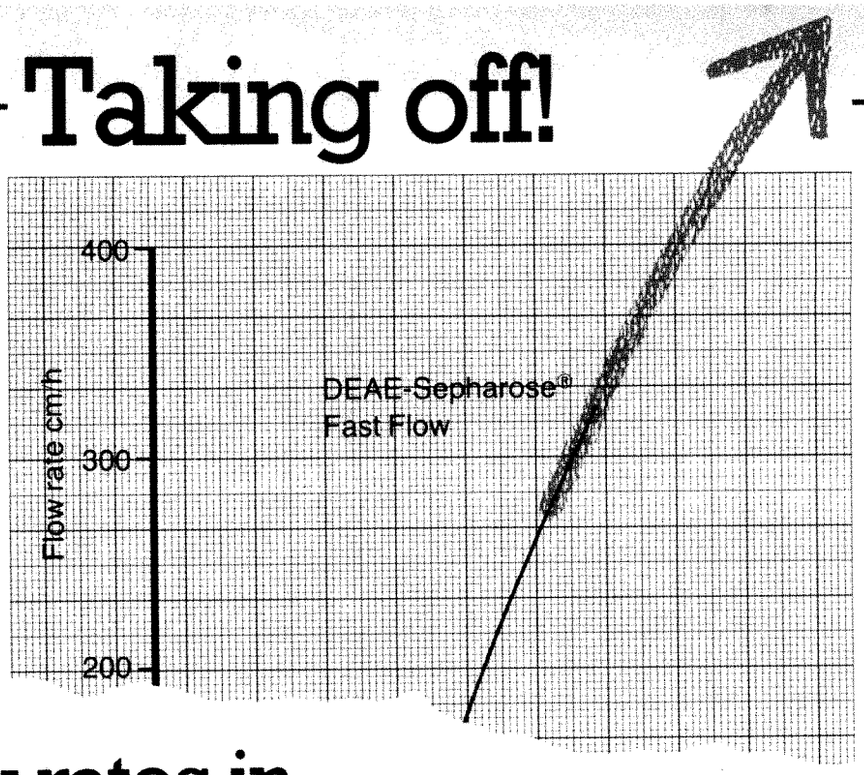
For further information about the separation power of the FPLC System, please contact your local representative of Pharmacia Fine Chemicals.

Pharmacia Fine Chemicals AB
Box 175
S-751 04 Uppsala 1
Sweden

Pharmacia Fine Chemicals

Circle No.52 on Reader Service Card.

Taking off!



Flow rates in large scale chromatography

The flow rates that you can get in laboratory, pilot and production scale ion exchange chromatography are taking off.

The reasons?

- *DEAE-Sepharose Fast Flow*
- *CM-Sepharose Fast Flow*

The results?

- *Faster processing and greater through-put*
- *Easier scale up and simpler operation*
- *Switch from batch to column techniques*

Both of these Fast Flow ion exchangers are new. They're based on the widely-used and well-documented DEAE- and CM-Sepharose CL-6B gels. Optimization of the cross-linking now allows you to use high flow rates at all scales of operation – up to 180 cm/h at pilot and production levels and much much more at the laboratory bench. This means well over 10 bed volumes per hour or up to 15 l/min in Sephamatic® GF 08-015 seventy five litre production columns under normal operating conditions.

Even in difficult processing situations, Fast Flow gels make life easier for you. For example, 500 litres of bacterial cell extract containing a biosynthetic product maintained at 4°C – and that means a fairly viscous solution – can be run through a 16 litre KS 370 Stack column containing DEAE-Sepharose Fast Flow in about 5 hours. (Data from KabiVitrum AB, Stockholm). And if you're currently operating batch ion exchange processes, these gels will probably allow you to change to more convenient and reproducible column techniques.

Scaling up processes becomes easier as well, now that you can use high flow rates all the way from the laboratory, through pilot plant and into production.

Some things haven't changed though. The first class chemical and physical stabilities of the CL-6B gels have been kept. You can re-equilibrate and wash *in situ* and you don't have to worry about fines breaking off and blocking the column. And when the new and old gels were assessed by independent producers in Europe and the United States, they found no differences in the characteristics of the purified products.

Act fast yourself. Order from your usual representative of Pharmacia Fine Chemicals ...

	500 ml	10 litre
DEAE-Sepharose Fast Flow	17-0709-01	17-0709-05
CM-Sepharose Fast Flow	17-0719-01	17-0719-05

... or request further information today.

Pharmacia Fine Chemicals

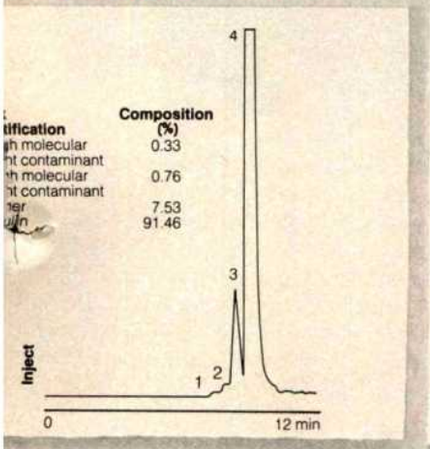
Pharmacia Fine Chemicals AB,
Box 175, S-751 04 Uppsala 1, Sweden

Circle No. 18 on Reader Service Card.

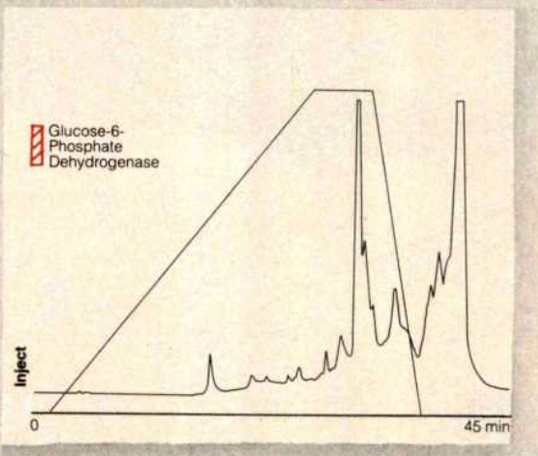
Now isolate and analyze proteins faster, with higher resolution and recovery.

Waters HPLC systems with new ion exchange, gel filtration and hydrophobic packings for biological separations.

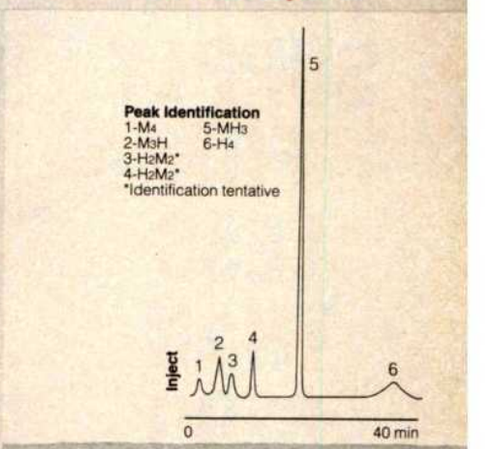
High speed gel filtration separation of insulin.



Total recovery of enzymatic activity from a crude bacterial extract using ion exchange.



High resolution ion exchange separation of isoenzymes.



Separation time: 12 minutes.

Waters new high speed gel ation methods allow rapid tionation of protein samples. s same gel filtration method lets profile sample composition, ate all components and verify purity of collected fractions.

ple: 10 µl Insulin, 5 mg/ml
mn: PROTEIN-PAK 125
nt: 0.1% Trifluoroacetic Acid, pH 2.1
Rate: 1.0 ml/min
ction: 214 nm, 0.5 AUFS

100% recovery of biologically active protein.

The large pore size of Waters polymeric ion exchange packings (1,000 Å) gives you recoveries of enzymatic activity approaching 100% for even high molecular weight enzymes.

Sample: 100 µl Bacterial Extract, 3 mg/ml
Activity: Glucose-6-Phosphate Dehydrogenase
Column: PROTEIN-PAK DEAE 5PW
Gradient Conditions
Eluent A: 0.02M Tris HCl, pH 8.5
Eluent B: 0.02M Tris HCl, pH 8.5 + 0.5M NaCl
0-100% B, curve 6, 25 min
Flow Rate: 0.5 ml/min
Detection: 280 nm, 0.1 AUFS
Specific Activity Increase: 4.9
Activity Recovery: 100%

Complete resolution of LDH isoenzymes.

The resolving power of Waters high performance ion exchange packings provides resolution of the closely related isoenzymes of lactate dehydrogenase.

Sample: 100 µl LDH Isoenzymes, 1 mg/ml
Column: PROTEIN-PAK DEAE 5PW
Gradient Conditions
Eluent A: 0.02M Tris Acetate, pH 8.0
Eluent B: 0.02M Tris Acetate, pH 8.0
+ 1.0M NaOAc
0-100% B, curve 6, 45 min
Flow Rate: 0.5 ml/min
Detection: 280 nm, 0.05 AUFS

Add the performance of Waters HPLC to your separations. And achieve greater success in protein isolation and analysis.

For a demonstration or additional information, contact your local Waters representative or call 617-478-2000 (X 2665).

Waters

MILLIPORE

Waters The Liquid Chromatography People®
34 Maple St./Milford, MA 01757/(617)478-2000

For a demonstration circle No. 27 on Reader Service Card
For further information circle No. 28 on Reader Service Card

NEW

AN E-C POWER SUPPLY FOR LESS THAN $200



The new $198 EC103 Minicell™ power supply is ideally suited for less-demanding electrophoretic separations. Miniaturized within EC's strict design and safety standards, the EC103 is only 8" L x 5" W x 4.5" H and weighs a mere 5 lbs. Digital display for voltage or current. Constant voltage output from 20 to 250 volts in 20 steps. Current output: 0-100 mA. Dual output jacks. Input: 115-230V; 50-60Hz. Only $198 f.o.b. St. Petersburg, FL. Precision at a truly affordable price. E-C Apparatus Corp., 3831 Tyrone Blvd. N., St. Petersburg, FL 33709.

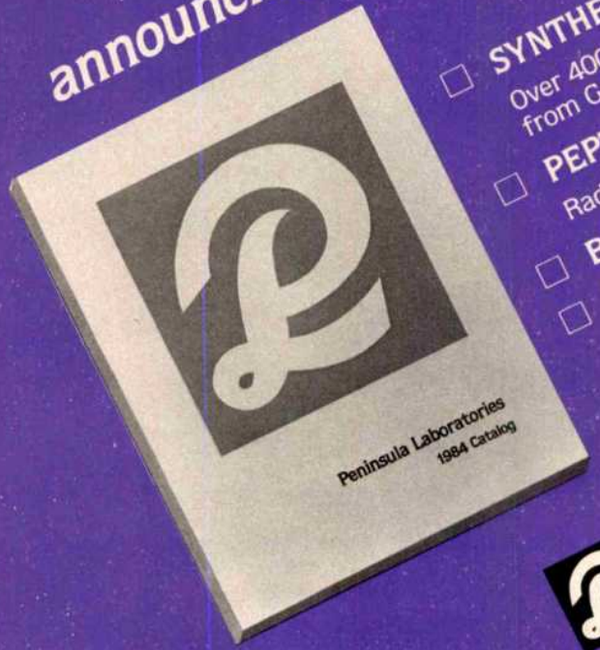
E-C

INFORMATION? *CALL TECHNICAL SERVICE COLLECT AT 813-344-1644*

Circle No.06 on Reader Service Card.

---

announcing...



- ☐ **SYNTHETIC PEPTIDES** — Over 400 biologically active peptides from Gastro-Intestinal to Neuro-Peptides
- ☐ **PEPTIDE ANTISERA** — Radioimmunoassay and Immunofluorescence Kits
- ☐ **BULK ANTISERA**
- ☐ **NUCLEOTIDE PRODUCTS** — Phosphoramidites, Phosphotriesters and protected Nucleosides
- ☐ **CUSTOM SYNTHESIS**

Call or write for our 1984 catalog:

Peninsula Laboratories, Inc.

611 Taylor Way, Belmont, California 94002
(415) 592-5392 Telex 172511 (PEN LABS BLMT)
Out of State Toll-free: (800) 922-1516

Circle No.14 on Reader Service Card.

Monoclonal Anti-Human Leukocyte Reagents

Are you differentiating cell types—myeloid, CALLA, lymphoid (B- or T-cell subsets)? If so, consider our new and thoroughly characterized reagents for building your panels.

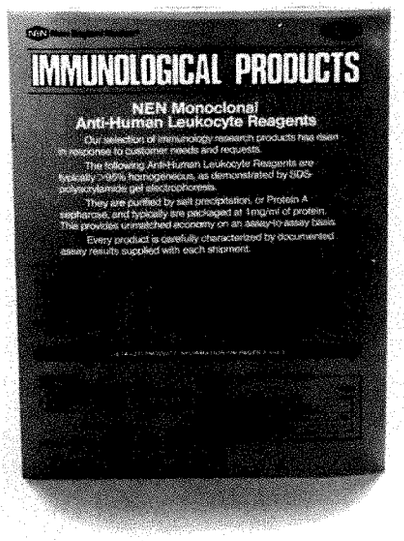
Our monoclonal anti-human leukocyte reagents are >95 percent homogeneous, as demonstrated by SDS-polyacrylamide gel electrophoresis. They are purified by salt precipitation, or on protein A sepharose, and are packaged at a protein concentration of 1 mg/ml. This provides unmatched economy on an assay-to-assay basis.

Equally important, every reagent is carefully characterized by lot-specific assay results supplied with each shipment.

Don't be without our catalog of reagents for immunological studies. In it you will find anti-human leukocyte reagents, as well as:

- Monoclonal antibodies specific for mouse immunoglobulin studies
- Allele-specific monoclonal anti-mouse lymphocyte reagents
- Enzymes and fluorescent-labeled conjugates of both monoclonal and polyclonal antibodies
- Hybridoma screening systems for murine immunoglobulins

Simply circle the inquiry number to receive the catalog without charge. We'll also keep you informed of future products as they're developed.



Not for use in humans or clinical diagnosis

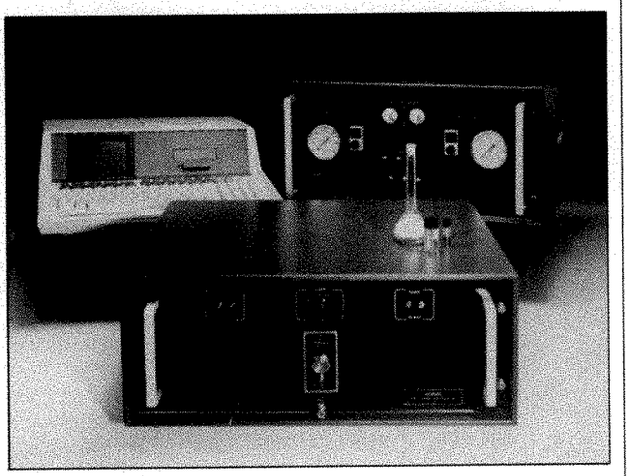
New England Nuclear
549 Albany Street, Boston, MA 02118
Call toll free: 800-225-1572, Telex: 94-0996
Mass. and Internat'l: 617-482-9595
Europe: NEN Chemicals GmbH D-6072, Dreieich, W. Germany
Postfach 401240, Tel. (06103) 803-0, Telex 4-17993 NEN D
NEN Canada: 2453 46th Avenue, Lachine, Que H8T 3C9
Tel. 514-636-4971, Telex 05-821808 ©1983 NEN

NEN New England Nuclear®

DUPONT

Circle No.29 on Reader Service Card.

RADIOACTIVITY DETECTION SYSTEMS FOR H.P.L.C.



A SYSTEM SPECIFICALLY DESIGNED FOR THE HOMOGENEOUS AND HETEROGENEOUS DETECTION OF BETA EMITTERS (^{14}C, ^{3}H, ^{35}S, ^{32}P) UNDER H.P.L.C. CONDITIONS.

Features include microprocessor-controlled signal-averaging, ultra fast coincidence circuitry and IEEE-488 interface. A versatile HP 85- based data system for data aquisition, storage and peak integration. The Precision Splitter/Mixer provides a sophisticated post-column accessory for homogeneous detection.

REEVE ANALYTICAL LTD

REEVE ANALYTICAL

73 CHAPEL ST.
GLASGOW, G20 9BD
TEL: 041-946 6303

Circle No.02 on Reader Service Card.

EDITORIAL OFFICES

London Office
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024

Washington Office
1134 National Press Building, DC 20045
Telephone: (202) 737-2355

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff: Alun Anderson, Tim Beardsley, Philip Campbell, Isobel Collins, Peter Gambles, Melanie Kee, Tim Lincoln, Naomi Molson, Sara Nash, Geoffrey North, Peta Pickering, Miranda Robertson, Robert Walgate, Charles Wenz, Nigel Williams, Guil Winchester

Washington Editor: Peter David
Washington Correspondent: Stephen Budiansky

Art and Design: Joan Godwin, Joel Chernin, Theresa George, Paul Norris, Marie Xeridat

Administration: Mary Sheehan, Jacqueline Cole, Ruth Cutts, Joanna Czechowska, Ruth Goldenberg, Sharon Isaacs and Penny Walker (Washington)

Publisher: Elizabeth Hughes

Marketing Director: Ray Barker

Promotion Manager: Felicity Parker

International Advertising Manager: Andy Sutherland
Features Advertising Manager: Marion Delaney
Group Classified Advertising Manager: John Barnes — Telephone: (01) 240 1101

Nature New York Office
15 East 26 Street, New York, NY 10010
Telephone: (212) 689-5900 Telex: 668497

General Manager: Gary Rekstad

American Advertising Manager: Henry Dale

Classified Advertising Manager: Miss Gene Fein

Circulation Manager: Mirta Soto

Canadian Display and Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1—(416) 690-2423

Display Advertising Representatives

New York: Nature Office
Chicago: Didier & Broderick, Inc.—(312) 498-4520
San Francisco: Jobson/Jordan/Harrison/Schulz, Inc.—(415) 392-6794
Los Angeles & Pasadena: Jobson/Jordan/Harrison/Schulz, Inc.—(213) 796-9200
Philadelphia: Daniel Adams Associates, Inc.—(215) 353-6191
Boston: CEL Associates—(617) 848-9306
Fort Lauderdale: Brinker & Brinker—(305) 771-0064
New Jersey: Mary Grunmeier—(201) 572 9018
Dalton, Pennsylvania: Donald E. Northup Associates—(717) 563 2572
West Germany and Austria: Franz Schrecklinger TMW Top Media Werbegesellschaft GmbH Frankfurt—(611) 726046
Holland and Belgium: Adele Struyck G. Arnold Teesing BV Amsterdam—(020) 2636 15
Switzerland: Werner Stähli Agentur Iff AG Schaffhausen—(053) 45821
Scandinavia: Andrew Karnig Andrew Karnig & Associates AB Stockholm—(08) 51 68 70
Japan: Mashy Yoshikawa Orient Echo Inc Tokyo—541-4923
Australia: Gus Bartel & Associates PO Box 1755, Southport, Queensland 4215 (075) 466 577

Nature ISSN 0028-0836
Registered as a newspaper at the British Post Office

© 1984 Macmillan Journals Ltd
Vol. 307 No. 5951 9 February 1984

nature

Reagan votes for basic research

The budget deals generously with basic science; the snags are that the election will tempt Congress to deny the President what he wants and that the strategy of budget deficits may blow up in his face.

PRESIDENT Ronald Reagan's pre-election budget is what his pollsters had been expecting and will not surprise even his would-be opponents next November. Between now and then, politicians and voters alike will be less concerned with what is called the bottom line — the huge deficit yet again in prospect — than with the details of spending plans for the financial year that will have just begun by election day. In the circumstances, it is commendable that the new budget is less like an electioneering document than it might have been. Moreover, other things being equal as the economists say, the budget could do much to strengthen basic research in the United States. So the important question is whether Congress will let good things come about. And while there is no reason to suspect that Congress has its knife into basic science, the danger is high that in the run-up to the election, large chunks of the budget will be so encumbered with spending plans the administration cannot accept that the business of the United States will be managed by continuing resolutions until after the votes have been counted. Those planning to order new equipment on the strength of last week's budget will prudently contain themselves in patience.

Of all the components of the budget, the most welcome because the most overdue is the proposal that federal support for agricultural research should be put on a more rational basis. The plan is that, from next October, the US Department of Agriculture should have $50 million to spend on research grants awarded competitively, twice as much again as in the year now under way. This shift of resources, in magnitude only modest, will be significant if it can be taken as a commitment by the administration to the principle that agricultural research in the United States should be reformed.

Opposition

Handing out large parcels of public money to the land-grant colleges was a powerful stimulus to self-conscious agriculture at the end of the nineteenth century, while the Department of Agriculture's own research establishments were fruitful sources of basic science as recently as half a century ago. But times have changed. It is now comically anomalous that agriculture, the most important single industry in the United States, should uniquely be deprived of more than token access to the groups of imaginative people in US universities willing and able to break a lance or two in the interests of the agriculture of the future — and without demanding a public service contract and pension in return. None of this is to pretend that competition for research grants, and the peer reviews that attend them, are free from blemish or to deny the value of what industrial companies are now spending — perhaps $100 million a year — on basic research in agriculture. But it will be a test of the administration's seriousness towards research that it should be prepared to battle for the modest proposal now put forward in the face of the entrenched opposition there will be in Congress.

The next most welcome development is the proposed generosity towards the National Science Foundation, still no better endowed in real terms than in the early 1970s. Again it makes sense that the only agency of government able to support projects not tied to what are called missions (public health included) should have more funds at its disposal when executive agencies and private industry are both busily spending more in the universities, helping to shape the pattern of research in the image that suits them best. Because the foundation's spending bears more directly on the quality of technical education, in secondary schools as well as in universities, the promised increase cannot fail to be a sound investment. The fact that they may materialize is a feather in the cap of Dr George ("Jay") Keyworth, director of the Office of Science and Technology Policy at the White House. So too is the decision not to leave to Congress the choice of a number to represent the budget of the National Institutes of Health, for there will now be less scope for the dreaded Waxmans to monkey with the constitution of the institutes.

Disappointments

For the rest, where research support is concerned, the new budget is the predicted mixture of small disappointments. The National Aeronautics and Space Administration will continue to gather information about the Solar System more quickly than it can afford to have the data analysed and digested. There will, indeed, be $150 million to be spent next year on the early design of a space station whose purpose has not yet been decided. There seems to be nothing in the budget to offset the decline of modest overseas collaboration threatened by the proposed withdrawal from the United Nations Educational, Scientific and Cultural Organization (Unesco). Star wars, or preparations for them, are to flourish. The fast breeder reactor has finally been abandoned but for the wrong reason; the Department of Energy should have recognized years ago that the Clinich River design is technically obsolete, but should this year have made provision for the "crash programme" of fast reactor development for which Congress will be crying when it gets to hear of the European plan for cooperation in this field.

These complaints are only minor minuses compared with the outstanding weakness of the federal budget for 1985 — the administration's plan to finance its operations during the coming year by the device on which it has relied since it came to office, the trick of paying its bills with borrowed money. The sums involved are vast, in round numbers $200 million a year or two-thirds of the enlarged defence budget that Congress is being asked to approve. The federal government's borrowing amounts to 6 per cent of the gross national product of the United States, which is another way of saying that the government used the whole of last year's growth to cover the gap between what it spent and what it raised in taxes. The debt that has accumulated after nearly a decade of this spendthrift way of running a government has risen so that annual interest payments now exceed 2 per cent of the gross national product, and are reaching the point at which no Secretary of the Treasury can hope to escape from them. The borrowed funds come from two sources, domestic savers (including corporations) in the United States who lend to the government funds that would otherwise be invested in productive industry, and investors overseas who find it profitable to put money into US financial securities while interest rates remain high and who have so far also thought it safe. As the months go by, they increasingly ask themselves whether it is wise to have lent so much on the chance that the federal government will one day be in balance again.

The symptoms of this imbalance are plain, at least from the outside. Because interest rates must be kept high to attract borrowers, the dollar is strong compared with other currencies, US

0028-0836/84/060489-01$01.00

© 1984 Macmillan Journals Ltd

manufacturers are less able to sell their products on world markets and the United States must run a huge deficit on its trade balance — $40,000 million last year, probably more in 1984. Even though the flexibility of the US economy has in the past year allowed for growth (by no less than 6 per cent) without accelerating inflation, even President Reagan agrees that this state of affairs cannot continue much longer. His offer last week to negotiate with Congress some way of moving towards a balance (by 1989) is unlikely to bear fruit in the run-up to the election. The question that will increasingly keep people awake at night is whether before then the house of cards will collapse, perhaps because overseas investors in the United States take fright, perhaps because still higher interest rates bring the economic recovery to a stop.

On the face of things, these arcane considerations may seem to have no bearing on the provisions in last week's budget for the support of basic science. But they are crucial. In due course, the federal government will have to balance its books more closely by increasing taxes or by spending less. Either way, the flickering economic revival will be impeded. If spending cuts are the chosen method, who can be sure that the generosity of this year's budget towards basic science can be maintained? If higher taxes bear the brunt, who can be sure that the funds now channelled from industry towards basic science will continue to flow. And if the administration, this or the next, chooses to fudge the issue by allowing inflation to run riot again, who will be safe? □

Time to move Greenwich

On the centenary of the choice of the zero of longitude, other places for it deserve a hearing.

IN the week in which Britain, once an imperial power on whose vast possessions, it was said, the Sun never set, has suffered the humiliation of being thoroughly beaten by upstart New Zealand at the national sport of cricket (like baseball, played with pieces of wood and bruising balls, but unlike baseball in that games can last for five days), it is natural that there should have been nostalgic celebration of the centenary of the choice of the meridian through Greenwich as the commonly agreed zero of longitude. Here at least, wishful argument seems to go, is proof that there was a time when the centrality of the British place in the world was generally acknowledged. But even that may be a slightly hollow comfort.

The truth is that Greenwich is far from being the most convenient place through which to trace the zero of longitude. In Western Europe, one of the most densely populated regions of the world, several countries (Britain, France, Spain and so on) find themselves straddling the meridian, unable to decide whether they belong to the Eastern or the Western Hemisphere. Plainly a better choice would have been to place the zero of longitude 10 or even 20 degrees west of where it is at present; the first choice would have left only New Zealand uncertain where it is, the second would have bisected the surface of the Earth in such a way that no country of importance was thus embarrassed. Astronomers, after all, are usually reasonably clever people, and would have been able to assimilate the notion that the zero of longitude should be in the mid-Atlantic just as astronomers in Potsdam and Pulkova have had to learn that they have been well to the east of it.

It is also important, at this centenary, modestly to acknowledge that the conference in Washington in 1884 that settled on Greenwich (with one dissenting vote) let slip a splendid opportunity to put on a more reasonable footing the measurement of angular displacement. The sad truth is that, from sheer indolence and inertia, generation after generation of people who acknowledge the good sense of measuring most quantities on a decimal scale have indolently allowed themselves to continue measuring angles in an arithmetic whose radix, for practical purposes, is 60. By doing so, they have lazily adopted a system variously attributed to the Babylonians and to the ancient cultures of India. It is true, of course, that changing to a system in which, say, radians would be the equivalent of 100 degrees would have been a considerable undertaking, and that it would have been necessary also to decimalize time. But that is no excuse for having done nothing. □

How not to test drugs

British and French anxiety about human volunteers in drug trials should be settled quickly.

THE British pharmaceutical industry, long used to the cultivation of an air of injured innocence in the face of complaints from central government about the prices of drugs sold to the public health services and from sections of the general public that its products are unsafe, has every reason to be aggrieved that some of its arrangements for testing a new drug in volunteers should have called in question the whole basis on which the toxicity of new compounds is assessed (see page 495). The only immediate comfort is that a similar issue has, by coincidence, arisen in France.

The circumstances in which this row has arisen are not nearly as complicated as they seem. In Britain as elsewhere, clinical trials to assess the efficacy and side effects in patients of a potential drug require formal approval from the regulatory body, the Department of Health acting on the advice of the Committee on the Safety of Medicines. But manufacturers, required by established procedures to provide evidence of the toxicity of a new drug by means of tests with animals, also have an incentive to provide evidence that the new material causes no obvious harm to people. The tradition that a person responsible for developing a new material will try it out on himself and his close colleagues (never satisfactory) has in the past few years become outdated as the need has grown for more and more systematic tests in human beings as part of the process of licensing a drug for clinical trials. The result is the practice of delegating testing in volunteers to commercial companies.

Except to those who hold or seem to hold that new medicines as such are an abomination, there is nothing wrong with these procedures, at least when they are followed sensibly. What has gone wrong with the study of a putative tumour inhibitor is that the good sense of the proposal has not been assessed by people at once knowledgeable enough to make an informed judgement, independent in the sense that no benefit accrues to them and publicly identifiable. A commercial company's internal ethical committee whose identity is not disclosed will not suffice when volunteers are recruited publicly, and invites complaints that participants have been less than fully informed of the risks they run. The failure of the company concerned to inform the volunteers' own physicians of what was afoot is another needless omission. But it is sheer humbug that many who should know better have been complaining that by offering participants a cash reward of £250, those mounting the study have been offering inducements great enough to sway a person's judgement of his own long-term interests. It could just as well be argued that a participant without the wit to demand a fee of this order is unlikely to be fit to give informed consent.

The danger now is that the Department of Health will panic at what appears a little like the discovery of a loophole in the arrangements for the regulation of drug testing, introducing another layer of paperwork with which to belabour the drug industry and, worse still, to impede the development of new medicines. The need, however, is far simpler — a publicly agreed set of guidelines by which all drug testers can be bound. The rules should be easily devised. The socially disadvantaged, especially prisoners and old people, should not be allowed to volunteer for drug tests unless, as the French ministry of health suggests, there is reason to think their health may benefit. People who are not sufficiently alert to give informed consent should similarly be excluded. Consent for all drug studies should be given by properly constituted ethics committees, whose names and credentials are known and whose opinions will be made publicly available. Nobody should complain if people or even university departments occasionally make modest sums of money out of these procedures. Now and again, ministers of health should summon up their courage and make public speeches pointing out the need for such activities, now too easily and too often counted as dispensable. □

0028-0836/84/060490-01$01.00

© 1984 Macmillan Journals Ltd

US budget

Military and basic science offered more rapid growth

Washington

PRESIDENT Reagan released his proposal for the 1985 federal budget last week, and left no doubts about the direction of his research and development policy: massive increases on the military side. The President proposed a record $33,852 million for military research and development, an increase of 22 per cent over current spending. And while total civilian research and development spending would be held almost constant under the President's plan, its basic research components would increase by 10 per cent overall, with particularly generous treatment for the National Science Foundation (NSF), the National Aeronautics and Space Administration (NASA) and the Department of Energy.

The new money for basic research is to come largely from deep cuts in applied research and — that particular bugaboo of the Reagan Administration's free-market philosophy — the engineering demonstration project. Energy projects, notably solar and fossil, but also (against the administration's will) the Clinch River fast breeder reactor, are the chief victims.

Basic research

Dr George Keyworth, the President's science adviser, has been taking pains to cast this budget as staunchly pro-science. For the first time, basic research is to receive a larger share of total non-military research appropriations than either applied research or development. Keyworth points with pride, too, to new funds for university instrumentation and training and to the fact that basic research support to universities has grown steadily during Reagan's term, reaching nearly $4,000 million in this year's proposal, an increase of more than 25 per cent in real terms from the level where it had stagnated under the previous administration.

Last week also provided some apparently good news about industry's contribution to US research and development. According to a forecast released by the Battelle Memorial Institute, industrial support is expected to grow by 10 per cent in 1984 to a point where it makes up slightly over half of all US research and development expenditure, public and private.

The emphasis on basic academic research in the President's budget may well stifle complaints that the real increases have been reserved for military projects. If the Reagan budget is adopted, military research and development will have more than doubled (in nominal dollars) in just four years. Most of the military spending supports the new weapons systems to which the Reagan Administration has committed itself, including the Trident II and MX missiles and the B-1 bomber. A significant share of the new funds, however — roughly $250 million — is earmarked for the ballistic missile defence programme (also known as Strategic Defense Initiative or star wars).

Science foundation

On the civilian side, NSF is once again in line to be the big winner, with a 14.6 per cent increase in its budget to come on top of last year's 17 per cent increase. Mathematical and physical sciences and engineering would receive the lion's share of the new money, which includes $10 million for the establishment of "Centers for Cross-Disciplinary Research in Engineering" to focus university research on problems faced by industry and to train graduate and undergraduate engineering students.

The NSF budget also includes a 22 per cent increase in support for scientific instrumentation — a total of $237 million, $122 million of which will be available under research grants to individual researchers with the remainder going to support university-based and national centres. Support for graduate students under research grants will increase by 15 per cent, allowing an additional 1,050 students to participate. And a new half-million-dollar experimental programme will provide women scientists with an opportunity to initiate research projects.

NSF has received the go-ahead to begin construction of the Very Long Baseline Array (VLBA), a network of radio telescopes across the United States. VLBA is expected to offer 100 times greater resolution than any existing radiotelescope.

A modest effort in supercomputing — far short of what advocates had hoped for — is also included in NSF's proposed budget. No new hardware will be purchased, but $20 million is to be made available to pay for access to existing supercomputers and to cover local user costs, such as terminals, software, networks and communication time. In a separate budget item, NSF plans to acquire a new supercomputer for the National Center for Atmospheric Research at Boulder, Colorado.

Energy

The Reagan philosophy is most evident in the proposed budget for the Department of Energy. While overall spending on research and development is to grow by only 1 per cent, basic research is to receive an 18 per cent increase.

Funds will be provided to start construction of the electron accelerator designed by the Southeastern Universities Research Association, which beat Argonne National Laboratory last year in a bitter political fight. Brookhaven National Laboratory, which last year lost another bitter fight to keep its illfated ISABELLE accelerator alive, is to receive its expected consolation prize of $15 million for a tunnel that will link its existing van de Graaf accelerators and synchrotron, creating a new heavy-ion facility. A plan to incorporate the ISABELLE ring to create a massive relativistic heavy-ion machine is apparently still several years away.

The energy budget also provides support for initial studies of the Superconducting Super Collider concept in order to establish its feasibility and then to produce a specific design in several years.

Keyworth's pet project at Lawrence Berkeley Laboratory, the Center for Advanced Materials, has survived a year of criticism and, having lost its synchrotron advanced light source and the prefix "National", is to receive $11 million with which to continue construction.

This year, the administration has taken a ▶

President Reagan's 1985 budget for research and development, fiscal years (1 October–30 September) 1984 and 1985

	1984		1985
	Reagan request	As passed	Reagan request
NSF	1,240	1,239	1,408
NIH	3,842	4,240	4,342
NASA	2,473	3,257	3,341
Defence-related	29,882	27,636	33,852
Energy	4,713	4,844	4,885
Agriculture	849	872	898
EPA	208	250	281
All others	2,589	2,941	2,769
Research and development facilities	1,195	1,439	1,306
Total	46,991	46,718	53,082

Source: Office of Management and Budget. All figures in millions of dollars.

0028-0836/84/060491-01$01.00

© 1984 Macmillan Journals Ltd

different approach to its perennial battle with Congress over the National Institutes of Health (NIH).

In past years, the tactic has been to ask for no increase for NIH in the knowledge that Congress in its rapture with that most popular of scientific agencies will add too much anyway. The administration has become increasingly concerned, however, that Congress's enthusiasm tends to be channelled into advocacy for narrow, disease-specific research, a tendency that threatens NIH's traditional freedom to support valuable but less glamorous basic research. "The 'disease-of-the-month club' syndrome is no longer a joke", Keyworth said recently.

This year, the administration is proposing to give NIH an additional $102 million, but specifically for areas of basic research that it believes have been neglected, such as neurobiology. Keyworth said he hopes that this proposal will "open a dialogue" with Congress and will give the administration a chance to air its views on the importance of basic research in the life sciences — particularly its role in underpinning US competitiveness in biotechnology.

The Environmental Protection Agency, which in past years has felt the full weight of the administration's budget-cutting, is in for a modest increase this year in research funding. The news may not cheer the environmental lobby, however, as much of the new money will go for further studies of acid rain at a time when many — including a panel that Keyworth himself assembled — have concluded that enough studies have already been done to support regulatory action, a step at which Reagan still baulks.

The National Oceanic and Atmospheric Administration (NOAA), the National Bureau of Standards (NBS) and the US Geological Survey are all in for major cuts in support for research and development on the principle that much of their research belongs in the private sector.

Particularly hard hit is NOAA, which is to lose a full one-third of its $242 million research and development budget. And NBS's fire and building research centres are once again under threat of closure.

Stephen Budiansky

Agricultural research

Towards grant competition

Washington

THE US agricultural system, which has been repeatedly criticized for fostering mediocrity and neglecting basic science, is in for a major shake-up if the Reagan Administration has its way. The 1985 budget proposal would triple the size of the small programme of competitive grants for agricultural research, bringing it up to $50 million, the maximum level currently authorized by law. The bulk of the new money would be reserved for basic research related to biotechnology.

Under the administration's proposal, most of the federal funds for sponsored research in agriculture would continue to be distributed to the country's land-grant colleges according to a state-by-state formula not involving peer review. But by rapidly expanding the competitive grants programme beyond its token level of $17 million, the administration hopes to effect a major change in the way agricultural research is carried out. The expanded programme is expected to attract researchers at non-land-grant universities (among them the leading US basic research centres, such as Harvard and Stanford Universities) which are shut out from the formula-fund system. It may also bring some scientific rigour, by way of peer review, to a system often accused of drifting more and more to applied research of dubious scientific merit.

The new money, £33 million, is to come in part from an overall increase in the research budget of the Department of Agriculture (USDA) and in part from the phasing-out of the so-called "special grants", funds earmarked, usually by Congress, for specific projects.

Congress has baulked at past efforts to expand the competitive grants programme, which the smaller land-grant colleges in particular see as a threat to their assured support from the formula funds. This time things may be different. Many of the larger and scientifically more competitive land-grant institutions have broken ranks and have declared themselves in favour of an enlarged competitive grants programme. And both Secretary of Agriculture John Block and presidential science adviser George Keyworth have taken a personal interest in the programme.

Besides the new effort in biotechnology (which would receive $28.5 million), the administration's proposal would establish a small effort in animal sciences ($4.5 million); existing components in plant sciences ($15 million) and human nutrition ($2 million) would continue. Approximately one-quarter of the competitive research funds would be expected to support graduate research assistants working under project grants.

The National Science Foundation's plant biology programme, which has traditionally taken up the slack in basic plant research, is also due for an increase under the President's budget — a 16 per cent rise, to $58 million.

The USDA formula funding would grow by 2 per cent next year; and Department of Agriculture's own in-house research arm, the Agricultural Research Service, would receive a 3 per cent increase. Beltsville is not to be frozen out.

Stephen Budiansky

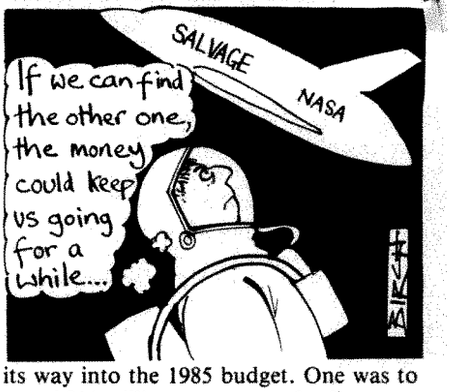
Space science

Panel's advice ignored

Washington

SPACE scientists are unlikely to give more than two cheers for the 1985 budget request for the National Aeronautics and Space Administration (NASA). The agency's basic research receives a hefty increase to $828 million and three important new projects — the Mars Geoscience/Climatology Orbiter, the Upper Atmosphere Research Satellite and the Scatterometer experiment — have been given the go-ahead. But NASA has unexpectedly baulked at a plan to inject urgently needed new funds into university research groups.

The plan, devised jointly last year by university space scientists and NASA officials, carried the *imprimatur* of Frank McDonald, NASA's chief scientist. Yet neither of its chief recommendations found



its way into the 1985 budget. One was to spend $11 million extra a year for five years to buy new equipment for universities — chiefly oscilloscopes, spectral analysers, computers, micro-ion probes and gas analysers. The other was to resurrect the NASA graduate fellowships that helped build up university space science in the 1960s.

Worse still, the budget contains no increase for basic research grants for university space science. Support for physics and astronomy is held at the same level as last year, while the amount for planetary science is reduced. Only one important recommendation of the McDonald report — more money to analyse mission data — has been incorporated in the 1985 request. Funds for space shuttle operations are due to rise from $81 million to $105 million, but that is not expected to be enough to make shuttle science significantly cheaper or faster; since 1978 there has been a backlog of experiments waiting to fly.

NASA's overall budget increase is a scant $274 million, or 4 per cent. That is enough, however, to keep alive major projects such as the Space Telescope (now renamed the Hubble Space Telescope), the Galileo mission to Jupiter and the Venus Radar Mapper. For 1985 only $150 million is allocated for research on the proposed space station.

Peter David

0028-0836/84/060492-01$01.00

© 1984 Macmillan Journals Ltd

US military research

Caltech to expel Army think-tank

Pasadena, California

A US Army think-tank set up at the California Institute of Technology (Caltech) is to be closed at the urging of the faculty. Growing concern over the think-tank, known as the Arroyo Center for Army Analysis, led last week to an emotional two-hour faculty meeting at which Caltech's president, Marvin Goldberger, was criticized for having allowed the centre to be established under what could be viewed as Caltech's auspices. The faculty urged Goldberger to sever ties with the centre "expeditiously and in a responsible fashion at the earliest possible time".

Goldberger said he would honour the faculty's wishes, but that the university would meet its existing commitments to the Army. The centre could thus keep its ties with the university for up to three years.

The centre is the Army's first outside think-tank. Its mandate is to examine future needs of the Army and to carry out objective analyses of policy questions. It was hoped that association with a leading university would give the centre an air of independence and attract good people.

The Army got its foot in Caltech's door by way of an expanding relationship between the Department of Defense and the Jet Propulsion Laboratory (JPL), the Pasadena space research centre operated by Caltech for the National Aeronautics and Space Administration (NASA). Three years ago, NASA slashed its support for JPL, most of whose planetary exploration missions were shelved. The Caltech faculty, saying it wished to preserve JPL's "unique" research teams, with some trepidation authorized JPL to accept up to 30 per cent of its support from the Department of Defense, and the proportion has indeed grown to 12 per cent (roughly $50 million of its $425 million budget).

Ironically, NASA's fortunes have rebounded in the past year, and JPL is now busy with a new mission to Venus and several smaller projects. Nonetheless, Goldberger and JPL's director, Lew Allen (a former Air Force chief of staff), have continued to encourage the military to invest in JPL's future. The Arroyo centre was seen as a "goodwill gesture" to the Army — and a direct link with the Army's upper echelon. The centre now has 23 employees, a director and a separate facility at JPL.

The Caltech faculty began to be concerned about the centre last autumn. "We were told everything was exploratory", said one faculty member, "but real commitments were being made." Last week's "Faculty Discussion" — a special session called only when serious matters need airing — concluded that the Army centre does not fit in with Caltech's strengths and expertise. A particular concern was that Caltech could be seen as lending its reputation to the policy recommendations made by the centre. Although 60 per cent of the Caltech faculty have individually consulted for the military, 95 per cent say it is wrong for the university to enter into an institutional arrangement with them.

Goldberger said that there had been a breakdown in communications with the faculty on the issue and that "all the worries should have come up sooner". He said that Caltech and the Army have yet to work out the details of how the centre will be operated in the light of the faculty's recommendations.

Sandra Blakeslee

US foundations

Kettering changes course

Boston, Massachusetts

A SMALL drama of scientific tradition confronted by unavoidable change is being played out in Ohio. As part of a major shift of focus of its research, the Charles F. Kettering Foundation of Dayton, Ohio, is arranging to hand over the Kettering Research Laboratory of Yellow Springs to the Battelle Memorial Institute, the large non-profit contract research organization in Columbus, Ohio.

The Kettering Laboratory has built its reputation over the past 50 years on basic research in the plant sciences, concentrating on nitrogen fixation and photosynthesis. It has some twenty senior scientists in a staff of eighty. In the past, 60 per cent of its financial support has been provided by the foundation, most recently to the tune of about $1.6 million a year, while the remainder has been made up of federal grants and some contracts.

The foundation announced the new arrangement in May 1983, and Battelle took over management of the laboratories last September. Assuming that the Internal Revenue Service approves the merger, the deal will be completed in the next few weeks. The Kettering Foundation will provide $8 million over the next five years to maintain current operations during the laboratory's adjustment to becoming part of Battelle.

Battelle, a public trust whose charter states that it must "benefit mankind", and whose net income is disbursed to charity through the Battelle Foundation, carries out applied contract research for clients in such areas as metallurgy, information sciences and organic chemistry. Battelle is otherwise similar to a profit-making business: it must generate income by selling its work to government and industry.

The expected changes are worrying the laboratory's staff, used to functioning independently in an academic atmosphere and focused primarily on long-term non-applied questions. One leading investigator asserts that he and about half of the senior staff at the laboratory are seriously hunting for other jobs. Under the new regime, members of staff will deal with novel concepts such as "deliverables" and "marketing", anathema to many of them, and will be responsible to Battelle management.

Albert Adelman, associate director of Batelle Columbus, claims that he and his staff are trying to make the changes as easy as possible for those at the laboratory. Battelle has asked everyone at the laboratory to stay on. It is consulting the staff about major decisions, particularly in the search for a new scientific director, and new people with more experience in business will be phased in gradually.

The question remains of why the Kettering Foundation decided to divest itself of the Kettering Research Laboratory. Kettering is an "operating foundation", meaning that more than 80 per cent of its endowment income is channelled into its own projects. In addition to the laboratory, the foundation has supported social science research, including an international affairs programme, the Institute for Development of Educational Activities and an urban management programme. All three have either been gutted or spun off since 1981, when David Matthews, Secretary of Health, Education and Welfare under President Gerald Ford, became president of the board of trustees.

According to Robert Daley, director of public affairs at the foundation, the decision to transfer the laboratory to Battelle was made by the trustees to "ensure the long-term growth of the laboratory". He claimed that Battelle was chosen because it would be better for the laboratory to be associated with a larger international company with wide experience in research management. He says that the laboratory staff were informed of the decision-making process, but at least two senior researchers insist that the announcement of the merger plan last May was a complete surprise.

Meanwhile, the Kettering Foundation has turned its attention to matters of public policy. It now supports a Domestic Policy Association whose goal is to foster public understanding of federal policies. It is also developing other programmes to educate the public about government, says Daley.

The Kettering Laboratory was founded in the late 1920s by the successful inventor and entrepreneur Charles F. Kettering "to sponsor and carry out scientific research for the benefit of humanity", particularly in plant science and agriculture. Despite the foundation's contention that it is maintaining Kettering's original trust, many staff members feel betrayed by what they see as abandonment by the foundation and its trustees of its essential purpose.

Christopher Earl

0028-0836/84/060493-01$01.00

© 1984 Macmillan Journals Ltd

European information technology

Only one dog left in manger

Brussels

WEST Germany will agree to contribute its share towards Esprit, the £1,000 million Brussels-inspired European research programme on information technology, at a meeting of research ministers later this month. So, at least, European Commission sources believe after the visit to Bonn by research commissioner Etienne Davignon two weeks ago.

In the past six months, Britain and West Germany together having been blocking progress on the project pending a wider European agreement on the budgetary problems of the European Communities. But now, it seems, Britain may be the only obstacle.

According to Brussels, West German opposition was limited to tying a release of Esprit funds to an offsetting reduction of the whole Brussels research budget (which is dominated by energy, including nuclear fusion). But, it seems, Bonn has been convinced that such a move would be counter-productive: the Brussels research directorate has recently been putting its house in order, and, Davignon argues, is ready to take on more work.

Thus, with West Germany ostensibly out of the way, Davignon is turning all his attention to Britain where the nut will certainly be harder to crack. Not only has the United Kingdom set up its own information technology programme under the Alvey directorate (on a scale roughly equivalent to what Britain might get out of Esprit), but the British Prime Minister insists on a complete solution to the European budget issue before new spending is approved. Davignon seeks to tackle this policy at its source.

Ironically, the Esprit sums for 1984 are already written into the current Brussels budget, but cannot be released until the ministers agree. Frustrated, the Commission is already unofficially preparing the official "call for tenders" that would follow ministerial approval. The hope is that after 28 February (when research ministers will meet in Brussels) the Esprit motor will be warmed up and running, just waiting to leap off the grid in its race against Japan and the United States.

Meanwhile, the European Commission has managed to get at least one new technology project under way: an eight-laboratory effort, costing £1 million, to produce the semblance of a working, optical computer within two years.

Esprit, although considered advanced and pre-competitive by industry, does not include "optronics", considered by some as the real future of computing and by others as a mere dream. But Esprit has now been all but taken over by the Brussels industry directorate, leaving the research directorate to look further ahead.

This it certainly has done. One goal of optronics is a thousandfold increase in computing speeds (to picosecond rather than nanosecond switching times) but there is at present no demonstrable read-write memory or clock working at these rates. But the optronics programme, which has been under way for little more than a month, will put Europe ahead of the United States and Japan in these fields, according to one of its co-directors, theorist Dr P. Mandel of the University of Brussels.

The eight groups involved, at Heriot-Watt University (Glasgow) (where Dr S.D. Smith is director of the experimental part of the European project), Milan, Pisa, Strasbourg, Frankfurt, Freibourg, Munich and Brussels, knew of one another before the Commission programme was mooted, but they are now working together with a will, claims Mandel. **Robert Walgate**

UK data protection

Reservations over data bill

THE British Government's Data Protection Bill successfully ran the gauntlet of its second reading debate in the House of Commons last week, despite Labour opposition and public reservations by several professional bodies.

The bill, which has been controversial from the start (see *Nature* **302**, 641; 1983), sets out to prevent the abuse of personal information held in machine-readable form so as to allow Britain to ratify the Council of Europe's convention on data protection. But many fear that the vague terms employed in the bill and the wide scope of its exemptions from this laudable aim will make it a burden on companies which would be required to register as "data users" while providing no worthwhile protection to people about whom data are held, "data subjects" as they are called.

Soviet computing

Aleksandrov urges speed

ATTEMPTS to computerize the Soviet economy could turn out to involve "vast and futile expenditure", Academician Anatolii P. Aleksandrov, president of the Soviet Academy of Sciences, has been warning. Writing in *Izvestiya*, Aleksandrov alleges that the Soviet Union is failing to make proper use of "even the comparatively small amount of computer equipment" that it manufactures.

The main bottleneck, according to Aleksandrov, is the shortage of trained personnel in the Soviet Union and a lack of awareness of the potential of computers among the population at large; overcoming these deficiencies will be a task comparable with "eleminating illiteracy" after the revolution.

Aleksandrov's article is ostensibly a call for a massive training programme, with the necessary computer manuals produced "before 1985", tuition in computer sciences (already partially implemented in tertiary education) at the secondary level and salary incentives for employees who complete computer familiarization courses.

Computers and automation equipment, Aleksandrov urges, must be "highly reliable", with at least 3-5 years of "trouble-free working". Accidental power failures, he said, should not lead to loss of information or to damage.

The situation with software is more complicated. The introduction of computers into the Soviet economy has taken place piecemeal, so that the various designers and ministries have produced equipment which, although "quite good for its time", is incompatible in software and components.

The need therefore is for a major overhaul of the Soviet computer industry so that all future hardware and software would be compatible. The logistical implications of such a revision of plans would be considerable — particularly in the Soviet context, where quarterly, annual and quinquennial production targets make no allowances for retooling or changes of production lines. If a switch to fully compatible hardware and software is decided, the planners will be hard pressed to incorporate it into the directives for the 1986–1990 five year plan.

The technical quality of existing Soviet software, to judge from Aleksandrov's remarks, is not entirely satisfactory. Existing tasks in the software sphere, he says, include the adaptation of software to automatic search systems and the protection of software against distortion due to voltage or frequency fluctuations, electromagnetic interference and the like.

For Aleksandrov, however, the task is clearly that of education. His programme for computer education (if the comparison with the literacy campaign of the 1920s has any real meaning) would embrace about half the population of the Soviet Union. Such a programme would itself require a major coordinated effort and, according to Aleksandrov, even the production of computer education manuals ("before 1985") must be preceded by work by the State Committee for Science and Technology, the Academy of Sciences, all ministries manufacturing computer equipment and automation facilities and the ministries of higher and secondary education, in order to "integrate" the available technical resources and software. **Vera Rich**

0028-0836/84/060494-01$01.00

© 1984 Macmillan Journals Ltd

The several amendments agreed by the House of Lords have not been enough to satisfy the critics. Holders of personal data stored in machine-readable form — subject to certain exemptions — would be required to register with a new Data Protection Registrar, who would keep records on the sort of information being held. (Personal data, as defined in the bill, include expressions of opinion about individuals but not indications of data users' intentions regarding those individuals.) Data subjects — subject to further exemptions — would be allowed access to information held about them and would be entitled to demand that any inaccuracies be corrected. Data users, for their part, would in general be prevented from unauthorized disclosure of personal information — again, subject to exemptions.

It is the exceptions to the general principles of the bill that arouse anger. The main concern of the British Medical Association is that computerized medical records would, if the bill became law, have to be registered, presumably by the health authorities, whose representatives — including non-medically qualified personnel — would then be empowered to pass on such records either to the police or to the Inland Revenue. The medical profession considers this quite unacceptable.

The British Medical Association (BMA) and a medical inter-professional working group on personal information have been negotiating with the Department of Health to find a solution to the problem. The department has offered some concessions based on voluntary codes that would in any case apply only while records were within its purview. BMA and the working party want medical records to be specifically exempted from the general provisions of the bill so that they could only ever be disclosed to an outside authority on the order of a crown court judge. At the same time the bill must not interfere with epidemiological research.

An earlier blanket exemption from the bill's subject access provisions and prohibitions on disclosure for data relating to immigration control has now been dropped, but it seems that the Home Secretary retains the power to exempt much government-held data from subject access "if they appear to him to be of such a nature that their confidentiality ought to be preserved". Universities are worried about the possibility of having to hand over their students' academic records on demand. And although data subjects who do manage to establish that inaccurate information about them is being held do not now have to prove damage in order to be awarded compensation, this does not apply if the data are improperly disclosed, a surprising omission picked up by Liberal Member of Parliament Mr Simon Hughes.

Quite apart from these sensitive issues, many fear that the proposed system for control of computerized personal data would be quite unworkable. The Data Protection Registrar would probably have a staff of about 20; the number of data users who would have to register would be of the order of hundreds of thousands, many of them holding entirely innocuous information. The definitions in the bill are so vague that it is unclear whether many computer systems will be required to be registered or not. The bill contains no detailed guidelines or codes of practice to help data users, and some Members of Parliament have suggested simple devices that would enable the letter of the bill to be complied with while breaking its spirit — for example, maintaining different (but unlabelled) lists for (say) creditworthy and uncreditworthy customers.

Those who defend the bill point out that as there is now no protection against the abuse of personal data in British law, the bill must at least be an improvement. But this would be to forget that once an important bill becomes law, major modifications are unlikely to be made for some years afterwards. Liberal and Social Democrat Members of Parliament who voted for the bill say they will be pressing for further changes in the committee stage. But the committee will be very limited in the expert opinion on which it will be able to call, and a Labour move to put the bill before a special standing committee was heavily defeated. However, the Home Secretary appears willing to accept that further modifications to the bill will be necessary.

Tim Beardsley

Fast reactors

Anglo-French accord at last

THE British and French electricity utilities have agreed on an outline programme of fast reactor development.

Sir Walter Marshall for the Central Electricity Generating Board (CEGB) and M. Jean Guilhamon for Electricité de France (EDF) signed a document on Tuesday which "sets out principles for long-term cooperation" covering the joint construction of fast reactors. According to CEGB, the first such joint reactor would be built in France, while the locations of future stations have yet to be decided.

In the short term, this implies CEGB support for the building of Superphénix II, a putative successor to the 1,300 MW Superphénix I now nearing completion near Avignon. Previously, EDF has shown itself cool to the Superphénix II project because of its probable cost (about twice that of an equivalent pressurized water reactor (PWR)).

Sir Walter Marshall said on Monday that the CEGB contribution to a Superphénix II would be "neither large nor trivial", and certainly more than 16 per cent. CEGB would receive electricity and revenues in proportion.

Assuming this more advanced reactor would cost no more than 40 per cent more than an equivalent PWR, the electricity so bought by CEGB would be cheaper than electricity made by burning coal in the United Kingdom, said Sir Walter.

According to M. Guilhamon, EDF is aiming to start Superphénix II in 1986. "Breeders are easier to operate than PWRs" said Guilhamon. Breeder operators suffer less exposure to radiation, and the thermal inertia of the sodium cooling circuit offers greater protection against accident, Guilhamon claimed. The sodium is unpressurized, and normally runs at 500°C; but it must reach 1,000°C before it boils. The water in a PWR, however, is superheated. This gives a PWR a 20 minutes safety margin but a fast breeder reactor two hours, he claimed.

Sir Walter would be happy to see just one British demonstration fast reactor before 1997 (his retirement date), he said. The main constraint was not cost but the "exhausting" prospect of a public inquiry probably longer and larger than the current Sizewell inquiry into the proposed British PWR. This has been "psychologically very difficult" said Sir Walter. Sizewell must be well out of the way before another such inquiry were contemplated.

Meanwhile, further agreements between British and continental agencies are expected, following the outline inter-state agreement on fast reactor cooperation signed in January. One between the UK Atomic Energy Authority (UKAEA) and its European partners is taking longer than promised — but as this shares out the research among signatories, and as research is the main component of fast reactor work at present, the delay is not surprising. A UKAEA spokesman pointed out wryly that while the CEGB–EDF agreement was between only two agencies, the research agreement involves several in a number of countries.

In fact, some years ago UKAEA had been seeking just such a bilateral agreement — but with the United States rather than France. The authority hoped that the trouble over federal support for the Clinch River project would lead the United States to fall into the arms of a British collaboration; but the US fast breeder programme was finally seen to be so confused and irretrievable that the decision was made to join in with Europe.

It may be considered significant that Sir Walter Marshall, who as the then chairman of UKAEA was behind the approach to the United States, has now also concluded a bipartisan agreement (though this time with France) as chairman of CEGB. Sir Walter approves of clarity, and it may have been against his taste to negotiate with a plethora of European agencies (as are involved in the existing European consortium behind Superphénix I).

Robert Walgate

0028-0836/84/060495-01$01.00

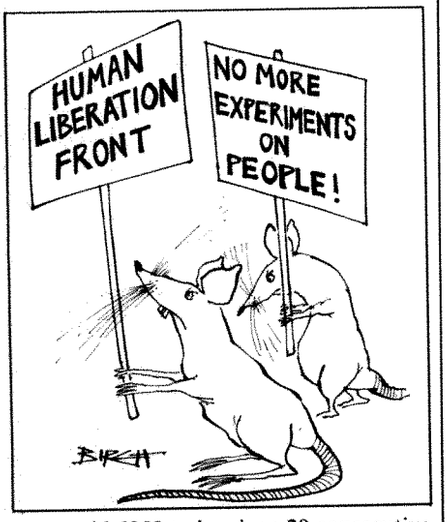
© 1984 Macmillan Journals Ltd

UK drug trials

Row over student trial

EVENTS have forced the British Medicines Commission into an urgent study of arrangements for testing new drugs on healthy volunteers. The issue has come to a head in the wake of complaints that plans for testing a new antitumour drug among London students are ethically objectionable and pay scant attention to the safety of the volunteers.

The disputed study, begun some months ago but now abandoned, was being carried out by a company known as Charterhouse Clinical Research Unit Ltd, on behalf of the manufacturer, Ortho-Cilag Pharmaceuticals Ltd. Volunteers, mostly students,



were paid £250 to be given 20 consecutive doses of up to 450 mg of hydroxyphenylretinamide (HPR), an analogue of vitamin A shown to be a tumour inhibitor in animals.

The study, designed to provide information of toxicity in human beings, has been criticized on the grounds that volunteers were not fully informed of the risks and that the testing company had failed to make adequate arrangements for monitoring participants' health. Inevitably, the result has been to reopen the contentious question of how clinical data on the effects of new drugs should be gathered — and of the role of academic pharmacologists in the process.

Under present arrangements in Britain, drug studies with healthy human volunteers are regarded as a private matter between consenting adults — the drug company and the volunteers. Since 1981, manufacturers have not been required to seek advance approval from the Committee on the Safety of Medicines (established, like the Medicines Commission, under the 1968 Medicines Act). One result has been the emergence of a number of companies linked with academic departments to provide human toxicity testing as a service to pharmaceutical manufacturers.

The whistle seems to have been blown on the Charterhouse trial by Professor Brian Rabin, professor of biochemistry at University College, London, who says HPR may plausibly accelerate the growth of some tumours, and who complains that Charterhouse had provided no information to the volunteers' own physicians and that it had no plans to follow up its volunteers to detect long-term untoward effects. He also attacks the information sheet given to volunteers for failing to list all possible side-effects of HPR and says the silence of Charterhouse's consent form on the matter of compensation in the event of damage being suffered is "totally disgraceful".

These criticisms are rejected by Professor Paul Turner, an adviser to Charterhouse and director of clinical pharmacology at St Bartholomew's Hospital Medical College. He says that "ill-considered remarks" and "destructive press coverage" have severely impeded research on a promising anti-tumour drug "showing no signs of toxicity".

Turner says that Charterhouse accepts work only on the condition that a written indemnification against damage claims is provided by the client company. A new consent form being drawn up by Charterhouse will aim to comply with a recommendation of the Association of British Pharmaceutical Industry that no-fault compensation should be offered explicitly to volunteers. Turner argues that new drugs such as HPR are best tested in an independent company separate from the manufacturer.

Although the HPR study had been approved by Charterhouse's own ethics committee, the deans of the London medical schools have now recommended that their students should take part only in studies that have been subject to independent scrutiny. Under a new agreement between Charterhouse and the Metropolitan Conference of Medical Deans, studies on students will in future be approved by the ethics committee of St Bartholomew's Hospital, with which Charterhouse is closely associated.

Although many British pharmaceutical manufacturers have in-house testing units, more and more volunteer studies have recently been carried out by small independent companies, often associated with pharmacology departments in medical schools, whose earnings can provide a welcome source of research funds. Charterhouse Clinical Research Unit Ltd was set up by Synthelabo, a non-profit charity linked to St Bartholomew's Hospital Medical College.

The demand for volunteer studies has grown rapidly since the introduction in 1981 of the Clinical Trials Exemption Scheme, which allows manufacturers with a good case for a drug to go into clinical trials without first facing a full-length scrutiny by the Department of Health's Committee on the Safety of Medicines. It is widely believed that a good volunteer study increases the chances of an exemption certificate being granted.

Practice varies from one company to another: some, such as Drug Development (Scotland) Ltd, linked to the University of Dundee, routinely notify volunteers' physicians about studies. Dr John McEwen, the company's medical director, says that information from volunteers' physicians has often proved useful to those running the studies. The company also offers no-fault compensation in the event of claims for damages, and is insured to cover any claim.

The Medicines Commission, which advises the government on matters related to medicines generally, now has some difficult questions to face. Members will certainly be mindful of Professor Turner's warning that if drug development is hampered in this country, the big manufacturers will simply pack their bags and go elsewhere.

Manufacturers feel hard done by because more is expected from them and from their pharmacological agents than from academic researchers in, say, physiology. Thus in the event of harm to a volunteer in an experiment by an employee of the Medical Research Council, the council would offer an *ex gratia* payment but there would be no legal redress unless negligence could be proved. The voluntary code on compensation to which most commercial companies adhere commits them to damages in a way the Medical Research Council and the Department of Health have been unwilling to apply to themselves.

Tim Beardsley

France seeks rules

THE testing of new drugs in France is to be tightened up, in a move by the ministry of health. No precise bill is yet on the table, but a "draft project" is under study, according to the French evening newspaper *Le Monde*.

French law on the matter is obscure, with some experts claiming that human tests are totally illegal. Testing has nevertheless been carried out, but often in a clandestine and unsatisfactory manner, making France — according to some — an effectively liberal regime in which multinationals can conveniently try out new formulas.

According to the ministry's draft, however, subjects and the authors of trials should sign clear written agreements with each other; payment should be according to published terms; authors would have the burden of proof; and prisoners, or members of the author's staff, would not be used as subjects unless it would be to the benefit of their health. Further, all human trials would be subject to the approval of a regional ethical committee — though the constitution of such a body remains, for the moment, undefined. Robert Walgate

0028-0836/84/060496-01$01.00

© 1984 Macmillan Journals Ltd

Sahel drought

Call for joint action

A CALL for the formation of a Sahel fund to combat the area's drought and desertification was issued last week at the end of the sixth meeting of the Permanent Interstate Committee for Drought Control in the Sahel (CILLS), at Niamey, the capital of Niger. The committee was formed ten years ago by eight West African governments (Cape Verde Islands, Chad, Gambia, Mali, Mauritania, Niger, Senegal and Upper Volta) to coordinate action during severe droughts. The sixth meeting was attended by heads of governments and the director-general of the Food and Agricultural Organization.

The World Meteorological Organization considers that the drought in Africa which began in 1968 has not yet ended, and is worst in the Sahel. Agencies working in the area report that although conditions in the Sahel are not everywhere as serious as in 1968–73, drought is now locally severe in the north. For example, in Mauritania, 85 per cent of the basic cereal crop has failed, with the result that half the population is now gathered in the capital of Nouakchott. In northern Mali, all cereals are badly affected. In Upper Volta, millet has failed entirely and the sorghum crop is poor. So a return to the conditions of the 1970s is feared. Ghana, with its third consecutive poor harvest and an increase of 10 per cent in population since the expulsion of Ghanaians from Nigeria, is expecting a fourfold increase in food prices within six months. In Senegal the cereal situation is disastrous, with animals dying and water holes drying up. But in the southern areas, harvests have been adequate and there are even some surpluses.

Water is the main problem. It is estimated that only 25–30 per cent of Sahelians have access to sufficient water. Desertification, which according to the United Nations Environment Programme, consumes 1.5 million hectares of potentially productive land a year, is exacerbated by population growth (2.7 per cent a year) and overgrazing.

Wood consumption (90 per cent of it for burning) is also a worry, especially because attempts at reafforestation have not been encouraging. The area could show a substantial surplus if consumption were reduced by even one per cent.

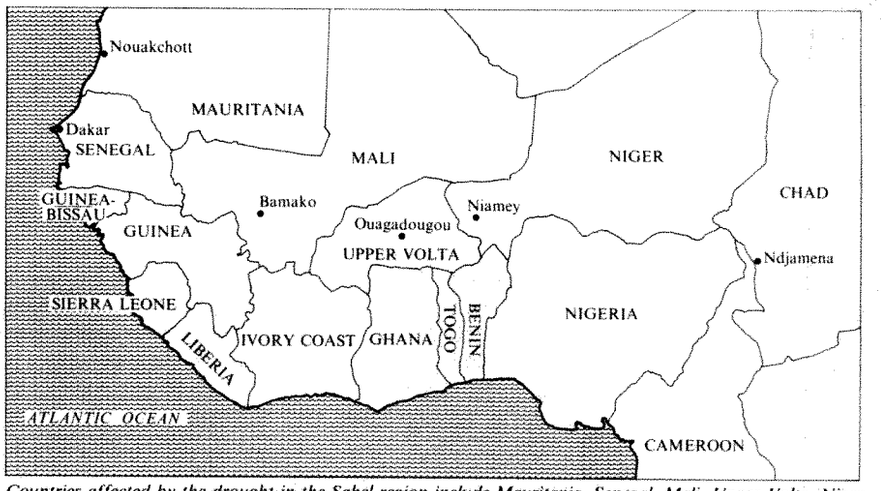
The effects of physical problems are made worse by the chronic social and economic conditions of the region, including inadequate transport, low farm prices, which discourage the production of food, and a general insufficiency of money. Emergency aid is difficult to deliver.

According to the Club du Sahel, formed by the Organization for Economic Cooperation and Development and several West African governments and various development agencies, aid to the area has doubled in ten years and now amounts to 15 per cent of the total gross national product in the Sahel states and more than 20 per cent of the gross national product in half of them. So dependent on aid has the region become that some agencies now fear that long-term massive food aid is sapping the ability of the region to acquire the resources necessary to respond to normal climatic swings, let alone to cope with disaster. Oxfam, the British charity, agrees with the UK Overseas Development Administration that the best help is the swift delivery of emergency food aid followed by agricultural aid, such as the supply of seed corn, as conditions improve.

One novel possibility is that climatic change and crop yield may be predictable from satellite data (see p.528 for a study of satellite results); this would be especially valuable if there is a recurring pattern comparable with the 10-year cycle now established for Botswana. A British project is currently under consideration by the Overseas Development Administration and the Food and Agricultural Organization. The World Meteorological Organization, however, considers that drought cannot be predicted and that there is no practical method of enhancing precipitation, but that much could be done to ameliorate the effects of droughts by improving agrometeorological monitoring services and the wide application of increased information on an interdisciplinary basis.

Conditions in the Sahel are part of a climatic threat to the world's poorest people. Nine Southern African states also made an emergency appeal last week. Oxfam, in common with other international agencies, has received more requests for help in the past six months than at any time in its entire 40-year history. Abnormal weather, Oxfam says, could lead to "the collapse of development across a whole sweep of countries so economically depressed that they cannot possibly cope themselves".

Sarah Tooze



Countries affected by the drought in the Sahel region include Mauritania, Senegal, Mali, Upper Volta, Niger, Nigeria, Chad and Ghana.

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 27 Jan.	Change
23¼	9¾	**Biogen (Switzerland)**	10¾	12½	+1¾
6¼	1⅝	**Bio-Logicals (Canada)**	1⅝	2	+⅜
16⅛	7¼	**Bio-Response (USA)**	10	11¾	+1¾
19	10½	**Cetus (USA)**	10⅞	13⅛	+2¼
15½	6½	**Collaborative Research (USA)**	6¾	8¼	+1½
39⅞	15	**Damon (USA)**	15⅜	17¾	+2⅜
34¼	16⅜	**Enzo-Biochem (USA)**	25	23	-2
18⅞	8⅝	**Flow General (USA)**	9½	8¾	-¾
49¾	25⅞	**Genentech (USA)**	34½	37¼	+2¾
17¾	7⅞	**Genetic Systems (USA)**	8⅞	8⅜	-½
23¼	12½	**Genex (USA)**	13	13½	+½
31	18¾	**Hybritech (USA)**	18¾	21	+2¼
22¼	10⅞	**Molecular Genetics (USA)**	12	14½	+2½
23¼	10½	**Monoclonal Antibodies (USA)**	12	12½	+½
73½	42	**Novo Industri A/S (Denmark)**	56	55⅜	-⅝
30¼	13⅝	**Pharmacia (Sweden)**	19⅜	18⅞	-½

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature*'s weighted index of biotechnology stocks stood at **181** on 27 January, compared with **175** a month earlier. Data from E.F. Hutton, Inc.

0028-0836/84/070497-01$01.00

© 1984 Macmillan Journals Ltd

The day before

SIR — In a leading article in *Nature* of 1 December 1983 (p.412) it is recommended that ABC show the film "The Day After" again, and then again, in order that people do not forget the effects of an all-out attack with megaton weapons which have been extrapolated from the known effects of the 20 kiloton explosions over Hiroshima and Nagasaki.

I was struck by what seem to me to be significant omissions about what happened the day before: the actors portrayed in the film made almost no organized effort to improve their chances of survival. There are two scenes showing men shovelling small quantities of earth against the foundations of a church and a farmhouse, while there are innumerable scenes showing people panicking in city streets, grocery stores and on the highways. In one scene Dr Oats, the senior surgeon-haematologist, washes his hands at a scrub sink while an orderly tells him, in a tone of voice appropriate for the revelation that skinned cats have been found in the hospital kitchen, and that there is a rumour that people are leaving Kansas City. The question is asked, "Where does one go from Kansas City?"

It would have been even-handed and more interesting had the producers of the ABC film shown that the people of Kansas City could have moved into the large complex of commercially developed caverns which underlie the city and its environs. These caverns were artificially excavated out of limestone to provide crushed rock for the Kansas and Missouri interstate highways. The excavated areas are covered by between 80 to 200 feet of bedrock and soil, have an even temperature (57–62° F), a large area (several hundred acres) and are connected to each other and to the surface by many miles of roads and railroads. The Monsanto and Libby food companies have stored very large quantities of canned food and frozen fruit juice in the caverns, and other companies have stored cars, tractors and vodka. It has been estimated that everyone in Kansas City is within 45 minutes of one of the entrances to these caverns, and that the caverns could be used for blast and fallout protection[1].

Few cities have the opportunities for evacuation comparable with those of Kansas City, but then few cities are located beside 150 Minuteman missile silos. It has been estimated that an all-out attack with megaton weapons would kill or injure 50–80 per cent of the unprotected population of the United States, and that the destruction of hospitals would make it impossible for physicians to "intervene effectively in the (resultant) human injury and death"[3]. These speculations have been repeated so often that many authors accept them as fact[3,4]. However, these estimates assume that urban populations remain unprepared and unprotected. Most of the casualties from megaton weapons would result from the blast effects, and by removing people from the likely target areas, evacuation could substantially reduce the number of deaths[5].

In a broader context, it is critically important that the superpowers and their allies keep talking in order to reduce as much as possible the number of nuclear weapons and the likelihood that any of those weapons will ever be used. Until such talks have borne generous fruit, the nations of the Northern Hemisphere would be wise to emulate Sweden, Switzerland and the Soviet Union and improve their civil defence capabilities.

ROBERT SHEPHERD
Royal Victoria Hospital,
687 Pine Ave West,
Montreal, Quebec, Canada

1. Halland, *J. Civil Defense* **12**, 22–25 (1979).
2. *Economic and Social Consequences of Nuclear Attacks on the United States*, 46 (U.S. Government Printing Office, Washington, 1979; Stock no. 052-070-04889-1).
3. Cassel, C. & Jameton, A. *Annls internal Med.* **97**, 426-432 (1982).
4. Bates, D.G. *Can. med. Ass. J.* **125**, pp 923-924 (1981).
5. *Defense Civil Preparedness Agency Attack Environment Manual*, Ch.1 (U.S. Government Printing Office, Washington, 1973; Stock no. CPG 2-1A1).

Crucifixion date

SIR — I read with great interest the review article by Humphreys and Waddington entitled "Dating the Crucifixion" (*Nature* **306**, 743-746; 1983). The date of 3 April, of the year AD 33, the 14 Nisan of the Jewish Calendar, appears to be well supported by their evidence. But one of their statements may be in error. They write ". . .we note . . . that the Crucifixion must have occurred on 14 Nisan and that the interpretation of the Last Supper as a Passover meal held at the official time cannot be correct". However, it is likely that the official time for the Passover meal in AD 33 was on the night of 13 Nisan. In support of this view I present the following paragraph from p. 326 of *Jesus of Nazareth* by Joseph Klausner (Macmillan, New York, 1944).

> "According to the ruling which was newly promulgated by the Pharisees in Hillel's time, the Passover was regarded as a *public* sacrifice; if, therefore, the 15th of Nisan fell on a Sabbath and the 14th on the eve of Sabbath, the Passover was sacrificed on the eve of Sabbath (the 14th of Nisan) at the moment "between the two days", even if this profaned the Sabbath; they used to argue that, like every public sacrifice, "the Passover abrogates the Sabbath rules." According, however, to an earlier ruling, which held good among the priestly party almost to the close of the period of the Second Temple, the Passover was regarded as a *private* sacrifice and one which might not abrogate the Sabbath rules; if, therefore, the 14th of Nisan fell on the eve of Sabbath, they sacrificed on the 13th instead of the 14th, so as not to profane the Sabbath (since they must sacrifice "in the evening at the moment of sunset.") Hence Jesus and his disciples celebrated Passover on the Thursday, the 13th of Nisan, and during the ensuing night of the 14th of Nisan (the night before Friday) they had to celebrate the "Seder", the Passover meal, with its unleavened bread and bitter herbs, instead of on the night of the 15th Nisan."

BORIS MAGASANIK
Department of Biology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139, USA

Animal rights

SIR — Since you have established from recent correspondence that animal rightists are not only cranks but humourless ones to boot, it is without relish that I enter the fray. With one conclusion of your leading article (*Nature* **306**, 527; 1983) I can entirely agree: there are no consistent or universal principles that imbue animals with "rights". A right, then, is not a given, an absolute, but is a function of the culture which accords it. Any right is a more or less widely accepted value built up over time and representing something of which we consider an animal or human should not be deprived.

While I can see no evidence for absolute natural rights of animals, to retain these for our own species while withholding them from other animal species is, in my view, antediluvian — or, to be more accurate, pre-Darwinian. What distinguishes this viewpoint from the ethnocentricity of those tribes whose members believe that they are the only humans and that people of neighbouring tribes can be killed like pigs?

Sir Andrew Huxley, president of the Royal Society, is reported to have asked what importance can be attached to the painless killing of purpose-bred laboratory animals that otherwise would not have lived. Man, according to this belief, has a right to destroy because he fancies that, like a god, he can create life. The surgeon Versalius used to say "I dressed him; God healed him." His antidote — a little humility.

STEWART BRITTEN
9d Stanhope Road,
London N6 5NE, UK

City slickers

SIR — The proposal for an Indian "technology city" (*Nature* **305**, 350; 1983) is welcome, but it is unfair to build such a city exclusively for Indian scientists working abroad. It is important to recruit the best of Indian scientific talent, no matter whether those concerned work in India or in the United States. While some of the best Indian scientists are working in India, not all Indian scientists working abroad are of high calibre.

I think the Government of India should go ahead with its plans but that it should appoint the best persons in the field, whether they are working in India or abroad. This could be achieved by a selection board comprising top ranking experts from India and other nations. There is always a risk that the very best scientists are not offered jobs in India for various sociopolitical reasons, but something has to be done if India is to keep pace with international developments.

ARUN KUMAR
Geology Department,
University of the West Indies,
Mona, Kingston 7,
Jamaica

Cell and cancer biology meld

The startling similarity of part of epidermal growth factor and the product of a viral oncogene is another leap forward in understanding the basis of cancer. A definite link to human cancer remains elusive.

FOR some time, there has been speculation that many viral oncogenes would turn out to be derived from cellular genes for growth factors or their receptors, so when Waterfield *et al.*[1] and Doolittle *et al.*[2] showed, seven months ago, that one of the two peptides that constitute platelet-derived growth factor (PDGF) is similar in sequence to the transforming protein encoded by the *sis* oncogene of simian sarcoma virus, there was eager anticipation of the next example. That has now materialized with the paper on page 521, in which Waterfield and his associates[3] reveal that an oncogene of a tumour virus of birds is almost certainly derived from the gene for the cell surface receptor for epidermal growth factor (EGF). The result is as dramatic as it will be important for research on normal and abnormal (cancer) growth of cells. But it is not as simple as it appears at first sight.

The EGF receptor gene has been a good candidate for a viral oncogene because[4] it shares with at least five viral oncogenes (of which the prototype is *src* of Rous sarcoma virus) the activity of a tyrosine phosphokinase, that is an enzyme which phosphorylates tyrosine residues in proteins. It has therefore been on the cards that one of the tyrosine phosphokinase encoding oncogenes would closely resemble the EGF receptor gene. If so, the argument went, the tumour virus would in effect be placing an EGF receptor in an inappropriate cell and/or at an inappropriate time. In the event, neither idea is exactly borne out.

The sequence of the *erb-B* oncogene of AEV-H, a strain of the avian erythroblastosis virus that causes erythroleukaemia and, less frequently, sarcoma in chickens, was published last November by Yamamoto *et al.*[5]. Unexpectedly, it contains a segment that is notably similar to the *src* oncogene and others with tyrosine phosphokinase activity. Nevertheless, at least three separate laboratories have since failed to demonstrate that the *erb-B* oncogene product has tyrosine phosphokinase activity of its own (an anomaly shared with the *mos* oncogene[6] and, very recently, the *fms* oncogene[7]). But while the tyrosine phosphokinase activity of EGF receptor has been known for some years, the sequence of the receptor has awaited its purification in sufficient quantities.

Starting either with human placenta or with the human epidermoid carcinoma cell line A431, and with the vital assistance of monoclonal antibodies against the EGF receptor, Waterfield's group has now determined the amino acid sequence of 14 fragments of the receptor. These scraps of information have been sufficient for a computerized data search to align six of the peptides with segments of the protein encoded by *erb-B*, with 74 identical amino acids out of 83. The other eight peptides could not be aligned with *erb-B*.

Waterfield *et al.* conclude that the *erb-B* oncogene represents only part of the EGF receptor gene — cautiously, they raise the formal possibility that it comes not from the receptor gene itself but from a closely related gene — and deduce that the missing part encodes the EGF-binding domain of the receptor which is positioned on the external surface of cells. What the gene retains is the short segment of EGF receptor that spans the membrane and the large segment on the internal surface of the membrane which has tyrosine kinase activity.

The first puzzle is why, then, does *erb-B* not have demonstrable tyrosine phosphokinase activity? One possibility is that it does, but that the activity has been missed, for lack of rigorous testing. If so, the next puzzle is how it can be stimulated when the *erb-B* version of the EGF receptor lacks the EGF-binding domain and the normal receptor has low tyrosine phosphokinase activity in the absence of EGF. A clue to the solution to both puzzles may come from the recent demonstration by Tamura *et al.*[8] of the activation of the tyrosine phosphokinase of insulin receptor by the proteolytic action of trypsin; perhaps the truncation of EGF receptor really does activate it.

It is even conceivable that the tyrosine phosphokinase activity, or lack of it, is not wholly relevant to the issue. Could it be that other activities of viral oncogenes/growth factor receptors are more relevant to cancer? For example, is the recently demonstrated induction of the *myc* gene of lymphocytes as an early response to PDGF[9] a more relevant clue or does that induction anyway depend on the tyrosine phosphokinase activity that PDGF induces in its receptor?

The next question that is likely to be answered by Waterfield and his associates is the intellectually more modest but technically formidable one of what the complete sequence of the EGF receptor looks like. The answer will be derived from the nucleotide sequence of its messenger RNA converted into cDNA. The sequencing is already well under way at Genentech under Axel Ullrich, an author of Waterfield *et al.* The sequence will reveal more clearly the exact relationship between the EGF receptor and the viral *erb-B* gene product. Moreover, a comparison between the cDNAs of EGF receptor and those of the cellular *erb-B* gene[10] will make it easier to decide whether the two genes are identical.

The relevance of the discovery of Waterfield *et al.* for cancers, particularly human cancer, not caused by tumour viruses simply cannot be answered at this stage. It is, at least, clear that activation of the cellular *erb-B* gene by avian leukosis virus, a tumour virus of chickens that does not carry its own oncogene, can cause a variety of neoplasms, particularly erythroblastosis[11]. Therefore, by analogy with *myc* genes[12,13], it is possible that the human cellular *erb-B* gene can be inappropriately activated by, for example, chromosome translocations. A thorough comparison of the expression of *erb-B* EGF receptor genes in healthy and tumour tissues should at least indicate how frequently they are expressed in abnormal amounts or in abnormal sizes (perhaps as a truncated protein) in tumours; the A431 cancer cell line was a good starting point for the EGF receptor purification precisely because it has at least fifty times more receptor than normal cells.

The discovery will also act as a spur to the purification and sequencing of other growth factor receptors, especially those with tyrosine phosphokinase activity — the PDGF, insulin and insulin-like growth factor-1 receptors. Perhaps it is from their genes that some other viral oncogenes have been derived. But it will be necessary to look further afield to account for them all, particularly if the recent identification[14] of about one-third of the gene for actin as part of the oncogene of a tumour virus of cats is relevant to its ability to produce fibrosarcomas.

Peter Newmark

1. Waterfield, M.D. *et al. Nature* **304**, 35 (1983).
2. Doolittle, R.F. *et al. Science* **221**, 275 (1983).
3. Downward, J. *et al. Nature* **307**, 521 (1984).
4. Ushiro, H. & Cohen, S. *J. biol. Chem.* **255**, 8363 (1980).
5. Yamamoto, T. *et al. Cell* **35**, 71 (1983).
6. Papkoff, J., Nigg, E.A. & Hunter, T. *Cell* **33**, 161 (1983).
7. Hampe, A., Gobet, M., Sherr, C.J. & Galibert, F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 85 (1984).
8. Tamura, S., Fujita-Yamaguchi, Y. & Larner, J. *J. biol. Chem.* **258**, 14749 (1983).
9. Kelly, K., Cochran, B.H., Stiles, C.D. & Leder, P. *Cell* **35**, 603 (1983).
10. Vennström, B. & Bishop, J.M. *Cell* **28**, 135 (1982).
11. Fung, Y-K.T., Lewis, W.G., Crittenden, L.B. & Kung, H-J. *Cell* **33**, 357 (1983).
12. Hayward, W.S., Neel, B.G. & Astrin, S.M. *Nature* **296**, 475 (1981).
13. Robertson, M. *Nature* **306**, 733 (1983).
14. Naharoo, G., Robbins, K.C. & Reddy, E.P. *Science* **223**, 63 (1984).

© 1984 Macmillan Journals Ltd

Neurobiology

More nerve growth factors?

from Joshua R. Sanes

THE discovery of nerve growth factor (NGF) by Levi-Montalcini and Hamburger[1] was the first major step in the molecular analysis of neural development, and remains the most influential achievement in this rapidly growing field. This soluble protein is required for the survival of sympathetic and sensory neurones *in vivo* and *in vitro*: in culture, it also promotes neuronal differentiation and chemotactically directs growing axons. NGF has been purified and sequenced, its genes are being cloned and mapped, and studies of its mechanism of action are underway. The current dogma is that NGF is produced by peripheral target tissues, picked up by nearby axons, and transported back to neuronal somata. Competition for limited supplies of NGF may determine which neurones live and which die during embryogenesis. In addition, NGF may act locally in the periphery as a stimulant and attractant, to regulate the size and shape of an axon's terminal field. Finally, NGF may be required for neuronal maintenance in adulthood, with loss of its supply — after axon injury, for example — signalling the neurone that adjustment or repair is required (reviewed in refs 2–4).

The early realization that only a few types of neurone are sensitive to NGF led to speculation that NGF was but one of a family of trophic agents[5]. If each of several cell types turned out to secrete limited amounts of a factor that supported its own innervation, retrograde trophic control would provide a general means to match the sizes of pre- and post-synaptic populations. Furthermore, a system of selective trophic sustenance might play some limited part in synaptic specificity: that is, in determining which cells or connections, as well as how many cells or connections, form or survive. Among the first steps in the hunt for other trophic factors have been the demonstration that extracts of appropriate targets support parasympathetic neurones *in vitro*[6,7], and the purification of a protein from brain that allows survival of some cultured neurones that do not respond to NGF[8].

From a cell biological point of view, the most useful new factor to obtain would be one that supports somatic motor neurones — a motor neurone growth factor (MNGF). The skeletal neuromuscular junction is the best studied of all synapses and would be a good system for studying factor uptake and action. Fortunately, there is reason to suspect that a MNGF exists, in that several aspects of neuromuscular synapse formation seem to reflect control by muscle-derived factors. In the embryo, spinal motoneurones (like many other neuronal types) are normally produced in excess; 30–50 per cent of them degenerate before the animal is born. Peripheral control of this process is indicated by the observation that the naturally occuring cell death can be increased by extirpation of the appropriate target, while neurones that would otherwise die can be rescued by grafting on extra target tissue. In the neonate, several axons innervate each skeletal muscle fibre. Excess inputs are withdrawn until each fibre receives a single input, and it has been proposed that even after a neurone's survival has been assured, its individual axonal branches continue to fight for the limited support that each muscle fibre can furnish. In the adult, partial denervation of a muscle induces the undamaged axons to sprout and reinnervate the denervated muscle fibres. Again, several aspects of the sprouting phenomenon suggest that it represents an axonal response to soluble factors released by denervated fibres. Thus, neuromuscular connectivity could be regulated in part by the supply and/or effectiveness of MNGF(s) throughout the life of an animal (reviewed in refs 9–12).

How, in turn, might the supply of such growth factors be regulated? Again, previous studies of muscle suggest a candidate: electrical (or contractile) activity. Paralysis of muscle rescues embryonic motoneurones destined to die, slows elimination of polyneuronal innervation in neonates and evokes sprouting in adults; conversely, chronic stimulation speeds synapse elimination and prevents sprouting[8-11]. Since many aspects of muscle metabolism are already known to be activity-dependent, it is tempting to suppose that denervated or inactive muscles secrete more MNGF than innervated, active muscles. Neurones could thus control and be controlled by their supply of factor, establishing a feedback loop.

Aware of these and other issues, many groups have begun to search for a MNGF. The most common approach has been to culture motor (or spinal cord) neurones in a minimal medium, and to assess the effects of adding muscle extracts or muscle-secreted material. Supplements have been found that increase levels of neurone survival, neurite extension, or transmitter synthetic capacity[13-21]. As one might have hoped, extracts are more active when made from neonatal or denervated muscle than from innervated adult muscle[18-21]. Early attempts at fractionation suggest that the extracts may contain several active factors, which differ in their modes of action (some seem to act in solution, others after first attaching to the substratum) and effects (for example, activities that promote neuronal survival and differentiation can be separated from each other). However, purification of the factor(s) has not yet been achieved.

So far, all attempts to identify a MNGF have relied on neurones in culture as bioassay. Now Gurney[22] reports, in this issue of *Nature* (p.546), results of an alternative immunological approach. He used a series of antisera (originally prepared at the MRC, London) to material secreted by organ-cultured denervated muscle. To carry out an assay he injected the sera into paralysed but innervated mouse muscles and looked for suppression of the sprouting that would normally occur. Gurney's paper contains three intriguing results. First, several antisera partially prevented sprouting *in vivo*. Second, each blocking serum recognized a 56,000-molecular-weight (56K) protein on immunoblots of muscle-secreted material. Third, and perhaps most astonishing, sera from patients with amyotrophic lateral sclerosis (ALS) not only blocked sprouting when injected but also recognized a 56K antigen on immunoblots. ALS is a disease in which many spinal motoneurones die and surviving motoneurones sprout poorly. Thus, the properties of ALS sera both provide a possible clue to the aetiology of this fatal disease and lend further support to the notion that a 56K protein may regulate sprouting.

Is the 56K antigen a MNGF? A promising preliminary result is that the antigen is present in an incompletely purified fraction that promotes neurite extension and survival of cultured spinal cord cells (ref. 22 and personal communication). If further work proceeds well, the idea that neurones of different types and of different ages are maintained in a trophic equilibrium will be open to new and exacting tests. □

Joshua R. Sanes is in the Department of Physiology and Biophysics, Washington University Medical Center, St Louis, Missouri 63110.

1. Levi-Montalcini, R. & Hamburger, V. *J. exp. Zool.* **116**, 321 (1951).
2. Thoenen, H. & Barde, Y.-A. *Physiol. Rev.* **60**, 1284 (1980).
3. Yanker, B.A. & Shooter, E.M. *A. Rev. Biochem.* **51**, 845 (1982).
4. Bradshaw, R.A. *Nature* **303**, 751 (1983).
5. Purves, D. in *Function and Formation of Neural Systems* (ed. Stent, G.S.) 21 (Dahlem Konferenzen, Berlin, 1977).
6. Manthorpe, M. *et al. J. Neurochem.* **34**, 69 (1980).
7. Nishi, R. & Berg, D.K. *J. Neurosci.* **9**, 505 (1981).
8. Barde, Y.-A., Edgar, D. & Thoenen, H. *EMBO J.* **1** 549 (1982).
9. Hamburger, V. & Oppenheim, R.W. *Neurosci. Comment.* **1**, 39 (1982).
10. Grinnell, A.D. & Harrera, A.A. *Prog. Neurobiol.* **17**, 203 (1981).
11. Brown, M.C., Holland, R.L. & Hopkins, W.G. *Rev. Neurosci.* **4**, 17 (1981).
12. Slack, J.R. & Pockett, S. *Muscle Nerve* **6**, 243 (1983).
13. Dribin, L.B. & Barrett, J.N. *Devl Biol.* **74**, 184 (1980).
14. Henderson, C.E., Buchet, M. & Changeux, J.-P. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2625 (1981).
15. Bennett, M.R., Lai, K. & Nurcombe, V. *Brain Res.* **190**, 537 (1980).
16. Giller, E.L., Neale, J.H., Bullock, P.N., Schrier, B.K. & Nelson, B. *J. Cell Biol.* **74**, 16 (1977).
17. Tanaka, H. & Obata, K. *Develop. Brain Res.* **4**, 313 (1982).
18. Smith, R.G. & Appel, S.H. *Science* **219**, 1079 (1983).
19. Henderson, C.E., Buchet, M. & Changeux, J.-P. *Nature* **302**, 609 (1983).
20. Slack, J.R. & Pockett, S. *Brain Res.* **247**, 138 (1982).
21. Nurcombe, V. *et al. Brain Res.* **291**, 19 (1984).
22. Gurney, M.E. *Nature* **307**, 546 (1984).

0028-0836/84/060500-01$01.00

© 1984 Macmillan Journals Ltd

Solar conversion

Non-aqueous electrolytes make wet cells work

from S. Dennison

IN the urgent search for photovoltaic devices for the direct conversion of solar energy to electricity, solid-state solar cells have in the past few years far outstripped their counterparts based on junctions between semiconductors and electrolytes. Although 'wet' photovoltaic cells may be more easily manufactured, they are far behind as practical converters. But now there is a prospect that this gap may be closed.

At the end of 1982, Stanford University chemists Chris Gronet and Nathan Lewis described (*Nature* **300**, 733; 1982) a cell based on an n-$GaAs_{1-x}P_x$ semiconductor in contact with a non-aqueous redox electrolyte with an efficiency of no less than 13 per cent, a doubling of the best previous efficiency. The problems that had plagued earlier devices were overcome by optimizing the semiconductor properties and preventing corrosion by the use of a non-aqueous electrolyte. Now, Gronet and Lewis, together with C. Lieber, have made an equally impressive step forwards by the construction of a 10 per cent efficient cell based on the rather more familiar material p-type silicon, in a non-aqueous electrolyte containing the cobaltocene–cobaltocinium redox couple (see this issue of *Nature*, p.533).

The efficiency of 10 per cent, obtained both with a laboratory lamp and direct sunlight, dwarfs that of previous cells based on p-silicon, putting it high in any league table of 'wet' photovoltaic cells. At the same time, the Stanford group has demonstrated a major departure from the expected behaviour of p-type silicon in contact with a non-aqueous electrolyte and have shown how certain intrinsic limitations on cell performance expected from the behaviour of the semiconductor–electrolyte interface are not apparent in their cell.

To a large extent, the major problems of the liquid-junction photovoltaic cell (see our discussion, *Nature* **300**, 687; 1982) have been solved by the replacement of aqueous with non-aqueous electrolytes. By the rigorous exclusion of water, semiconductor corrosion is almost completely suppressed. These solvents also allow a far wider range of redox couples to be used, so that difficulties of poor electron-transfer kinetics can be overcome. But the use of non-aqueous media introduced a further complication — an apparent limitation of the photovoltage obtainable from a cell, a phenomenon now known as 'Fermi level pinning'.

The phenomenon is this. With a number of semiconducting materials, the observed photovoltage seems to be more or less constant for each material and independent of the redox couples in solution with which it may be in contact. But on the conventional model of such an interface, the photovoltage should reflect the difference between the redox potential in solution and the Fermi level of the semiconductor. The observation that the photovoltage is substantially constant thus implies a constant difference between the redox potential and the Fermi level, which in turn suggests that the redox couples in the non-aqueous medium are in some way 'pinning' the Fermi level.

The explanation lies in the charge distribution at the interface. On the conventional view, there should be a depletion layer beneath the surface of the semiconductor across which most of the potential difference between the bulk semiconductor and the bulk electrotype should be dropped. A change in the redox potential should then alter the potential gradient within the depletion layer and thus the photovoltage. But if there are electronic states at the semiconductor surface which can be charged and discharged, the potential gradient at the surface will be substantially different from that expected and the interface more like the junction between a metal and an electrolyte. Such surface states may arise from bulk defects in the crystal lattice, from dangling bonds at the surface or by the adsorption of charged species onto the surface. The overall result is a characteristic photovoltage for each junction between a semiconductor and an electrolyte which is independent of the redox couple. It has recently been thought that behaviour of this type is common among small band-gap semiconductors, precisely those which are of most use for solar conversion, p-type silicon in particular. So Fermi pinning and the consequent loss of photoconversion efficiency has been regarded as the last nail in the coffin for liquid-junction photovoltaic devices.

The latest results from the Stanford group are particularly important because they show that device performance is not necessarily limited by the intrinsic properties of the interface between p-Si and the non-aqueous electrolyte. The explanation is not yet clear, but it seems likely that there is an obverse phenomenon to 'Fermi pinning' which might be called 'redox-pinning'. In a photovoltaic device of the type described, both oxidized and reduced forms of the couple would accumulate at the surface. If the components of the redox couple can communicate with the bulk of the semiconductor much faster than the surface states, the filling of these states will be effectively determined by the relative concentrations of the components of the redox couple in solution. And if these vary little with the degree of bias on the semiconductor, then the junction will behave as a classical device.

It now remains to be seen whether these important findings can be translated into a working cell configuration without loss of conversion efficiency. □

S. Dennison is at the Inorganic Chemistry Laboratory, University of Oxford, Oxford OX1 3QR.

Molecular biology

Selfish DNAs with self-restraint

from W.F. Doolittle, T.B.L. Kirkwood and M.A.H. Dempster

TRANSPOSABLE elements are small discrete genetic elements which can be replicated independently of the chromosomes in which they are embedded. Mechanisms of independent replication may vary, but the results are almost always the same — new copies appear at new genomic positions without loss of original copies[1]. Models of how transposition could occur have been with us for some time and now we are beginning to see models of how the process could be regulated.

In a recent issue of *Cell*, Kleckner and her colleagues describe the inwardly and outwardly directed promoters of IS10-Right, one of the two copies of the insertion sequence IS10 which form the terminal repeats of the bacterial transposon *Tn*10 (refs 2,3). Transcription of the IS10-encoded transposase gene initiates at the weak inward promoter pIN. The stronger pOUT is responsible for activation of genes distal to the site of IS10 or *Tn*10 insertion. The two are transcriptionally independent, but active pOUTs inhibit, in *trans*, the expression of transposase genes. This translational inhibition is mediated, Simons and Kleckner[3] suggest, "by direct pairing between the transposase messenger RNA and a small, complementary, regulatory RNA specified by the IS10-encoded pOUT promoter".

This molecular model makes excellent sense of the genetic phenomenon of multicopy inhibition — the suppression of the transposition-related functions of a single *Tn*10 transposon by multiple copies of its IS10 terminal repeats elsewhere in the cell. It also makes evolutionary sense. Transposable elements are viewed by some as

0028-0836/84/060501-02$01.00

© 1984 Macmillan Journals Ltd

molecular parasites[4,5]. Good parasites might wish to be gentle with hosts. (This is, for instance, the textbook explanation for the rapid loss of effectiveness of the myxomatosis virus introduced to control Australian rabbit populations in the 1950s — an attenuated parasite spreads more slowly but more surely through a larger and healthier host population[6].)

The eukaryotic transposable element P, an inhabitant of the *Drosophila* genome, provides another intriguing example of self-restraint at the molecular level[7]. Some 30–50 partial or complete copies are borne by *Drosophila* strains designated P. The mating of P males with females from element-free M strains produces sickly and nearly infertile ('dysgenic') hybrids as a direct result of unrestrained P replication. Hybrids which do survive produce, over several generations, flies of increasing vigour with increasing tolerance to mating with P males. The hybrid stock has itself become P, and is said to have acquired P cytotype.

There are many ways this could be explained but the recent model of O'Hare and Rubin[8] has particular appeal. P encodes, they suppose, a transposase and a regulator of transposition. The latter not only affects transposase activity (or synthesis) negatively, but its own activity (or synthesis) positively. Thus "in a dysgenic cross, P factor DNA is introduced into a background where there is neither transposase nor regulator, and expression of both functions leads (at least initially) to transposition. With time, positive feedback by the regulator of its own level of activity produces enough regulator to shut off transposition and the P cytotype is established".

The evolutionary value of self-restraint in transposable elements seems obvious intuitively, but it is always reassuring to have intuition confirmed by theory. The mathematics can be made fairly simple[9]. If χ is the average copy number of the element per cell, the net rate of multiplication for each cell in a (potentially) exponentially growing host population can be written as an expression in three terms, $\varrho(\chi) = \phi(\chi) - \gamma \, d\chi/dt - \omega$. $\phi(\chi)$ is the rate of cell division, which may be diminished as the element load increases; γ is the probability that each new transposition causes lethal mutation; and ω is the basal level of cell mortality independent of the element load. For an element without any form of restraint, both χ and $d\chi/dt$ will increase exponentially with time and in due course $\varrho(\chi)$ becomes negative. This may be due partly to declining $\phi(\chi)$, but mainly to rapidly increasing $\gamma d\chi/dt$. Once $\varrho(\chi)$ is negative, host cells are dying faster than they are born so the host cell population is driven to extinction and with it the element itself. On the other hand, if the element acquires the trick of restraining its transposition so that its copy number is limited to n, then (provided $\phi(n) > \omega$) the cell multiplication rate remains positive as $d\chi/dt$ goes to zero. The upshot is that, although the element may impose some small loss of fitness on its host, measurable as $\phi(0) - \phi(n)$, both of them continue to exist.

It takes little insight to deduce that, given these two alternatives, the better strategy for the transposon in the long run is to exercize self-restraint. The selection mechanism by which this outcome is reached is not entirely straightforward, however, since the force at work is the comparatively weak evolutionary process of group selection[10]; that is, it is through competition between groups, or clones, of cells containing both types of element that the unrestrained ones are eliminated and the restrained ones arise. Should competition arise within a clone, as would happen if a *Tn*10 pIN mutated to become unresponsive to the inhibitory effect of pOUT, the unrestrained mutant would enjoy a temporary but strong selective advantage until the clone was extinguished. Theory predicts that this type of behaviour should from time to time be observed, and it will be interesting to see if this is the case. □

W.F. Doolittle and M.A.H. Dempster are in the Departments of Biochemistry and Mathematics respectively, Dalhousie University, Halifax, Nova Scotia B3H 4H7. T.B.L. Kirkwod is with the National Institute for Medical Research, The Ridgeway, London NW7 1AA.

1. Shapiro, J.A. (ed.) *Mobile Genetic Elements* (Academic, London, 1983).
2. Simons, R.W., Hoopes, B.C., McClure, W.R. & Kleckner, N. *Cell* **34**, 573 (1983).
3. Simons, R.W. & Kleckner, N. *Cell* **34**, 683 (1983).
4. Doolittle, W.F. & Sapienza, C. *Nature* **284**, 604 (1983).
6. Ayala, F.J. & Vantine, J.W. *Evolving: the Theory and Process of Organic Evolution* (Cummings, Menlo Park, California, 1979).
7. Bregliano, J.C. & Kidwell, M.G. in *Mobile Genetic Elements* (ed. Shapiro, J.A.) 363 (Academic, London, 1983).
8. O'Hare, K. & Rubin, G.M. *Cell* **34**, 25 (1983).
9. Kirkwood, T.B.L., Dempster, M.A.H., Rose, M.R. & Doolittle, W.F. (in preparation).
9. Maynard Smith, J. *Q. Rev. Bio.* **51**, 277 (1976).

Waves and particles: the diffraction of electrons

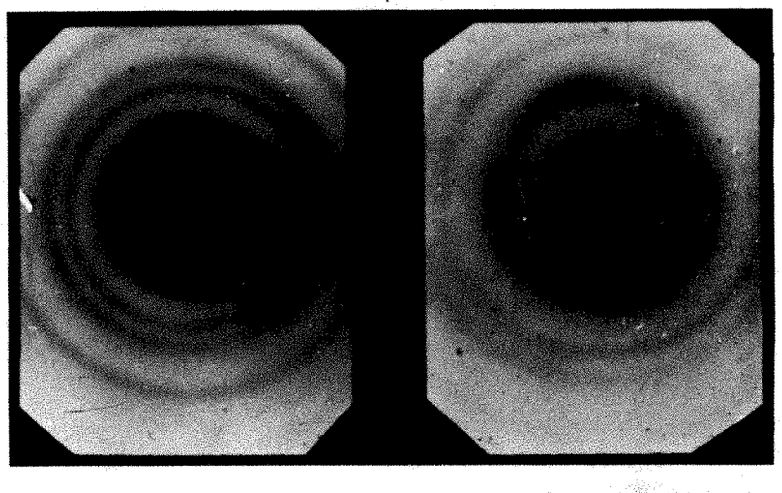
LOUIS DE BROGLIE sparked one of the most productive controversies in twentieth-century physics when he suggested that a frequency could be associated with a fundamental particle of matter as though it consisted of a group of waves. Could wave-particle duality be experimentally demonstrated?

Two independent experiments performed in 1927 implied that it could: the first, a study by Clinton Davisson and Lester Germer, of the scattering of a narrow beam of electrons off a nickel crystal; the second, by George Paget Thomson, of the diffraction of electrons transmitted through thin films. Their respective detectors were a galvanometer and a photographic plate. In Thomson's first experiment, electrons from a cathode-ray tube traversed an extremely thin film of celluloid 10 cm in front of the photographic emulsion, the whole apparatus being evacuated to avoid attenuation of the electron beam by air molecules. A pattern of concentric rings certainly appeared, but the argument for electron diffraction was not quite watertight since the atomic structure of cellulose was not yet accurately known.

Gold foil was substituted to provide a crystalline lattice of known structure, and shown here are the photographs which Thomson initially obtained (October 1927 at the University of Aberdeen). Larger rings (right) are associated with lower energies, as measured by the spark gap of the induction coil. The patterns, wrote Thomson later, "at once gave rings which agreed quantitatively with the theoretical expectation for a face-centred cubic lattice of side 4.06 Å". He concluded that the detailed agreement between his studies of electron diffraction and the de Broglie theory "must, I think, be regarded as strong evidence in its favour".

The photograph is reproduced by courtesy of the Science Museum (Natural History), where Sir George Thomson's original apparatus is also displayed. □

Selected from Beyond Vision *by Jon Darius (Oxford University Press, April 1984).*



0028-0836/84/060502-01$01.00

© 1984 Macmillan Journals Ltd

Gene expression

DNA methylation — how important in gene control?

from Adrian P. Bird

Is DNA methylation a key element in the regulation of vertebrate gene expression, or does it play a minor part? There is support for both views in the recent literature, for while it is now clear that *in vitro* methylation inhibits the transcription of several genes (see *News and Views* **296,** 602; 1982)[1], some *in vivo* studies raise doubts that reduced methylation is always a necessary precondition for gene expression.

Experiments with mouse retroviruses have provided strong evidence that DNA methylation can suppress transcription. Integrated viral sequences that are endogenous to mice are non-infectious and heavily methylated. This natural situation can be mimicked by introducing unmethylated proviral DNA into preimplantation embryos or embryonal carcinoma cells[2]. No infection results, and the surviving integrated copies of the provirus are found to be heavily methylated. Purified DNA from each of these sources is non-infectious, but infectivity can be restored by treating the cells with 5-azacytidine, an inhibitor of methylation, or by recovering the integrated provirus after cloning in bacteria[2–4]. Since molecular cloning causes loss of methylation at CpG sequences — the major site of methylation in animals — it seems likely that lack of infectivity is caused by methylation.

Confirmation is provided by the observation that remethylating the cloned proviral DNA with a rat liver methyltransferase abolishes infectivity once more[5]. As well as rounding off the experimental logic, these studies make the important point that methylation is inhibitory only at certain CpGs, as methylation of a subset of CpGs with the *Hpa*II methylase does not inhibit infection. Since *Hpa*II is commonly used to analyse methylation of genomic sequences, it is salutary that, in this case, *Hpa*II sites alone were not relevant to expression.

Experiments by Busslinger *et al.*[6] also stress the importance of specific CpG sequences. Members of the human β-globin gene family were methylated at CpG by using the ingenious method pioneered by Stein and co-workers[7]. The globin gene is cloned in a single-stranded M13 vector and a second strand is synthesized in which all cytosine residues are methylated. Upon transformation of this DNA into mouse cells, cellular methyltransferases can act on the unmethylated strand only at CpG. Thus when the transformed clone grows, the methylation at CpG sequences is maintained while methylation at cytosine residues in other sequences is lost. The initial experiments showed that cells which have been transfected with both a methylated γ-globin gene (which becomes methylated at all its CpG sequences) and an unmethylated β-globin gene (which remains unmethylated) were able to express the β-globin gene but not the γ gene. Thus methylation appears to be inhibiting transcription.

To extend this result, Busslinger *et al.* synthesized molecules in which certain segments were protected from methylation. When regions upstream of the 5′ end were left unmethylated, but the transcribed region was methylated, transcription of the γ-globin gene was seen. But when this pattern of methylation was reversed, so that the 5′ flanking regions were methylated, transcription was abolished. These and other experiments establish that somewhere between 760 base pairs (bp) upstream and 100 bp downstream of the 5′ end of the γ-globin gene are one or more CpG sequences whose methylation prevents transcription in mouse cells. Methylation at CpG sequences elsewhere is apparently irrelevant to transcription in these cells.

Of course, in order to be sure that loss of methylation is really part of globin gene activation *in vivo*, it is necessary to find out whether the relevant CpG sites are ever methylated in cells which do not synthesize globin. This is particularly important given that the hamster adenosine phosphoribosyl transferase gene is inhibited *in vitro* by methylation[8], but has recently been discovered to be unmethylated at the relevant sites in most, if not all, tissues[9]. Consistently unmethylated domains, regardless of expression, have also been observed at the 5′ end of the mouse dihydrofolate reductase gene[9] and the chicken α2(I) collagen gene[10], suggesting that changing levels of methylation are not a component of regulation for these genes either.

The existence of genes with what may be constitutively unmethylated 5′ domains does not contradict the idea that DNA methylation interferes with transcription, but merely emphasizes that absence of methylation is not sufficient to ensure transcription. There is, however, a case where heavy methylation of a purified gene appears to have no effect on transcription[11]. At the time in embryogenesis when the genes neighbouring them become active, ribosomal RNA genes (rDNA) of *Xenopus laevis* lose CpG methylation at certain spacer sequences. In sperm rDNA, though, the CpG sequences of the spacer region, including all 19 sites in the promoter, are highly methylated. Even so, sperm rDNA is transcribed just as efficiently as cloned (that is, unmethylated) rDNA upon injection into the nuclei of *Xenopus* oocytes; and transcription does not seem to be due to loss of methylation following injection. By contrast, methylation of two viral genes inhibits their transcription in the oocyte[12,13]. Although rDNA may not qualify as a typical vertebrate gene, methylation and transcription clearly are not always inimical.

Other exceptional genes have been hinted at by experiments in which methyl-sensitive restriction endonucleases were used to follow hormonally induced changes in gene expression. In the case of the chicken vitellogenin gene, transcription after treatment with oestrogen is associated with loss of methylation at a CCGG site about 600 bp upstream of the 5′ end[14–17]. Other sites in the vicinity of the gene remain methylated regardless of transcription. What is surprising, though, is that loss of methylation at this *Hpa*II site is gradual and follows, rather than precedes, the peak of vitellogenin gene transcription. Moreover, the same *Hpa*II site is gradually demethylated in oviduct, a tissue that does not transcribe the vitellogenin gene. In another species, *X. laevis,* all 20 testable CpG sequences at the A2 vitellogenin gene are as highly methylated in hormone-stimulated liver as in unstimulated liver and blood cells[18].

On the face of it these findings seem to violate the conventional view that undermethylation is a necessary precondition for transcription. There is, however, an acknowledged weakness in this kind of experiment — only a minority of CpG sequences are within a sequence that can be recognized by one of the methyl-sensitive restriction endonucleases. Thus failure to find a correlation between expression and methylation may arise simply because the

1. Felsenfeld, G. & McGhee, J. *Nature* **296,** 602 (1982).
2. Jahner, D., Stewart, C.L., Stuhlman, H., Harbers, K. & Jaenisch, R. *Cold Spring Harb. Symp. quant. Biol.* **47,** 611 (1982).
3. Harbers, K., Schnieke, H., Stuhlmann, H., Jahner, D. & Jaenisch, R. *Proc. natn. Acad. Sci. U.S.A.* **78,** 7609 (1981).
4. Hoffmann, J.W. *et al. J. Virol.* **44,** 144 (1982).
5. Simon, D. *et al. Nature* **304,** 275 (1983).
6. Busslinger, M., Hurst, J. & Flavell, R.A. *Cell* **34,** 107 (1983).
7. Stein, R., Gruenbaum, Y., Pollack, Y., Razin, A. & Cedar, H. *Proc. natn. Acad. Sci. U.S.A.* **79,** 61 (1982).
8. Stein, R., Razin, A. & Cedar, H. *Proc. natn. Acad. Sci. U.S.A.* **79,** 3418 (1982).
9. Stein, R., Sciaky-Gallili, N., Razin, A. & Cedar, H. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2423 (1983).
10. McKeon, C., Ohkubo, H., Pastan, I. & de Crombrugghe, B. *Cell* **29,** 203 (1982).
11. Macleod, D. & Bird, A.P. *Nature* **306,** 200 (1983).
12. Vardimon, L., Kressmann, A., Cedar, H., Maechler, M. & Doerfler, W. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1073 (1982).
13. Fradin, A., Manley, J.L. & Prives, C.L. *Proc. natn. Acad. Sci. U.S.A.* **79,** 5142 (1982).
14. Wilks, A.F., Cozens, P.J., Mattaj, I.W. & Jost, J-P. *Proc. natn. Acad. Sci. U.S.A.* **79,** 4252 (1982).
15. Burch, J.B.E. & Weintraub, H. *Cell* **33,** 65 (1983).
16. Folger, K., Anderson, J.N., Hayward, M.A. & Shapiro, D.J. *J. biol. Chem.* **258,** 8908 (1983).
17. Meijlink, F.C.P.W., Philipsen, J.N.J., Gruber, M. & A.B.G. *Nucleic Acids Res.* **11,** 1361 (1983).
18. Gerber-Huber, S. *et al. Cell* **33,** 43 (1983).
19. Maxam, A.M. & Gilbert, W. *Meth. Enzym.* **65,** 499 (1980).
20. Urieli-Shoval, S., Gruenbaum, Y., Sedat, J. & Razin, A. *FEBS Lett.* **146,** 148 (1982).
21. Bird, A.P. & Taggart, M.H. *Nucleic Acids Res.* **8,** 1485 (1980).

0028-0836/84/060503-02$01.00

© 1984 Macmillan Journals Ltd

endonucleases do not detect the relevant sites.

How can the inherent weaknesses of the restriction enzyme approach be overcome? The answer may lie with an unlikely-sounding, but recently perfected, technique for sequencing unique stretches of DNA in unfractionated genomic DNA. George Church of Harvard University has used a sophisticated blend of blot hybridization and Maxam–Gilbert[19] sequencing to locate all 5-methylcytosine residues in a region of the globin gene of transformed mouse cells (see ref. 4). It would now be interesting to use this technique to monitor other CpG sequences during activation of the vitellogenin genes.

In evaluating recent data on gene expression and DNA methylation, it may be helpful to take an evolutionary perspective, starting from the observation that the level of CpG methylation is considerably higher in vertebrates than in invertebrates. Apart from the much quoted case of *Drosophila*, in which methylated cytosine residues have not been detected at all[20], other invertebrates generally have a minor fraction of heavily methylated DNA in their genome[21]. No clear example of a methylated gene has yet been detected in invertebrates. Since these animals nevertheless exhibit cellular determination and differentiation, it seems likely that, in animals, DNA methylation was not involved in the evolution of these basic mechanisms.

Why, then, are the majority of vertebrate genes methylated? One possibility is that DNA methylation in invertebrates has a purely suppressive effect — simply being used to condemn sequences to transcriptional silence — while in vertebrates, the spread of methylation has elicited mechanisms that allow genes to tolerate and, in some cases, to exploit methylation for the control of expression.

There are three ways in which a gene might circumvent suppression by DNA methylation. First, key sequences might be maintained in an unmethylated condition by rendering them refractory to methylation, as in the case of genes whose 5′ domains are unmethylated in expressing and non-expressing tissues alike[8,9]. Second, genes may tolerate methylated bases at their regulatory sequences, as the ribosomal RNA genes of *Xenopus* appear to do. Last, expression of the gene may be suppressed by methylation in inappropriate tissues, but in tissues where the gene is to be expressed transcription can proceed following demethylation. It is this role for methylation that has hitherto attracted most interest, and it is to this category that the globin genes may belong.

A demethylation step is usually envisaged as a pre-requiste for transcription of genes regulated by loss of DNA methylation. A weakness of this view is that to regulate genes separately, a distinct mechanism is required to demethylate each kind of gene — it is difficult to see why this should have evolved. An alternative control mechanism, and one whose evolution is perhaps easier to envisage as an ancestral gene moved from the unmethylated to the methylated condition, is that activation of the gene begins with the gene still in a methylated state. Activation then leads to demethylation which, in turn, relaxes control of the gene. Here, demethylation is not thought of as a mechanism to facilitate initial activation of the gene, but to simplify continued expression once activation has occurred, perhaps by bringing the gene under more 'everyday' control mechanisms.

Although we do not yet know how any eukaryotic gene is regulated, preliminary evidence suggests that the degree of involvement of DNA methylation is variable. This is consistent with the view that methylation of genes was a relatively late occurrence in animal evolution, and it suggests that we should not expect a coherent unified mechanism for control of gene expression based on DNA methylation. In order to learn just how much of gene control is due to modulation of DNA methylation patterns, we must await future studies on a gene-by-gene basis. □

Adrian P. Bird is at the MRC Mammalian Genome Unit, King's Buildings, West Mains Road, Edinburgh EH9 3JT.

Biotechnology

Time for plant cell culture?

from M. W. Fowler

WITH the announcement last year by Mitsui Petrochemical Industries of a process for the production of the pharmaceutical and dye shikonin by large-scale plant cell culture, plant cell biotechnology at last became a reality on the industrial scene. Although the scale of activity (some 750 litres bioreactor capacity) is small in comparison with large-scale microbial fermentation processes, the shikonin process nonetheless marks an important first step forwards in plant cell biotechnology. Hitherto this area of biotechnology had been considered by many to be 'just another way of looking at plant biochemistry' rather than as a genuine alternative way of manufacturing plant natural products.

Plant cell culture may prove of particular value in the future in situations where a new chemical structure with interesting properties has been identified but the source plant is particularly difficult to grow. Of course, the possibility of deriving the compound by organic synthesis must be considered first — but there again there are now several reports of the synthesis by cell cultures of chemical structures that are quite unique. Before cell culture sets off in new directions, however, the technology must first be proved on an existing product.

In Japan, the Japanese Salt and Tobacco Monopoly has made particular progress towards the development of a tobacco biomass system and has used conventional stirred tank bioreactors (fermenters) at volumes in excess of 6,000 litres. Pharmaceuticals have been the target in West German laboratories and substantial success has been achieved at the University of Tübingen in the development of a process for the biotransformation of low-value digitoxin to the important and high-value cardiatonic digoxin.

Plant cell cultures are maintained in liquid culture in much the same way as yeasts and other microbial cells, but there are some major differences: plant cells grow much more slowly than microbial cells and doubling times of the order of 20–150 h are typical. Sub-culture periods are thus much longer. Also, it is easy to maintain microbial species in a culture bank, either lyophilized or cryopreserved, but similar techniques are only just being developed for plant cell cultures. In consequence, large liquid culture banks are needed to support plant cell cultures. Even if cell preservation techniques do become viable, the long time required to grow up plant cell cultures still means that careful planning is essential to initiate large-scale cultures.

On present-day costings, plant cell culture is likely to be considered only for high-value low-market-volume products. In general, products have to have a whole sale value of at least $250-500 per kg to make them worth considering as targets for a cell culture process. Once one or two processes have been developed successfully, however, the increased know-how and confidence are likely to lead to a widening of the target window.

What are likely to be the key developments in the years ahead? The comparatively few large-scale systems that are being actively tested have not encountered major problems. Fairly sophisticated technologies such as fluidized bed and immobilized cell systems are also showing rapid development. One area which is in urgent need of consideration is downstream processing, the unglamorous but often highly expensive end of the business. It is back in the cell however that the biggest problems still face us. Lack of the fundamental biochemical and genetic information that would enable control of product yields is the chief constraint on the development of plant cell biotechnology and there is much fundamental research that needs to be done. □

M. W. Fowler is Director of the Wolfson Institute of Biotechnology at the University of Sheffield, Sheffield S10 2TN.

0028-0836/84/060504-01$01.00

© 1984 Macmillan Journals Ltd

Deep seismicity and modes of deformation in Tonga subduction zone

Domenico Giardini & John H. Woodhouse

Department of Geological Sciences, Harvard University, Cambridge, Massachusetts 02138, USA

The geometry of deep (>400 km) earthquakes in the subducting western edge of the Pacific plate, when related to the local distribution of seismic activity, reveals a complex pattern of planes of shear failure, enabling the modes of deformation by which the descending plate shortens and thickens to be identified. The deepest part of the slab is displaced laterally, suggesting that it fails to penetrate the discontinuity at 670 km.

DEEP earthquakes occur in steeply dipping zones—Benioff zones—which, with the advent of plate tectonics, have been recognized as the trajectories of oceanic lithosphere subducted deep into the Earth's mantle[1]. Although most Benioff zones terminate at shallower depths, there is an absolute cutoff in activity at 650–700 km, and this has often been taken as *prima facie* evidence that subduction does not penetrate into the lower mantle. It is possible, however, that the cessation of seismic activity corresponds to the point in pressure–temperature space where catastrophic failure ceases to be a possible mechanism of deformation, in which case no inference can be drawn concerning the subduction of material to greater depths[2,3]. The question of whether or not subduction continues into the lower mantle is of great importance for our understanding of mantle convection and for models of the chemical evolution of the Earth because of its bearing on the question of whether the upper and lower parts of the mantle are isolated, separately convecting systems, or whether they are parts of a single, mantle-wide and chemically mixed convective flow[4–7]. For this reason we have studied the seismicity in one of the deepest and most active Benioff zones—the Tonga Arc—to obtain information concerning the deformation of material at great depth.

There has been much research related to the possible mechanisms of deep earthquakes. It has long been recognized that under conditions of temperature and pressure appropriate to the mantle, brittle failure is an unlikely mechanism for deep earthquakes and other mechanisms by which shear failure can occur have been put forward[8–11]. The occurrence of sudden phase transformations in the subducting lithosphere has been considered as a possible mechanism[12–15], or as a triggering mechanism[16], for deep events; the seismic evidence, however, favours a mechanism dominated by shear failure, as is the case for shallow earthquakes[17–19].

From seismic radiation it is possible to identify a pair of orthogonal planes at the focus—the nodal planes—which are the possible planes of shear failure. Extensive studies have revealed that the orientation of these planes is indicative of systematic shortening or extension in the down-dip direction[17,20]. However, the existence in deep Benioff zones of persistent planes of failure, or faults, has been an open question. Such planes may be expected to manifest themselves as lineations in the spatial distribution of hypocentres, but evidence of this kind has been obtained for only a few events[21–24].

In the Benioff zone associated with the Tonga trench, earthquakes almost invariably indicate down-dip shortening. We have determined[25–27] the moment tensors of 49 events in Tonga at depths >400 km, using the 'centroid-moment tensor' (CMT) technique of Dziewonski *et al.*[28]. This technique uses digitally recorded waveforms from the Global Digital Seismograph Network (GDSN) including all body wave phases; this enables us to obtain reliable results for the moment and the geometry of seismic sources using, in some cases, as few as six stations. An adequate digital data set has been available only since 1977 and the 49 CMT events constitute all earthquakes in Tonga deeper than 400 km large enough for the CMT technique to be applied.



Fig. 1 Map view of the south-west Pacific showing earthquakes with body wave magnitude $m_b > 4$ from the ISC catalogue 1964–79. All the events in the Tonga Benioff zone deeper than 300 km have been relocated using the technique of joint hypocentre determination.

To obtain an accurate representation of the seismic activity in the neighbourhood of each CMT event we have relocated some 2,200 events, deeper than 300 km, using P-arrival times reported by the International Seismological Centre (ISC, 1964–1979). Relocation was performed using the technique of joint hypocenter determination[29,30] (JHD). Estimates of the reliability of each hypocentre were obtained, and events having poorly determined coordinates were rejected.

One of our principal observational findings is that for more than 40 of the 49 CMT events it is possible to identify planar alignments in the pattern of relocated hypocentres in

0028-0836/84/060505—05$01.00

© 1984 Macmillan Journals Ltd

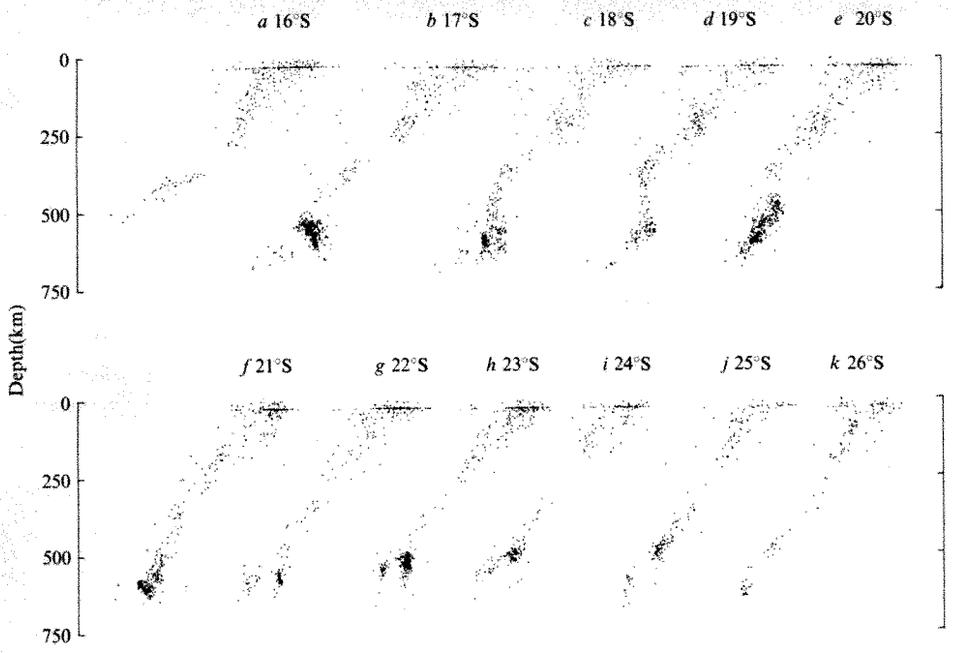


Fig. 2 Vertical sections through the Tonga Benioff zone between 16 and 27° S. The sections are along parallels of latitude, viewed from the south. Each is 1° thick and includes seismicity to the south of the indicated parallel.

approximate agreement with one, or occasionally both of the nodal planes; from this we infer the existence of planes of shear failure. The complex pattern of shear zones identified by this technique enables us to begin to elucidate the structural geology of the subduction zone.

Morphology

The Tonga and New Hebrides subduction zones form a highly complex part of the Melanesian boundary between the Indian–Australian plate and the Pacific plate (Fig. 1). The Benioff zone studied here is associated with the subduction of the Pacific plate to the west beneath the Indian–Australian plate and both the characteristics of the seismicity[31–35] and the nature of stress release in the subducting slab[6,17,35–37] have been studied. The inclined seismic zone terminates in the characteristic 'hook' shaped region of intense deep activity. All of the CMT events analysed in this study are located within this 'hook' (Fig. 4).

Figure 2 shows a sequence of vertical sections through the Benioff zone, having a thickness of 1° in latitude. Notable here is the extreme distortion and variable geometry of the zone. The separation of the seismicity into double seismic features, at great depth, is evident in several sections (Figs 1, 2*b*, *c*, *g*–*j*). Figure 3 shows a projection onto a vertical meridional plane, looking from the west, and displays some aspects of the vertical distribution of events.

For our later discussion we identify four principal regions within the zone of deep activity (see Fig. 3):

The northern region (17°–19° S): Intense seismic activity at the northern end of the 'hook' forms an inclined column. Its vertical extent can be seen in Fig. 3. A weak secondary line of activity flanks the region to the south-west (Fig. 1).

The central region (19°–21.5° S): This is a region of diffuse seismicity dipping at a shallower angle than other parts of the deep seismic zone (Figs 2*d*–*f*, 3).

The southern region (21.5°–27° S): This region is characterized by two parallel bands of seismicity (Fig. 1). The weaker band lies to the west and penetrates to greater depths, particularly in the south (Fig. 2*g*–*k*).

The gap (22.3°–23.2° S): The principal band of deep seismicity is interrupted by a distinct gap (Figs 1, 3). This gap appears to correspond with the gap in shallow activity (Fig. 1) which separates the Tonga and Kermadec Trenches, and coincides with the intersection of the Louisville Ridge with the trench at ~25° S.

Moment tensor solutions

We shall discuss here a set of 17 moment tensor solutions and their implications for deformation within the subduction zone. Many of these are representative of families of solutions showing the same behaviour. The moment tensor is a second rank symmetric tensor, which may be characterized in terms of its principal axes and its eigenvalues. The moment tensors determined in this study are constrained to have zero trace, corresponding to a source without volume change, and thus the sum of the eigenvalues is zero. A positive eigenvalue represents extension at the source and a negative eigenvalue corresponds to shortening. The largest eigenvalue is positive and is associated with the tension axis (T-axis) and the smallest eigenvalue is negative and is associated with the compression axis (P-axis). The intermediate eigenvalue is smallest in absolute value. In agreement with previous studies[6,17,35] we find that in Tonga the P-axis is usually in the down-dip direction, indicating net shortening of the zone. This can be seen in Fig. 4, where the P-axis is indicated by '+'. The moment tensor solutions are represented here in terms of an equal area projection of the lower focal hemisphere. The intermediate axis is at the intersection of black and white quadrants.

A source represented by a shear dislocation has a vanishing intermediate eigenvalue and P and T eigenvalues of equal magnitude, with opposite signs. Such a source is said to have a 'double couple' mechanism. We find that in Tonga the compressive eigenvalue tends to be the largest in absolute value (though other subduction zones exhibit different behaviour[26,38]). Here, however, we shall neglect this departure from the condition for plane shear failure, and concentrate on the geometry of the 'best double couple' solution.

The planes separating black from white in Fig. 4 are orthogonal nodal planes, which intersect in the intermediate axis and are inclined at 45° to the other two axes. The best double couple solution may be interpreted in terms of shear failure on either of these planes, in which relative motion is perpendicular to the intermediate axis and is in the sense which would take a material particle from a white into a black quadrant.

In Fig. 5 we have taken, as the centre of our field of view, events for which CMT solutions have been found, and displayed the local pattern of seismicity as seen from the direction of the intermediate axis. From this direction any lineations in the pattern of seismicity associated with planes of shear failure should be apparent, and should be aligned with one of the nodal

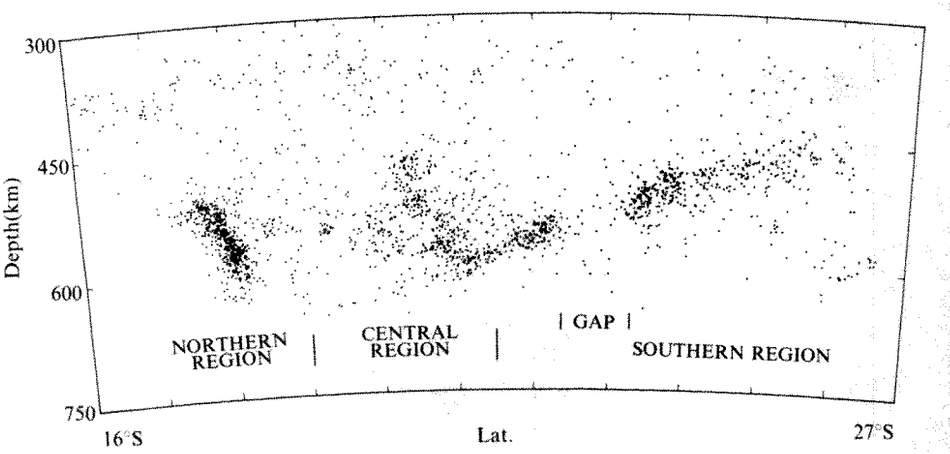


Fig. 3 Lateral projection of the seismicity on a vertical meridional plane viewed from the west. The subdivisions correspond to subregions of the Benioff zone identified in the text.

planes. We find that such lineations are nearly always discernible in the seismicity patterns of the relocated events, and we interpret these lineations as zones of shear failure. Viewed from the direction of the intermediate axis the nodal planes appear as straight lines which divide the circle into quadrants. The direction of maximum shortening (the P-axis) lies in the white quadrants and the T-axis lies in the blackened quadrants. These are orthogonal and are inclined at 45° to the nodal planes.

In each part of Fig. 5 the latitude, longitude and moment of the event are indicated, together with the direction of view and the scale of the box. Figure 5*a*, for instance, is centred on an event of 21 January 1977 (moment, 10^{25} dyn-cm), viewed from an azimuth of 158°, with the line of sight inclined slightly upwards, at an angle of 3° to the horizontal. The sides of the box are of length 300 km and the depth of the box in the direction of the line of sight is 160 km. A set of criteria on size, reliability of location, and azimuthal coverage of stations has been specified in each part of Fig. 5 to clarify the feature which we believe to be associated with the CMT event. In a few cases it has proved difficult to separate such features from the background seismicity which presumably is indicative of other modes of deformation; in such cases the time sequence of events has often convinced us of the reality of the lineation.

Northern region

Figure 5*a–c* shows events located in the northern region (Figs 3, 4). In Fig. 5*a* we would identify the approximately vertical lineation as a plane of shear failure. Figure 5*b* shows the same cluster of events seen from a steep angle. The events on the right in Fig. 5*c* are coincident with some of the events of Fig. 5*a*, but here the CMT event lies in the weak secondary zone of seismicity lying to the south-west (Fig. 4). In this case there are rather few events to define the lineation; the pattern is very similar, however, to the patterns observed in southern Tonga to be discussed below (Fig. 5*l–r*).

Central region

CMT events in the central region (Figs 3, 4) may be classified into two distinct families. Figure 5*d–f* shows representatives of the first family, and indicates a shearing deformation approximately in the plane of the Benioff zone. The second family (Fig. 5*h, i*) shows out-of-plane shear. Events in the first family (Fig. 5*d–f*) all show motion in the same sense within three subhorizontal lineations, gently dipping to the north and located at different depths (event in Fig. 5*f* is located in the lowermost lineation). This indicates a mode of deformation of subhorizontal shearing of the zone. A cross-cutting conjugate lineation is also apparent (Fig. 5*d*). The events of Fig. 5*f, g* show motion on different planes which are internal to the lowermost shear zone and are indicative of an organized internal structure. Events of the second family are shown in Fig. 5*h, i*. A complex structure of subhorizontal planes dipping at ~30° to the north-west becomes apparent.

We infer that in this central zone there are simultaneously active modes of shear deformation both in and out of the plane of the Benioff zone. Both the moment tensor solutions and the seismicity patterns indicate thickening and bottoming out of the descending material, and suggest that subduction experiences great resistance in penetrating the 670-km discontinuity.

As we have noted above, the gap in shallow seismicity which separates the Tonga subduction zone from the Kermadec zone is associated with the intersection of the Louisville Ridge with the trench and this gap appears to correspond to the gap in deep seismicity (Figs 1, 3). If, however, the ridge were embedded in a rigid subducting slab it would intersect the deep seismic activity much farther to the north. It is possible to identify the gap in deep activity with the Louisville Ridge if the entire zone

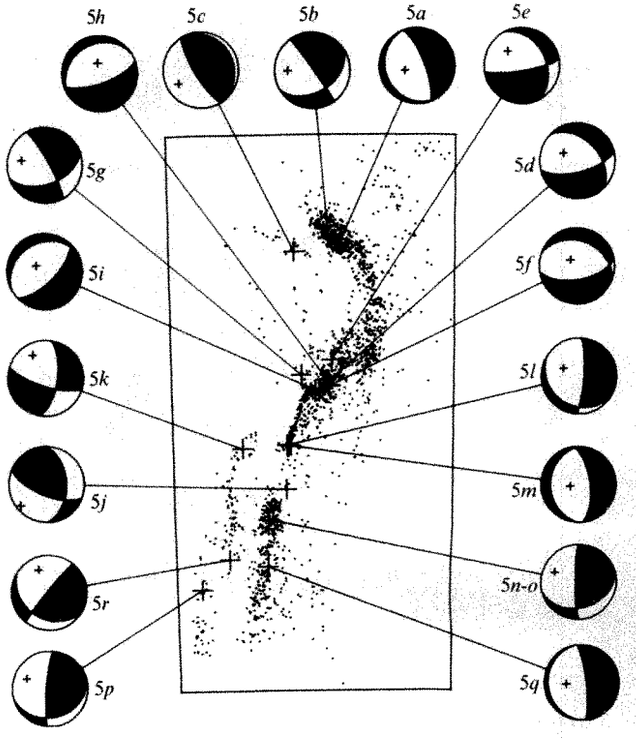
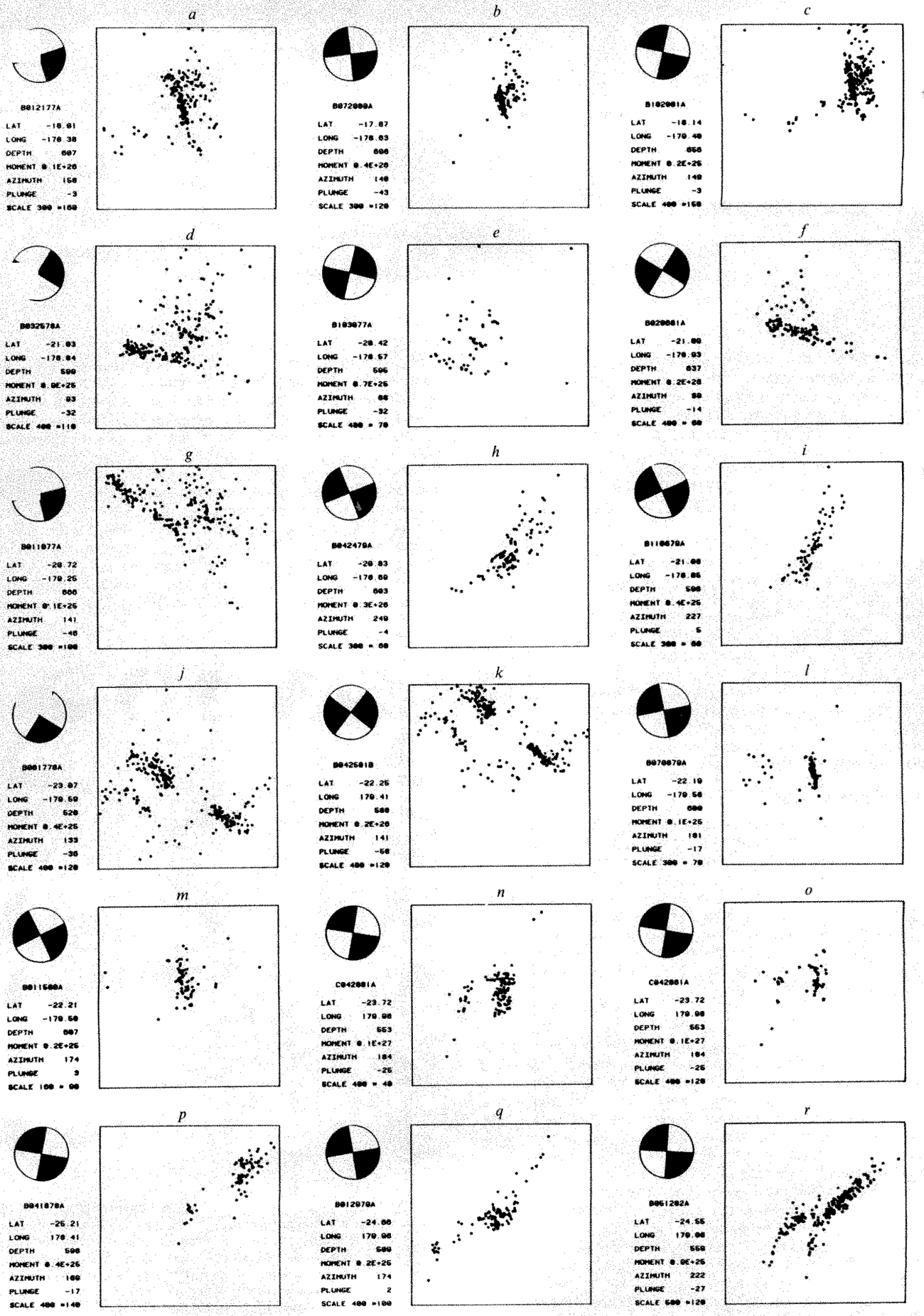


Fig. 4 Focal mechanisms of the selected CMT solutions. For each event the equal area projection of the lower focal hemisphere is shown, viewed from above; '+' represents the compressional axis and the black sectors indicate extension at the source. Relocated seismicity is plotted, deeper than 300 km and with latitude between 27 and 16° S and longitude between 178° E and 176° W. Each CMT event is identified by a number corresponding to events detailed in Fig. 5.


a
B012177A
LAT -18.81
LONG -178.38
DEPTH 607
MOMENT 0.1E+26
AZIMUTH 158
PLUNGE -3
SCALE 300 =150
b
B072099A
LAT -17.87
LONG -178.83
DEPTH 606
MOMENT 0.4E+26
AZIMUTH 148
PLUNGE -43
SCALE 300 =120
c
B102981A
LAT -18.14
LONG -179.48
DEPTH 656
MOMENT 0.2E+26
AZIMUTH 148
PLUNGE -3
SCALE 400 =150
d
B032578A
LAT -21.83
LONG -178.84
DEPTH 590
MOMENT 0.9E+25
AZIMUTH 83
PLUNGE -32
SCALE 400 =110
e
B103077A
LAT -20.42
LONG -178.57
DEPTH 596
MOMENT 0.7E+26
AZIMUTH 86
PLUNGE -32
SCALE 400 = 70
f
B020881A
LAT -21.00
LONG -178.93
DEPTH 637
MOMENT 0.2E+26
AZIMUTH 98
PLUNGE -14
SCALE 400 = 60
g
B011077A
LAT -20.72
LONG -178.25
DEPTH 606
MOMENT 0.1E+25
AZIMUTH 141
PLUNGE -46
SCALE 300 =100
h
B042479A
LAT -20.83
LONG -178.68
DEPTH 603
MOMENT 0.3E+26
AZIMUTH 249
PLUNGE -4
SCALE 300 = 60
i
B110679A
LAT -21.08
LONG -178.85
DEPTH 590
MOMENT 0.4E+26
AZIMUTH 227
PLUNGE 5
SCALE 300 = 60
j
B081778A
LAT -23.07
LONG -179.59
DEPTH 520
MOMENT 0.4E+25
AZIMUTH 133
PLUNGE -38
SCALE 400 =120
k
B042681B
LAT -22.26
LONG 179.41
DEPTH 580
MOMENT 0.2E+26
AZIMUTH 141
PLUNGE -58
SCALE 400 =120
l
B070879A
LAT -22.19
LONG -179.58
DEPTH 600
MOMENT 0.1E+25
AZIMUTH 181
PLUNGE -17
SCALE 300 = 70
m
B011680A
LAT -22.21
LONG -179.58
DEPTH 607
MOMENT 0.2E+25
AZIMUTH 174
PLUNGE 3
SCALE 100 = 90
n
C042881A
LAT -23.72
LONG 179.98
DEPTH 563
MOMENT 0.1E+27
AZIMUTH 184
PLUNGE -25
SCALE 400 = 40
o
C042881A
LAT -23.72
LONG 179.98
DEPTH 563
MOMENT 0.1E+27
AZIMUTH 184
PLUNGE -25
SCALE 400 =120
p
B041078A
LAT -25.21
LONG 178.41
DEPTH 598
MOMENT 0.4E+25
AZIMUTH 100
PLUNGE -17
SCALE 400 =140
q
B012978A
LAT -24.66
LONG 179.98
DEPTH 588
MOMENT 0.2E+26
AZIMUTH 174
PLUNGE 2
SCALE 400 =100
r
B061282A
LAT -24.55
LONG 179.98
DEPTH 559
MOMENT 0.8E+26
AZIMUTH 222
PLUNGE -27
SCALE 500 =120

has suffered horizontal shearing in which deeper material moves relatively south. This is the same sense as the shear inferred from Fig. 5*d–f*.

The gap

Figure 5*j, k* shows two events associated with the gap in deep activity. These are anomalous in that they do not show down-dip shortening and are of opposite sense. We note, however, that they are on opposite sides of the gap, and are both consistent with motion of the material in the gap, approximately in the down-dip direction, at a faster rate than the surrounding material.

Southern region

Figure 5*l–r* shows events in the southern region, both to the north (Fig. 5*l, m*) and to the south (Fig. 5*n–r*) of the gap. The almost vertical lineation is very clear in Fig. 5*l*; this lineation indicates a backward bending of the deepest part of the descending flow (see Fig. 2*g*). Figure 5*m* shows another event in the same cluster; the enlarged scale reveals a pattern of approximately conjugate alignments.

Figure 5*n–r* shows events associated with the double seismic feature in the south. Figure 5*n, o* shows the same event; in Fig. 5*n* a very thin slice has been taken and in Fig. 5*o* only larger events are shown. The seismic zone is very narrow for large events and the more diffuse pattern of Fig. 5*n* appears to be associated with the occurrence of smaller events on conjugate planes (see Fig. 2*g, h*). The vertical alignments in Fig. 5*n–o, r* indicate shear deformation in which material to the east moves relatively down. The event shown in Fig. 5*p* is in the secondary region of seismicity and shows motion in the same sense. The separated double seismic zone is interrupted by a connecting 'bridge' of seismicity at ~25° S (see Fig. 2*i*). Figure 5*q* shows an event located in this 'bridge', and here both horizontal and vertical alignments can be seen. The pattern of parallel, subvertical shear zones in which the sense of motion is such that trenchward material moves relatively down is reproduced by other solutions not presented here. Similar behaviour in the northern region has been noted above (Fig. 5*a–c*).

Figure 5*r*, which we interpret as shear on the central vertical plane, also shows this behaviour and appears to present us with a snapshot of the slab breaking and being displaced sideways as it reaches the bottom of its trajectory. The double seismic feature at the base of the Tonga subduction zone would represent, in this case, the debris of slab unable to penetrate deeper into the mantle.

Discussion

The correlations we have shown between the orientation of the nodal planes of deep earthquakes and the local pattern of seismicity can be found only if the nodal plane is known very precisely. Accurate hypocentral locations are also necessary, but in fact all of the features we have shown are identifiable using published ISC coordinates; the relocation of events serves principally to bring the patterns into better focus.

This analysis enables us to understand the apparently accidental orientations of the nodal planes in terms of fairly well defined modes of deformation—vertical shear in the northern and southern regions, and subhorizontal shear in the more diffuse central region. In each case shortening occurs in the down-dip direction and material is displaced laterally.

The concept of a subduction zone as a smoothly bending, coherent slab, possessing a uniform thickness defined by the seismicity is in need of modification. When seismicity is analysed in detail, we see a complex, cross-cutting pattern of interacting 'shear bands', and we are led, rather, to view the Benioff zone as that part of the convective flow which, by virtue of temperature, composition and strain rate accomplishes its deformation through episodes of shear instability. Viewed on a time scale of several million years the zone would appear to deform smoothly—and perhaps to no lesser extent than the surrounding mantle.

If, over geological time, the nodal planes were randomly oriented with respect to the P-axis, the result would be thickening and shortening without distortion. If, on the other hand, certain orientations remain dominant over long periods, they may lead to distortion and separation of parts of the slab such as we see in Tonga.

The transition to horizontal shear or to lateral separation in the deepest part of the seismic zone is evidence of the strong resistance experienced by the subducted material to penetration of the 670 km discontinuity. We observe horizontal displacement of the seismically active portion of the slab, and infer that material moves laterally rather than vertically at the base of the deep seismic zone. This favours the view that subduction does not continue into the lower mantle, although we clearly cannot infer the fate of the subducted material after it ceases to be seismically active.

We thank Adam M. Dziewonski for discussions, and for providing us with the ISC data sets. We also acknowledge his very large contribution to the development and implementation of the CMT technique, and the free use we have made of a number of computer programs. We also thank Jim Dewey for his computer program for joint hypocenter determination, Richard O'Connell and Brad Hager for discussions and constructive criticism, and Peter Molnar for his valuable comments. This research was supported by NSF grants EAR82-13330 and EAR83-07603.

Received 21 June; accepted 7 October 1983.

1. Isacks, B., Oliver, J. & Sykes, L. R. *J. geophys. Res.* **73,** 5855–5899 (1968).
2. Toksoz, M. N., Sleep, N. H. & Smith, A. T. *Geophys. J. R. astr. Soc.* **35,** 285–310 (1973).
3. Wortel, R. *Nature* **296,** 553–556 (1982).
4. Davies, G. F. *Geophys. J. R. astr. Soc.* **49,** 459–486 (1977).
5. O'Connell, R. J. *Tectonophysics* **38,** 119–136 (1977).
6. Richter, F. M. *J. geophys. Res.* **84,** 6783–6795 (1979).
7. Peltier, W. R. in *Proc. Enrico Fermi Int. Sch. Phys.* Vol. 78 (eds Dziewonski, A. M. & Boschi, E.) 361–431 (North-Holland, Amsterdam, 1980).
8. Orowan, E. *Geol. Soc. Am. Mem.* **79,** 323–345 (1960).
9. Griggs, D. & Handin, J. *Geol. Soc. Am. Mem.* **79,** 347–373 (1960).
10. Griggs, D. & Baker, D. W. in *Properties of Matter under Unusual Conditions* (eds Mark, H. & Fernback, S.) 23–42 (Wiley, New York, 1969).
11. Byerlee, J. D. & Brace, W. F. *Science* **164,** 713–715 (1969).
12. Randall, M. J. & Knopoff, L. *J. geophys. Res.* **75,** 4965–4976 (1970).
13. Ringwood, A. E. *Earth planet. Sci. Lett.* **14,** 233–241 (1972).
14. Schubert, G., Yuen, D. A. & Turcotte, D. L. *Geophys. J. R. astr. Soc.* **42,** 705–735 (1975).
15. McGarr, A. *J. geophys. Res.* **82,** 256–264 (1977).
16. Sungs, C. M. & Burns, R. G. *Earth planet. Sci. Lett.* **32,** 165–170 (1976).
17. Isacks, B. & Molnar, P. *Rev. geophys. Space Phys.* **9,** 103–174 (1971).
18. Mendiguren, J. thesis, Massachusetts Inst. Technol. (1972).
19. Khattri, K. *Earth Sci. Rev.* **9,** 19–63 (1973).
20. Isacks, B. & Molnar, P. *Nature* **223,** 1121–1124 (1969).
21. Oike, K. *Bull. Disas. Prev. Res. Inst.* **21,** 153–178 (1971).
22. Fukao, Y. *Phys. Earth planet. Inter.* **5,** 61–67 (1972).
23. Billington, S. & Isacks, B. *Geophys. Res. Lett.* **2,** 63–66 (1975).
24. Billington, S., Isacks, B. & Barazangi, M. *Geology* **5,** 699–704 (1977).
25. Dziewonski, A. M. & Woodhouse, J. H. *J. geophys. Res.* **88,** 3247–3271 (1983).
26. Giardini, D. *Geophys. J. R. astr. Soc.* (in the press).
27. Dziewonski, A. M., Friedman, A., Giardini, D. & Woodhouse, J. H. *Phys. Earth planet. Inter.* (in the press).
28. Dziewonski, A. M., Chou, T. A. & Woodhouse, J. H. *J. geophys. Res.* **86,** 2825–2852 (1981).
29. Douglas, A. *Nature* **215,** 47–48 (1967).
30. Dewey, J. W. thesis, Univ. California, Berkeley (1971).
31. Sykes, L. R. *J. geophys. Res.* **71,** 2981–3006 (1966).
32. Isacks, B., Sykes, L. R. & Oliver, J. *Bull. seism. Soc. Am.* **57,** 935–958 (1967).
33. Sykes, L. R., Isacks, B. & Oliver, J. *Bull. seism. Soc. Am.* **59,** 1093–1113 (1969).
34. Isacks, B. & Barazangi, M. in *Island Arcs, Deep Sea Trenches and Back-arc Basins* (eds Talwani, M. & Pitman, W. C. III) 99–114 (American Geophysical Union, Washington DC, 1977).
35. Billington, S. thesis, Cornell Univ. (1980).
36. Isacks, B., Sykes, L. R. & Oliver, J. *Bull. geol. Soc. Am.* **80,** 1443–1470 (1969).
37. Wyss, M. & Molnar, P. *Phys. Earth planet. Inter.* **6,** 279–292 (1972).
38. Giardini, D. *Proc. Enrico Fermi Int. Sch. Phys.* Vol. 85 (eds Kanamori, H. & Boschi, E.) (North-Holland, Amsterdam, in the press).

Fig. 5 (Opposite) Each part shows the CMT solution and the associated seismicity viewed from the direction of the intermediate axis; the CMT event is located in the centre of the box. The hypocentral position and the scalar moment are indicated for each solution. The azimuth and plunge identify the direction of the intermediate axis. The scale indicates the lateral dimension and thickness of the box of plotted seismicity, both in km. The nodal planes appear as perpendicular lines which divide the focal hemisphere into quadrants of compression (white) and extension (black) at the source. The compressional axis is located in the white quadrants, at 45° to the nodal planes. Only relocated seismicity is shown.

Nd–Sr isotopic composition of granulites and constraints on the evolution of the lower continental crust

Dalila Ben Othman, Mireille Polvé & Claude J. Allègre

Laboratoire de Géochimie et Cosmochimie (LA 196), Institut de Physique du Globe et Département des Sciences de la Terre, Universités de Paris VI et VII, 4 Place Jussieu, 75230 Paris Cedex 05, France

In a statistical study of granulite facies rocks, we have analysed 32 samples with ages ranging from 2,900 to 200 Myr for Sm, Nd, Rb and Sr and $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios. The Sm/Nd ratio is found to be constant compared with the highly variable Rb/Sr ratio. Using the model age formalism, we show that, in many cases internal differentiation of continental crust follows external differentiation with a large time interval.

SEISMIC studies of the continental crust show that the lower continental crust has quite specific physical properties. From geological observations and studies of drilled samples it has been established that the upper continental crust is composed of sedimentary rocks which overlie a basement mainly composed of metamorphic and granitic rocks. The nature of the lower crust is more controversial. The seismic velocities of lower crustal levels (ranging from 6.6 to 7.2 km s^{-1})[1] correspond to those measured for metamorphic and igneous rocks equilibrated in the granulite facies. Indeed, high pressure experiments show that such metamorphic assemblages are generated in pressure–temperature conditions similar to those prevailing in the lower continental crust. These observations have led several authors (for example refs 2–9) to abandon the old concept of a lower basaltic crust and to consider a lower crust mainly composed of granulites (in the sense of granulite facies rocks). Such granulites are mixtures of meta-sedimentary and meta-igneous (acid and basic) rocks, both metamorphosed in the same (high P, high T) conditions.

Heat flow interpretations in continental environments and direct measurements of U, Th and K concentrations in granulites have led several authors[10–13] following Heier[4] to postulate that the lower crust is depleted in U, Th, K and Rb. Such a depletion would explain the enrichment of the upper crust in these elements. According to these authors, this depletion would result from a general differentiation into an upper and a lower level within the continental crust in association with crustal anatexis and/or fluid migration; this would represent a fundamental process of continental crust evolution.

Thus, when considering the formation and development of the continental crust one must distinguish two potentially distinct geological processes: first the process by which a piece of new continental crust differentiates at the expense of the mantle (referred to as external differentiation of continental crust or EXCOD) and second, the process by which the upper and lower levels differentiate within the continental crust (referred to as internal differentiation of the continental crust or INCOD). What are the exact relationships between EXCOD and INCOD? Do they occur simultaneously or independently in time? What are their relationships with the orogenic processes? Are the answers to those questions unique? Any contribution to these problems may be critical to solving our understanding of the development of the continental crust.

Recently[14], the genesis of upper continental crust was investigated on a large scale through the study of granitoid rocks of different ages, using the coupled Sr and Nd isotopic tracers. The main result of this statistical study was that, in general, the Precambrian granitoids have a signature relatively close to that of the mantle whereas clear evidence was found for recycling processes being involved in the genesis of young granitoids. Although this approach was based on a limited number of samples, it has been confirmed by several case studies of granitoid rocks throughout the world: western US[15,16], Caledonian[17] and Hercynian[18] granites in Europe, Namibian of Africa[19], Western Australia[20]. The genesis of old granitoids is not so simple because the Nd[21] and Sr isotopic growth curves of the Archaean mantle are still debated and because lead isotope studies suggest that the genesis of old granitoids is similar to that of young ones[22].

Using a similar approach for the lower continental crust, the present study surveys various types of granulites of different geological ages. It will be followed by some detailed case studies.

The study of granulites is more complex than that of granitoids because they are not direct igneous products with a well defined age of intrusion. They rather frequently correspond to polymetamorphic assemblages with quite complex tectonometamorphic histories; some were originally igneous rocks, some others were sediments, as testified by the presence of abundant marbles in granulitic formations. Therefore isotopic results in granulite terranes generally require detailed discussion, integrating geological constraints.

In a strict petrological sense, granulite facies rocks correspond to regional high grade metamorphism with rare hydrous minerals, high rock densities (3 g cm^{-3}) and high temperature–high pressure paragenesis. Common features are the presence of hypersthene and clinopyroxene in calcic assemblages, the presence of myrmekite, greyish blue quartz and pleochroic hypersthene in acidic assemblages, with plagioclase and K-feldspar.

We have selected several samples of various ages on the criterion that they correspond to an unambiguous definition of the granulite facies (as quoted above) for which different geological and petrological studies (done previously) yield independent information on their history. The largest part of our samples were obtained through the courtesy of P. Hurley and were mainly studied by Spooner[23], and Spooner and Fairbairn[24]. These are acid and intermediate varieties of pyroxene granulites following the definition of Spooner[23] (instead of 'charnockite' according to Holland's definition[25]). The other samples come from In'Ouzzal (Ahaggar), Ansignan and Agly (Pyrenees), from several different areas in Norway, and also include xenoliths from Le Bournac (France), kinzigites from Beni Bousera (Moroccan Rif) and the Ivrea zone (Italian Alps). In each case, the petrographic description can be found in the literature referenced with a brief description of each area in the caption of Fig. 4.

Chemistry of granulite rocks

For each sample we have determined the Nd isotopic composition and the Sm–Nd concentrations through isotope dilution. For some samples, we have determined the Rb–Sr concentra-

0028-0836/84/060510—06$01.00

© 1984 Macmillan Journals Ltd

tions and Sr isotopic compositions, while in some others, we have used the values published by Spooner and Fairbairn[24]. The analytical techniques used have been described in detail elsewhere[14,26,27], but we wish to emphasize the particular point of zircon dissolution. For every sample, after dissolution in an open Teflon beaker, heavy minerals are dissolved in a closed, pressured Teflon bomb at 205 °C. Indeed, since heavy minerals contain high proportions of rare earth elements (REE)[28], such a technique is crucial for granitoids as well as for granulites. Some workers either do not use bomb dissolution or do not give any indication about their analytical procedure[29–35]. All the corresponding results are dubious and should be used with caution. However, due to a lack of enough reliable analyses in literature, we have used these results in our statistics. However, this incomplete dissolution of zircons may enlarge the real spread of the $^{147}Sm/^{144}Nd$ and the $^{143}Nd/^{144}Nd$ ratios. All the experimental results are reported in Table 1, with error limits.

Our selection represents only 32 specimens of typical granulites and therefore cannot be considered as a fully representative worldwide sample of this facies. However, a comparison between these results and those obtained on granitoids as referred to lower and upper continental crust respectively is of great interest.

In relation to petrological variations within the granulites, Nd and Sm concentrations vary from 3 to 65 p.p.m. and 0.4 to 11 p.p.m. respectively (Table 1). The average values of 28.2 p.p.m. for Nd and 5.3 p.p.m. for Sm are relatively close to those obtained for the upper continental crust and comparable with the average values of the main components of the North American continental crust: for shales, Nd = 31 p.p.m.; for the Precambrian shield composite (north Quebec + Saskatchewan + south-west Quebec) Nd = 30.7 p.p.m. More significant is the relative clustering of the $^{147}Sm/^{144}Nd$ ratios at around 0.121 (Fig. 1*a*). The average value for granitoid rock is around 0.113 and is close to the value for granulites (Fig. 1*b*). In addition, the average value for the whole crust estimated by De Paolo and Wasserburg[36] is 0.116 and estimated by Jacobsen and Wasserburg[37] is 0.119, which are both remarkably close to our average. However, the $^{147}Sm/^{144}Nd$ ratios in the continental crust are not strictly constant since the measured values vary from 0.06 to 0.20 in granulites and from 0.07 to 0.16 in granitoids. When considering basic ortho-derived granulites and xenoliths, the spread largely overlaps the mid-ocean ridge basalt (MORB) field. However, from a statistical point of view, the distinction between acidic continental material and MORB (Fig. 1*c*) is remarkable.

Both the absolute contents and the ratios of Rb and Sr are highly variable (Table 1). Rb and Sr contents vary from 2.4 to 117 p.p.m. (a factor of 49), and from 31 to 870 p.p.m. (a factor of 28) respectively; Sr varies more than the REE. $^{87}Rb/^{86}Sr$ ratio varies from 0.09 to 4.8 (a factor of 55). We emphasize that all values are equal to or larger than the bulk Earth value which is around 0.09. Therefore there is no doubt that the bulk continental crust (lower + upper crust) has a higher Rb/Sr ratio than that of the bulk earth.

Amongst several authors, Heier[4] claimed that rocks of the granulite facies are strongly depleted in Rb relative to Sr. This is true of the Lewisian granulites[38,39] and a few others[24] but does not appear to be a general rule as shown by Leyreloup *et al.*[40], Allègre *et al.*[41] for Bournac granulites and by Spooner and Fairbairn's[24] extensive study. Available Rb/Sr ratios data for granulites are shown in Fig. 2. The observed scatter indicates that some areas are depleted in Rb whereas others are not. The cause of this difference is not understood but must be taken as a fact.

The raw data for the $^{87}Sr/^{86}Sr$ and $^{143}Nd/^{144}Nd$ isotopic ratios (Table 1), uncorrected for age, have been directly reported on the Sr–Nd correlation diagram (Fig. 3). In addition, we have plotted the field defined for granitoids taken from the literature. In their study of Lewisian granulites, O'Nions' group[33,42] have defined a field for granulite with low $^{87}Sr/^{86}Sr$ and negative ε_{Nd}. However, many granulite-facies rocks plot outside this field. In fact, the field for all the granulites would largely overlap that for granitoids.

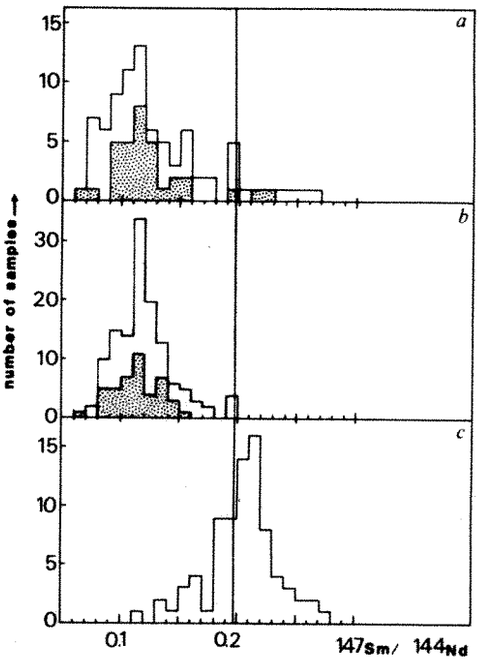


Fig. 1 Histograms of the $^{147}Sm/^{144}Nd$ ratios. *a*, In granulites (data (▩) are from this paper, the others (□) are from refs 29, 30, 32–35). *b*, In granitoids (data (▩) are from refs 14, 20, 52, 74, the others (□) are from 15–19). *c*, In MORB.

Without discussing the detailed age relationships and considering only the geological age we must point out that, as for granitoids, ε_{Nd} values are mainly a function of the rock ages due to fairly constant Sm/Nd ratios of the continental crust. On the other hand, the $^{87}Sr/^{86}Sr$ ratios are more variable but the scatter seems to increase with age. This also reflects the great variability of Rb/Sr ratios.

Model age systematics

The results will now be discussed in terms of model ages of our samples, as defined by McCulloch and Wasserburg[31]. Since continental crust material ultimately derives from the mantle, a model age relative to their mantle source will be estimated. In doing so, the evolution curve of the depleted mantle, as defined by MORB data will be chosen[14,15]. The details of the calculations are given in Table 1 legend.

As the Sm/Nd ratio of the continental crust is fairly constant, the Nd model age is expected to be 'robust' relative to a complex evolution of the continental crust. In contrast the Rb/Sr fractionation history of the crust prevents Sr model age from being geologically significant. A comparison of both model ages will be very useful in deciphering the history of each particular segment of the continental crust.

For each area we have reported the radiometrically determined geological ages of the granulites. In most cases these ages are obtained by whole rock Rb–Sr (often poorly defined) isochrons (Liberia, Tanzania, Columbia, Venezuela, Bournac, Norway, Rogaland and Beni Bousera). In some cases U–Pb ages are available from zircon studies (In'Ouzzal, Amitsoq, Bournac, Ivrea zone, Venezuela). Last, in a few cases, ages have been obtained by whole rock Pb–Pb dating (Venezuela).

Because the Rb–Sr and U–Pb systems are often perturbed by metamorphic events, ages must be interpreted with caution. When a granulite is ortho-derived, the meaning of its age is clear; it probably represents the time of intrusion of its progenitor. When the rock is para-derived, the meaning is more conjectural. Does it correspond to a large-scale rehomogenization contemporaneous with an orogeny[43], or to mixing or the

Table 1 Analysis of typical granulites

Samples			Nd	Sm	$^{147}Sm/^{144}Nd$	$^{143}Nd/^{144}Nd$	Rb	Sr	$^{87}Rb/^{86}Sr$	$^{87}Sr/^{86}Sr$	ε_0^{Nd}	ε_0^{Sr}	Nd Model ages (Myr)	Geological ages (Myr)	Metamorphism ages	Sr model ages
Beni Bousera																
BB6*		Kinzigite	28.3	5.35	0.113	0.51206±3	76.4	183.4	1.19	0.72259±16	−10.9	253	1,493	20	20	1,200
BB9*		Kinzigite	31.7	6.35	0.120	0.51198±3	77.7	128.8	1.72	0.72445±11	−12.5	279	1,737	20	20	900
BB10*		Kinzigite	36.2	6.54	0.108	0.51199±3	139.6	208.6	1.91	0.72468±10	−12.3	282	1,524	20	20	818
BB12*		Kinzigite	35.9	6.37	0.106	0.51206±5	44.2	222.2	0.575	0.71958±18	−10.9	210	1,397	20	20	2,198
M6 482*		Kinzigite	23.8	4.65	0.117	0.51206±3	165.2	229.5	2.05	0.72120±30	−10.9	233	1,555	20	20	640
Ivrea zone																
ST 8*		Acid granulite	24.9	5.17	0.125	0.51237±5	32.9	228.4	0.417	0.713521±31	−4.9	124	1,164	480	260	1,978
ST 10*		Basic granulite	15.7	3.68	0.142	0.51226±4	4.45	248.0	0.052	0.71014±40	−7.0	76	1,672	480	260	—
ST 13*		Kinzigite	11.8	3.08	0.158	0.51237±3	101.3	105.0	2.79	0.726183±17	−4.9	304	1,854	480	250	596
AGLY 1		Leptinitic gneiss	16.869	4.017	0.1432	0.51232±4	117‡	328‡	1.048‡	0.73911‡	−5.8	487	1,569	553	300	2,539
Norway																
NOR 15		Larvikite	45.138	9.632	0.1290	0.51240±4	91.3‡	183.4‡	1.44‡	0.7256±1*	−4.3	289	1,165	1,000	1,000	1,137
NOR 73		Arendalite	14.192	4.990	0.2126	0.51271±4					1.7		—	1,000	1,000	
NOR Rog.	65/75	Charnockite	31.117	4.903	0.0953	0.51191±4					−13.8		1,464	1,000	1,000	
	39/73		48.427	8.658	0.1081	0.51118±35					−28.1		2,732	1,000	1,000	
Lapland		Metagabbro	24.10	5.13	0.1290	0.51221±3	39‡	263‡	0.428‡	0.71638±7‡	−8.0	165	1,505	1,000	1,000	2,466
Le Bournac	801	Paragneiss	36.69	7.011	0.1156	0.51210±2			0.563†	0.7131†	−10.1		1,471	1,000	380	1,360
	802	Orthogneiss	44.726	6.744	0.0915	0.51237±2					−4.9	118	851	1,000–200	380	
Bal	9	Paragneiss	65.03	11.512	0.1071	0.51197±3					−12.6		1,540	1,000–200	380	
Bal	142	Orthogneiss	52.99	9.899	0.1129	0.51223±2			1.63†	0.7274†	−7.6	320	1,236	1,000–200	380	1,081
Columbia	7276		41.32	7.984	0.1169	0.51213±2	210	124	4.814	0.8027	−9.6	1,389	1,443	1,270–1,370	1,200	1,471
Tanzania	7051	Charnockite	8.62	1.815	0.1260	0.51131±2	71‡	410‡	0.459‡	0.72118±6‡	−25.6	268	3,090	2,232–2,692	2,600	3,143
							67	427	0.504	0.7237		232				3,228
Uganda	7012	Aplitic granulite	2.970	0.390	0.0794	0.51057±5	135	31	13.241	1.1969	−40	6,982	2,848	2,600		2,636
	7020	Aplitic granulite	15.582	2.999	0.1164	0.51077±4	207	78	8.003	1.0081	−36.1	4,304	3,688	2,600		2,701
India	7217	Basic charnockite	4.993	1.611	0.1957		2.4‡	80‡	0.087‡	0.7045±6‡				2,563	2,500–2,400	2,684
									0.147	0.7077						3,650
	7246	Charnockite	13.369	3.478	0.1572	0.51206±3	14.5	201	0.208	0.70910±5‡	−10.9	61	2,723	2,560	2,500–2,400	2,678
Liberia	NR 6164		18.014	2.910	0.0977	0.51028±3	88.6‡	272‡	0.952‡	0.74643±4‡	−45.6	591	3,744	2,642–2,692	2,700	3,406
	GCM 6224		22.318	4.424	0.1199	0.51106±4	62.4‡	209‡	0.871‡	0.73936±3‡	−30.4	491	3,315	2,596–2,643	2,700	3,124
Venezuela	8100A2					0.51081±1					−35.3		2,800		2,000	
			15.862	2.479	0.0941	0.51087±8	74.5	100.6	2.162	0.7968	−34.1	1,305		2,020–2,800	2,000	3,130
						0.51077±4					−36.1				2,000	
	7494		33.225	3.817	0.0695	0.51060±1				0.70857±4‡	−39.4	618	2,611	2,020–2,800	2,000	
Guyana	8204		65.663	10.751	0.0990	0.51140±2	67.1‡	870‡	0.225‡	0.70854±3‡	−23.8	54	2,201	1,975–2,133	2,000–2,100	2,150
In'Ouzzal	M408	Charnockite	6.274	1.454	0.1391	0.51156±7					−20.7		3,123	3,100-Pb	2,100	
	L101	Charnockite	39.471	7.668	0.1176	0.51092±6	73‡	55.7‡	3.90‡	0.870‡	−33.2	2,344	3,473	2,946-Sr	2,100	3,055
Surinam	8322				0.108	0.51146±2			0.088	0.7048	−22.6	0.28	2,305	2,055–2,760	2,000–2,760	

The Rb–Sr analyses are done either by D. Rousseau (‡), J. Hamet (†) or M. Polvé (*), the others are done by the MIT Group (see references). Blanks for the Rb and Sr analyses done either by D. Rousseau, J. Hamet or M. Polvé are around 70 pg and 160 pg respectively. Blanks for Nd and Sm done either by D. Ben Othman or M. Polvé, are around 100 pg and 70 pg respectively. $^{81}Sr/^{86}Sr$ ratios are normalized using $^{86}Sr/^{88}Sr = 0.1194$; errors are quoted at $2\sigma_m$. Nd is run as Nd+. $^{143}Nd/^{144}Nd$ ratios are normalized to $^{146}Nd/^{144}Nd = 0.7219$, errors are quoted as $2\sigma_m$. The average measured value of Nd standards from Johnson Mattey is 0.511981±0.000015. Nd model ages are given by the intercept between two curves: $(^{143}Nd/^{144}Nd)_{sample} = (^{143}Nd/^{144}Nd)_T + (^{147}Sm/^{144}Nd)_{sample}(e^{\lambda T} - 1)$ and $(^{143}Nd/^{144}Nd)_{DM} = AT^2 + BT + C$. *DM*: Depleted mantle, with $A = 1.53077 \times 10^{-5}$; $B = -0.22073 \times 6.54 \times 10^{-3}$; $C = 0.513078$. *A*, *B*, *C* are chosen to fit the evolution curve of a depleted mantle defined by data on MORB, ophiolites (Bay of Island, Semail), Precambrian mafic volcanics (Kambalda, Kanowna, Onverwacht), komatiites (Cape Smith, Abitibi), Isua supracrustals and meteorites (Angra dos Reis, Juvinas)[64–71]. Sr model ages are given by the intercept between two curves $(^{87}Sr/^{86}Sr)_{sample} = (^{87}Sr/^{86}Sr)^T + (^{87}Sr/^{86}Sr)_{sample}(e^{\lambda T} - 1)$ and $(^{87}Sr/^{86}Sr)^T_{DM} = A'T^2 + B'T + C'$ with $A' = -1.54985776 \times 10^{-4}$; $B' = -1.6007234 \times 10^{-4}$; $C' = 0.70273029$. *A'*, *B'*, *C'* are chosen to fit the evolution curve of a depleted mantle defined as for Nd by data on MORB, ophiolites (Zambales, Toba, Guatemala, Bay of Island), komatiites (Cape Smith, Abitibi), BABI and Allende[64,69,72,73].

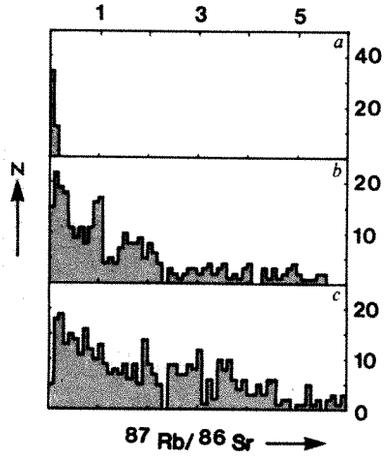


Fig. 2 Histograms of the $^{87}Sr/^{86}Sr$ ratios. *a*, In granulites (data are from this paper and refs 11–13, 23, 24, 34, 38–40, 45, 47, 49, 54, 63, 75–88 and J. Hamet and C.J.A., in preparation). *b*, In granitoids (data are from refs 14, 16, 17, 19, 20, 53, 54, 74, 89–99). *c*, In MORB.

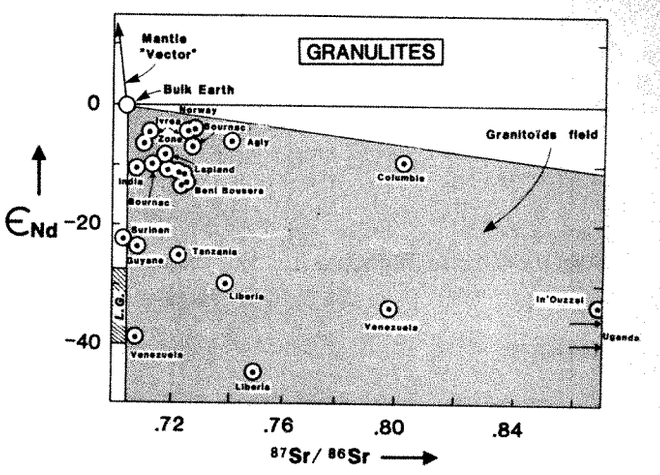


Fig. 3 Data from granulites plotted in the ε_{Nd} versus $^{87}Sr/^{86}Sr$ diagram in comparison with the fields of granitoids and Lewisian gneisses.

age of the sediment sources? In the case where the granitoids present in the same region have the same age, this age is most probably related to a regional episodic event. We will call this age the geological age. Aware of these possible complications, we have plotted the geological age versus the Nd model ages in Fig. 4*a* for the granulites. Additional data are for Lewisian gneisses[33], Lofoten, Norway[30], Australian samples[34] and Antarctic[29].

As can be seen, the Nd model ages are in most cases greater than the geological ages. This is especially true for the younger samples: Bournac, Norway, Ivrea zone, Beni Bousera, and for the older African gneisses (Liberian, Uganda, Tanzania, In'Ouzzal).

For some older samples such as those from India, Australia, Columbia, Guyana and Scotland, the Nd model age is close to the 'geological age'. For two samples from Venezuela, the 'geological age' is greater but is also poorly determined.

In several cases, it is possible to determine with reasonable accuracy the time at which the rocks were metamorphosed to granulite facies. The geological significance of this event is more clear than the previously discussed geological ages. This is the case for samples from In'Ouzzal (Ahaggar), Imataca (Venezuela), Lofoten and Bamble (Norway), Bournac (France), Ansignan (France), Beni Bousera (Morocco), the Ivrea zone (Italy), and the Lewisian gneisses (Scotland).

The garnet-whole rock Sm–Nd age for In'Ouzzal (D.B.O. and C.J.A., in preparation), the zircons of some metasedimentary rocks[44] and petrological studies all give an age of 2,100 Myr which also corresponds to the age of the Tassendjanet pluton and of a main orogenic episode in this area.

In the Imataca area (Venezuela) studied by Hurley *et al.*[45,46] and Gaudette *et al.*[47] granulite-facies metamorphism occurred at 2,000 Myr which also corresponds to an important tectono-metamorphic event of this area.

The Lewisian complex studied by Hamilton *et al.*[33] yields an age of about 2,600 Myr for granulite-facies metamorphism which is the age of a major orogenic event associated with granitoid injection.

The Lofoten occurrence in Norway studied by Jacobsen and Wasserburg[30] gives an age of 1,700 Myr for granulite facies metamorphism. This age corresponds in Norway to a major tectono-metamorphic event with associated plutonism.

In the Bamble area, also in Norway, the granulite facies metamorphism is dated at 1,000 Myr, an age which also corresponds to the major tectono-metamorphic event in this area, again associated with important plutonism[49–51].

In the Bournac case, a Sm–Nd garnet whole-rock isochron[52] gives an age of 380 Myr apparently linked to the end of the Caledonian orogeny and slightly older than the intrusion of the Velay granite, the latter being 300 Myr old[53].

The Agly granulite was formed 300 Myr ago during the Hercynian orogeny and is associated with granitoid intrusions such as Querigut and Milhas[54].

The Ivrea zone granulites are 260 Myr old (M. Polvé and C.J.A., in preparation), the same age as the granitic intrusions in the neighbouring amphibolite facies zone[55,56].

The Beni Bousera granulite-facies metamorphism is quite young (20 Myr) (M. Polvé and C.J.A., in preparation). Such a metamorphic event seems to be linked with the regional tectonics[57,58]. However, no important plutonism is presently known in the area.

Comparison of these ages for granulite metamorphism with the Nd model age clearly shows that they are generally much younger. This information is almost the same as the one given by the geological age. The Venezuela case seems to be an exception. However, the geological age is very uncertain. In our opinion the most probable age is 2,800 Myr. The smallest time interval (200 Myr) is for the Lewisian gneisses. In other cases the time interval between Nd model age and granulite facies metamorphism is from 500 to 1,200 Myr.

On the other hand, age determinations seem to indicate that granulite-facies metamorphism seems always to be linked to major orogeny. It does not reflect a continuous reequilibration process at depth stopped by the upwelling of a piece of lower crust to the surface during some event not related to any large orogenesis. If such reequilibration at depth followed by 'upwelling' occurs, this event is related to a major orogenesis.

Geological consequences

Following McCulloch and Wasserburg[31] and O'Nions *et al.*[59], we interpret the Nd model age as the age of segregation from the mantle (EXCOD). The other ages, either the global geological ages or the better defined metamorphic ages, that we use for comparison with the Nd model ages, are clearly linked to orogenic processes and are sponsored to date INCOD. If this dual identification is correct we can draw the following conclusions: EXCOD has preceded INCOD by a large time interval for young granulites and for many of the older ones too. In these cases it seems that EXCOD and INCOD are two separate phenomena with INCOD operating on preexisting continental crust. This is only true if rocks have not been multiply metamorphosed in granulite facies.

For some Precambrian samples, ages of EXCOD and INCOD are similar. EXCOD and INCOD are still possibly two separate processes but would follow each other with a rather small time interval. The model proposed by Moorbath[43] for a single episode

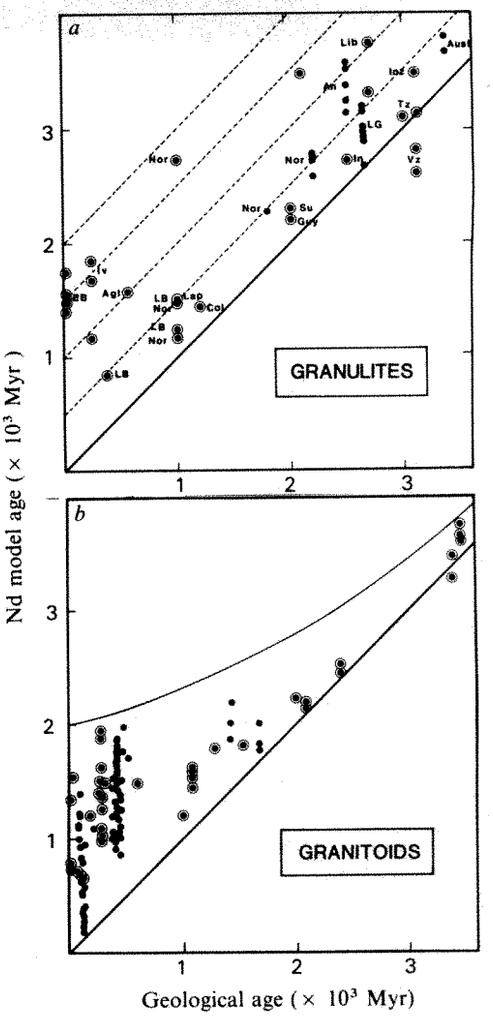


Fig. 4 Nd model age versus geological age diagram. *a*, For the granulites, our data (◉) are plotted together with other data (●) from refs 29, 30, 32–35. Underlined name samples correspond to well-known INCOD. *b*, For the granitoids, our data (◉) are from 14, 20, 52, 74, the others (●) from refs 15–19. Inz: In'Ouzzal formation (Ahaggar)[100–105] the primary age of these rocks is 3,300 to 3,100 Myr (ref. 41 and C.J.A. and D. Rousseau, in preparation) and the granulite metamorphism is 2,100 Myr[105] old. This age corresponds roughly to the age of a major orogeny in this area and to the age of granitic intrusions (Tassendjanet pluton). Lib: Liberian granulites, from Nimba Range and Grand Cape Mountains. The major tectonometamorphic event in this province is 2,800 Myr to 2,700 Myr old[46,89] but 2,000 Myr ages corresponding to the Eburnean orogeny can be found. Granitoids in this area are also 2,600 to 2,700 Myr old[46,89]. Vz: Imataca complex (Venezuela)[45–47] the geological age of the basement is 2,800 to 2,700 Myr old[47] the major metamorphic event is 2,000 Myr old. Granitoids intrusions occurred at 2,700 Myr[45,46] and 2.1 Myr. Guy: Guyana complex (Kanuku and Bakhuis Mountains)[47,48,75,106]. Basement is 2,060 Myr for the Bakhuis Mountains[106] and 2,180 Myr old for the Kanuku formation[75]. The major tectonometamorphic event and the related large granitic complexes is the 2,100–2,000 Myr transamazonian event[75]. Ug: Western Nile complex (Uganda)[107–109]. The granulites had been dated, not very accurately, at 2,600 Myr[88] and granitoid intrusion is 550 Myr old[109]. Tz: Tanzania granulites from Msagali area. A poorly defined isochron gives an age of 2,690 Myr[88] but older ages may be possible. Granitoids from the north (Tanganikan shield) and south (Ubedian granites) are 1,800–2,000 Myr old[108]. The whole system seems to have been affected by the Mozambican orogeny: 1.1–0.65 Myr ago[108]. In: Granulites from Mysore and Madras State (India)[98]. The granulites and probably the granitoids are 2,700 Myr old[23,24,110]. The age of the granulite facies metamorphism is still debated: 2,000 Myr[111] or about 2,500 Myr[112]. Col: Santa Martha Sierra (northern Columbia). The granulites are 1,270–1,370 Myr old[76,113], granitoids in this area are about 1.0 Myr old. Nor: Granulites from south Norway (Bamble, Rogaland). Rogaland: granulites are dated at 1,000–1,200 Myr[114] and granulite metamorphism is supposed to be 1,000 Myr old[115] but a possible metamorphic event may have occurred at about 1,400–1,500 Myr[78,79,115]. Later intrusions took place at about 900–950 Myr[116,117]. Bamble: [50,51,118] This region has suffered two major metamorphic events: 1,600 Myr (Svecofennian) and 1,000 Myr (Sveconorvegian) the latter being HP event[49]. Granites are about 1,000 Myr[50,51]. Lap: Valjok area (Lapland). Granulite metamorphism is supposed to be 1,000 Myr old (J. Touret, personal communication). LB: Bournac pipe, Massif Central, France(refs 40, 41, 63, 119 and J. Hamet and C.J.A., in preparation). An age of at least 1,000 Myr has been found for the first (granulitic) metamorphic event, followed by an hercynian event. Agl: Ansignan and Agly para- and ortho-derived granulites (Pyrenees, France)[54]. A first metamorphic event occurred at 550 Myr. Later the granulite metamorphism is probably hercynian, related to the emplacement of the Pyrenean granitoids (350 Myr). BB: Beni Bousera granulites (Rif, Morocco) (refs 120, 121 and M. Polvé and C.J.A., in preparation). U–Pb, Sm–Nd, Rb–Sr dating show that the recycled material is very old and the granulite facies is young (20 Myr). Iv: Valle Strona–Ivrea zone (Italian Alps). This complete section of upper mantle, lower and intermediate crust is well known 122–127. The recycled material of the para-derived samples is 1,600 Myr–2,500 Myr old (ref. 128 and M. Polvé and C.J.A., in preparation). Two metamorphic events are known around 475 Myr[90] and 260 Myr (M. Polvé and C.J.A., in preparation). Granitic intrusions in the neighbouring zones are 270–275 Myr old[90].

of crustal differentiation may work in special cases but does not seem to be generally the case. A similar diagram can be constructed for granitoids for which the geological ages are well defined and correspond to the age of the intrusion[14–17 19,20] and compared with that for granulites (Fig. 4*b*). This comparison emphasizes two points: for young samples the two diagrams are very similar and thus recycling processes appear to be important in both cases. For old samples they are largely more scattered in the granulite cases than in the granitoid one.

A direct comparison between lower and upper crust is difficult at this stage, since upper crust contains large amounts of granitoid rocks but also a large proportion of gneisses and metamorphic terranes, for which the relative volumes are unknown. Granitoids seem to have a history that is easier to decipher and to systematize than that of granulite rocks, but we must remember that granitoids are produced by the melting of large volumes of rocks (including crustal rocks). Therefore such processes may homogenize relatively large volume of rocks giving a kind of statistical regional average.

Nd model ages are not sensitive to crustal events. Comparing Sr and Nd model ages, a Rb systematic depletion in lower crust should correspond to Sr model ages superior to Nd model ages. Our data (Table 1) show that this case exists but the reverse (Sr model ages < Nd model ages) also exists, sometimes within the same area. This demonstrates the non-systematic depletion of Rb.

Conclusions

Granulite-facies rocks have Sm/Nd ratios identical to those of granitoids from the upper crust. These values are roughly constant for continental crust material (about 0.121 for $^{147}Sm/^{144}Nd$). Thus internal differentiation of the continental crust apparently involves no fractionation of Sm and Nd. The Rb/Sr ratios are more variable and there is no unique signature characterized by systematic depletion in Rb. In some areas, rocks appear depleted in Rb while in others they are not. Such variability in the lower crust should be considered seriously. If on the average, the lower crust is more depleted in K, Rb, U and Th than the upper crust, as shown by heat flow studies[60] and/or budget calculations[61,62], the reason may be that the lower crust contains a larger proportion of basic rocks than the upper crust. If this hypothesis proves to be true, we shall have to change our estimates of the lower crust composition by taking

an average of acid and basic components. For the Sm/Nd ratios this is unlikely to change dramatically the estimates based on the present study, since basic intrusions, either tholeiites, alkaline or calcalkaline basalts, have a light REE enriched pattern resembling that of granitoids[63]. On the opposite, the average Rb/Sr ratio of the lower crust will probably be considerably lowered. Future work on granulites should aim to estimate the respective proportions of basic and acid rocks in the lower crust.

The model-ages relationship clearly shows that recycling processes are important factors for building the lower continental crust. In most cases the lower crust is made up of preexisting igneous or sedimentary material. As shown by Touret[51], granulitic rocks also contain a large proportion of metasediments. If these characteristics are true, and assuming that granulites are really the lower part of the continental crust, it is possible to propose a model for continental development: most of the new continental segment is developed on a basin, located at the edge of the continent but with a continental basement. This basin is stressed, folded, intruded by mantle-derived melts, subjected to anatexis at several levels, and then transformed in a new continental segment. This segment has then the property that its upper part is made of new continental material intruded by anatectic granites which are partly reworked lower crust, partly locally derived, with its lower part made of older continental material. A small basin is a possible location for such an episode of continental growth. This model fits the constraints given by the petrogenesis of granitoids and granulites as determined by geology, petrology and isotope studies.

We thank D. Demaiffe, S. Fourcade, A. Michard, J. Touret and particularly Professor P. M. Hurley who provided us with the samples analysed in this study, together with relevant information. J. F. Minster and C. Jaupart critically read the manuscript. This is IPGP contribution 673.

Received 14 February; accepted 31 October 1983.

1. Gutemberg, B. *Physics of the Earth Interior* (Academic, New York, 1959).
2. Green, D. H. & Lambert, I. B. *J. geophys. Res.* **70,** 5259–5268 (1965).
3. Ringwood, A. E. & Green, D. H. *Tectonophysics* **3,** 383–427 (1966).
4. Heier, S. K. *Geol. For. Stock. Förh.* **87,** 251–289 (1965).
5. Heier, S. K. *Phil. Trans. R. Soc.* **A273,** 429–442 (1973).
6. Fyfe, W. S. *Phil. Trans. R. Soc.* **A273,** 457–461 (1973).
7. Tarney, J. & Windley, B. F. *J. Geol. Soc., Lond.* **134,** 153–177 (1977).
8. Christensen, N. I. *J. geophys. Res.* **70,** 6147–6164 (1965).
9. Christensen, N. I. & Fountain, D. M. *Bull. geol. Soc. Am.* **86,** 227–236 (1975).
10. Shaw, D. M. *Geochim. cosmochim. Acta* **32,** 573–609 (1968).
11. Lambert, I. D. & Heier, S. K. *Lithos* **1,** 30–53 (1968).
12. Sighinolfi, G. P. *Contr. Miner. Petrol.* **21,** 346–356 (1969).
13. Sheraton, J. W. *Earth planet. Sci. Lett.* **8,** 301–310 (1970).
14. Allègre, C. J. & Ben Othman, D. *Nature* **286,** 335–342 (1980).
15. De Paolo, D. J. *Science* **209,** 684–687 (1980).
16. De Paolo, D. J. *Nature* **291,** 193–196 (1981); *J. geophys. Res.* **86,** 10470–10488 (1981).
17. *Hamilton, P. J., O'Nions, R. K. & Pankhurst, R. J. Nature* **287,** 279–284 (1980).
18. Ben Othman, D., Fourcade, S. & Allègre, C. J. *Earth planet. Sci. Lett.* (submitted).
19. Hawkesworth, C. J., Kramer, J. K. & Miller, R. M. G. *Nature* **289,** 278–282 (1981).
20. MacCulloch, M. T. & Chappell, B. W. *Earth planet. Sci. Lett.* **58,** 51–64 (1982).
21. MacCulloch, M. T. & Compston, W. *Nature* **294,** 322–327 (1981).
22. Gariépy, C., Dupré, B. & Allègre, C. J. *Trans. Am. geophys. Un.* **63,** 367 (1982).
23. Spooner, C. M. thesis, Massachusetts Inst. Technol. (1969).
24. Spooner, C. M. & Fairbairn, H. W. *J. geophys. Res.* **75,** 6706–6713 (1970).
25. Holland, T. H. *Mem. geol. Surv. India* **28,** 158 (1900).
26. Richard, P., Shimizu, N. & Allègre, C. J. *Earth planet. Sci. Lett.* **31,** 269–278 (1976).
27. Birck, J. L. & Allègre, C. J. *Earth planet. Sci. Lett.* **39,** 37–51 (1978).
28. Fourcade, S. & Allègre, C. J. *Contr. Miner. Petrol.* **76,** 177–195 (1981).
29. De Paolo, D. J., Manton, W. I., Grew, E. S. & Halpern, M. *Nature* **298,** 614–618 (1982).
30. Jacobsen, S. B. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **41,** 245–253 (1978).
31. MacCulloch, M. T. & Wasserburg, G. J. *Science* **200,** 1003–1011 (1978).
32. MacCulloch, M. T., Arculus, R. J., Chappell, B. W. & Ferguson, J. *Nature* **300,** 166–169 (1982).
33. Hamilton, P. J., Evensen, N. M., O'Nions, R. K. & Tarney, J. *Nature* **277,** 25–28 (1979).
34. De Laeter, J. R., Fletcher, I. R., Rosman, K. Jr, Wilhaus, R. & Libby, W. G. *Nature* **292,** 322–324 (1981).
35. Rogers, N. W. & Hawkesworth, C. J. *Nature* **299,** 409–413 (1982).
36. De Paolo, D. J. & Wasserburg, G. J. *Geochim. cosmochim. Acta* **43,** 615–627 (1979).
37. Jacobsen, S. B. & Wasserburg, G. J. *J. geophys. Res.* **84,** 7411–7428 (1979).
38. Drury, S. A. *Precamb. Res.* **7,** 237–257 (1978).
39. Weaver, B. L. & Tarney, J. *Earth planet. Sci. Lett.* **51,** 279–296 (1980).
40. Leyreloup, A., Dupuy, C. & Andriambolona, A. *Contr. Miner. Petrol.* **62,** 283–300 (1977).
41. Allègre, C. J., Hamet, J. & Leyreloup, A. *Abstr. 3rd R.A.S.T.* Montpellier (Soc. Geol. Fr., 1975).
42. Carter, S. R., Evensen, N. M., Hamilton, P. J. & O'Nions, R. K. *Science* **202,** 743–747 (1978).
43. Moorbath, S. *Nature* **254,** 395–397 (1975).
44. Lancelot, J. R. thesis, Univ. Paris VII (1975).
45. Hurley, P. M., Kalliodoski, J., Fairbairn, H. W. & Pinson, W. H. *Bol. Geol. Publ.* Suppl. 6, 431–433 (1972).
46. Hurley, P. M., Fairbairn, H. W. & Gaudette, H. E. in *The Early History of the Earth* (ed. Widley, B. F.) 511–521 (Wiley, New York, 1976).
47. Gaudette, H. E., Hurley, P. M., Fairbairn, H. W., Espejo, A. & Dahlberg, E. H. *Bull. geol. Soc. Am.* **89,** 1290–1294 (1978).
48. Montgomery, C. W. & Hurley, P. M. *Earth planet. Sci. Lett.* **39,** 281–290 (1978).
49. O'Nions, R. K. & Baadsgaard, H. *Contr. Miner. Petrol.* **34,** 1–21 (1971).
50. Touret, J. *Norg. Geol. Unders.* **215,** 120–139 (1962).
51. Touret, J. thesis, Univ. Nancy (1969).
52. Ben Othman, D. thesis, Univ. Paris VII (1982).
53. Caen-Vachette, M., Couturié, J. P. & Didier, J. *C. r. hebd. Séanc. Acad. Sci., Paris* **294,** 135–138 (1982).
54. Vitrac-Michard, A. & Allègre, C. J. *Contr. Miner. Petrol.* **50,** 257–285 (1975).
55. Köppel, V. *Contr. Miner. Petrol.* **43,** 55–70 (1974).
56. Köppel, V. & Grünenfelder, M. *Mem. Soc. Geol.* **33,** 257 (1979).
57. Priem, H. N. A. *et al. Contr. Miner. Petrol.* **70,** 103–109 (1979).
58. Zindler, A., Staudigel, H., Hart, S. R., Endres, R. & Goldstein, S. *Nature* **304,** 226–230 (1983).
59. O'Nions, R. K., Hamilton, P. J. & Hooker, P. J. *Earth planet. Sci. Lett.* **63,** 229–240 (1983).
60. Jaupart, C., Sclater, J. G. & Simmons, G. *Earth planet. Sci. Lett.* **52,** 328–344 (1981).
61. De Paolo, D. J. *Geochim. cosmochim. Acta* **44,** 1185–1196 (1980).
62. Allègre, C. J., Hart, S. R. & Minster, J. F. *Earth planet. Sci. Lett.* (in the press).
63. Dupuy, C., Leyreloup, A. & Vernières, J. *Phys. Chem. Earth.* **11,** 401–415 (1978).
64. Allègre, C. J. *Tectonophysics* **81,** 109–132 (1982).
65. Jacobsen, S. B. & Wasserburg, G. J. *J. geophys. Res.* **84,** 7429–7445 (1979).
66. McCulloch, M. T., Gregory, R. T., Wasserburg, G. J. & Taylor, H. P. *Earth planet. Sci. Lett.* **46,** 201–211 (1980).
67. McCulloch, M. T. & Compston, W. *Nature* **294,** 322–327 (1981).
68. Hamilton, P. J., Evensen, N. M., O'Nions, R. K., Smith, H. S. & Erlank, A. J. *Nature* **279,** 298–300 (1979).
69. Zindler, A. thesis, Massachusetts Inst. Technol. (1980).
70. Hamilton, P. J., O'Nions, R. K., Evensen, N. M., Bridgwater, D. & Allaart, J. H. *Nature* **72,** 41–43 (1978).
71. Lugmair, G. W., Marti, K., Kurtz, J. P. & Scheinin, N. B. *Geochim. cosmochim. Acta* Suppl. 7 (1976).
72. Papanastassiou, D. A. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **5,** 361–376 (1969).
73. Gray, G. M., Papanastassiou, D. A. & Wasserburg, G. J. *Icarus* **20,** 213–239 (1973).
74. Ben Othman, D. & Allègre, C. J. *Nature* (submitted).
75. Spooner, C. M., Berrangé, J. P. & Fairbairn, H. W. *Bull. geol. Soc. Am.* **82,** 207–210 (1971).
76. McDonald, W. D. & Hurley, P. M. *Bull. geol. Soc. Am.* **80,** 1867–1872 (1969).
77. Weaver, D. L. *Contr. Miner. Petrol.* **71,** 271–279 (1980).
78. Jacobsen, S. B. & Heier, S. K. *Lithos* **11,** 257–276 (1978).
79. Field, D. & Raheim, A. *Earth planet. Sci. Lett.* **45,** 32–44 (1979).
80. Heier, S. K. & Thorensen, J. K. *Geochim. cosmochim. Acta* **35,** 89–99 (1971).
81. Gray, C. M. *Contr. Miner. Petrol.* **65,** 79–89 (1977).
82. O'Nions, R. K. & Pankhurst, R. J. *Earth planet. Sci. Lett.* **22,** 328–338 (1974).
83. Moorbath, S., Welke, H. & Gale, N. H. *Earth planet. Sci. Lett.* **6,** 245–256 (1969).
84. Lambert, R. S. J. & Holland, J. G. in *The Early History of the Earth* (ed. Widley, B. F.) 191–202 (Wiley, New York, 1976).
85. Gasparini, P. & Mantovani, M. S. M. *Earth planet. Sci. Lett.* **42,** 311–320 (1979).
86. Matejoska, O. & Vankova, V. *Studia geophys. geod.* **24,** 285–301 (1980).
87. Hubbard, F. H. & Withley, J. E. *Lithos* **12,** 1–11 (1979).
88. Spooner, C. M., Hepworth, J. V. & Fairbairn, H. W. *Geol. Mag.* **1,** 128–129 (1970).
89. Hurley, P. M., Leo, G. W., White, R. W. & Fairbairn, H. W. *Bull. geol. Soc. Am.* **82,** 3483–3490 (1971).
90. Hunziker, J. C. & Zingg, A. *Schweiz miner. petrogr. Mitt.* **60,** 181–213 (1980).
91. Videl, Ph. thesis, Univ. Rennes.
92. Roddick, J. C. & Compston, W. *Earth planet. Sci. Lett.* **34,** 238–246 (1977).
93. Hamet, J. & Mattauer, M., C. R. *Somm. Soc. Geol. Fr.* **2,** 80–84 (1977).
94. Hamet, J. & Allègre, C. J. *Contr. Miner. Petrol.* **38,** 291–298 (1973).
95. Hamet, J. & Allègre, C. J. *Bull. geol. Soc. Am.* **87,** 1429–1442 (1976).
96. Arth, J. G. & Hanson, G. N. *Geochim. cosmochim. Acta* **39,** 325–362 (1975).
97. Farmer, G. L. & De Paolo, D. J. *J. Geophys. Res.* **88,** B4, 3379–3401 (1983).
98. Schärer, U., Hamet, J. & Allègre, C. J. *Earth planet. Sci. Lett.* (in the press).
99. Schärer, U. *Earth planet. Sci. Lett.* **49,** 205–218 (1980).
100. Lelubre, M., *Bull. Serv. Carte Geol. Algérie* **22,** (1952).
101. Giraud, P. *Rep. 22nd Session India,* New Delhi Pt 3, Proc. Sect. 13, 1–20 (1964).
102. Caby, R. thesis, Montpellier Univ. (1970).
103. Fourcade, S. & Javoy, M. *Contr. Miner. Petrol.* **42,** 235–244 (1973).
104. Ferrara, P. & Gravelle, M. *Earth planet. Sci. Lett.* **1,** 319–324 (1966).
105. Allègre, C. J. & Caby, R. *C. r. hebd. Séanc. Acad. Sci., Paris* **275,** 2095–2098 (1972).
106. Priem, H. N. A. *et al. U.S. Geol. Surv. Open File* **78–701,** 341–343 (1978).
107. Hepworth, J. V. *Geol. Survey of Uganda Report* No. 10 (1964).
108. Cahen, L. & Snelling, J. *The geochronology of Equatorial Africa* (North-Holland, Amsterdam, 1966).
109. Hepworth, J. V. & MacDonald, R. *Nature* **210,** 726–727 (1966).
110. Pichamuthu, C. A. *The Precambrian of India, The Precambrian* Vol. 3, 1–96 (Interscience, New York, 1967).
111. Crawford, A. R. thesis, Australian National Univ. (1968).
112. Shackleton, R. M., in *The Early History of the Earth* (ed. Widley, B. F.) 317–322 (Wiley, New York, 1976).
113. Hurley, P. M., Melcher, G. C., Pinson, W. H. & Fairbairn, H. W. *Can. J. Earth Sci.* **5,** 633–638 (1968).
114. Pasteels, P., Demaiffe, D. & Michot, J. *Lithos* **12,** 199–208 (1979).
115. Pasteels, P. & Michot, J. *Norsk Geologik Tidsskrift* **55,** 111–134 (1975).
116. Pasteels, P., Michot, J. & Laneau, J. *Geochron. Isot. Ann. Soc. Bel.* **93,** 453–476 (1970).
117. Pedersen, S. & Falkum, T. *Chem. Geol.* **15,** 97–101 (1975).
118. Pineau, F., Javoy, M., Behar, F. & Touret, J. *Bull. Miner.* **104,** 630–641 (1981).
119. Vitrac-Michard, A., Albarède, F. & Allègre, C. J. *Nature* **291,** 460–464 (1981).
120. Kornprobst, J. *Contr. Miner. Petrol.* **23,** 283–322 (1969).
121. Reuber, I., Michard, A., Chalouan, A., Juteau, T. & Jermouni, B. *Tectonophysics* **82,** 231–251 (1982).
122. Giese, P. *Schweiz. miner. petrogr. Mitt.* **48,** 261–284 (1968).
123. Berckhemer, H. *Schweiz. miner. petrogr. Mitt.* **48,** 235–254 (1968).
124. Sighinolfi, G. P. & Gorgoni, C. *Chem. Geol.* **22,** 157–176 (1978).
125. Dostal, J. & Capredi, S. *Lithos* **12,** 41–49 (1979).
126. Menhert, K. R. *Neues Jb. Miner. Abh.* **125,** 156–199 (1975).
127. Fountain, D. & Salisbury, M. H. *Earth planet. Sci. Lett.* **56,** 263–277 (1981).
128. Koppel, V. & Grünenfelder, M. *Bull. Suisse Miner. Petrogr.* **51,** 385–409 (1971).

Effect of chromosomal position on amplification of transfected genes in animal cells

Geoffrey M. Wahl, Bruno Robert de Saint Vincent* & Margaret L. DeRose

The Salk Institute, PO Box 85800, San Diego, California 92138, USA

A single protein (CAD) contains the first three enzymatic activities of de novo *uridine biosynthesis. The chromosomal location of CAD genes introduced into Chinese hamster ovary cells significantly affects the frequency and cytogenetic result of their amplification. The amplification frequency in one transformant is 100-fold that of the others; in another, amplification of donated genes inserted near a centromere results in chromosome instability and rearrangements.*

GENE amplification in mammalian tissue culture cells occurs spontaneously, with high frequencies, and is a common mechanism of resistance of animal cells to diverse agents[1]. Phenotypic reversion of mutations engendering temperature-sensitive[2] or kinetically altered[3] proteins also occurs by gene amplification. Cytogenetically gene amplification results in expanded chromosomal regions which band abnormally by the trypsin–Giemsa method (often called homogeneously staining regions, or HSRs) or acentric extrachromosomal elements (called double minute chromosomes, or DMs) (see ref. 4 for a review). Since the amplified regions are 100–2,000 kilobases (kb)[4–10], far longer than the structural genes under selection, it is likely that chromosomal sequences or structures distant to the selected gene are involved in amplification. Since large amounts of DNA are co-amplified with the selected gene, 'sequence' amplification is often a more accurate term.

The abnormal chromosomal structures typical of gene amplification in drug-resistant tissue culture cells also occur in untreated spontaneous tumour cells[11–15]. Cellular analogues of retroviral oncogenes are sometimes amplified in HSRs and DMs[11,14,15]. Amplified proto-oncogenes are overexpressed in several tumour lines[12,14,15] supporting the hypothesis that elevated expression of cellular oncogenes could be involved in tumorigenesis (see refs 16–18 for reviews).

The numerous examples of gene amplification emphasize that it can occur in many chromosomal regions. As in bacteria[19], the chromosomal position of a mammalian gene can determine the amplification frequency and mechanism, and the size and stability of the resulting amplified regions. The various examples of eukaryotic sequence amplification differ significantly, reflecting the differences between cell lines and the selective agents used as well as the chromosomal positions of the genes. In order to assess directly the contribution of chromosomal position in sequence amplification, we studied the amplification of a cloned CAD gene inserted by gene transfer into different locations in the chromosomes of CAD-deficient Chinese hamster ovary cells (CHO) cells. [CAD is a multifunctional protein which contains the first three enzymatic activities of *de novo* uridine biosynthesis: carbamyl phosphate synthetase, aspartate transcarbamylase and dihydroorotase.]

High-frequency amplification

Purified DNA sequences introduced into mammalian cells integrate into chromosomes in an apparently random fashion[20–22]. If chromosomal position affects gene amplification, it should be possible to use gene transfer to screen for chromosomal sites which engender unusual forms of amplification. Two parameters which could be altered by changing gene position are the frequency and the cytogenetic consequences, for example, rearrangements associated with amplification, of its amplification.

To establish whether either of these parameters is affected by changing gene position, we introduced cloned genomic copies of the Syrian hamster CAD genes[20] into the chromosomes of CAD-deficient (Urd-A)[23] mutants of CHO cells using the calcium phosphate precipitation technique in the absence of carrier DNA[24,25]. Transformants expressing the donated Syrian hamster CAD genes can be identified by their ability to grow in the absence of uridine[20]. Four transformants were isolated which contain approximately three to six functional donated CAD genes. The CAD activity in each transformant was approximately the same as that of the wild-type CHO K1 cells (see Table 1). This agrees with our previous finding that the Syrian hamster CAD gene is expressed at approximately one-quarter the level of the Chinese hamster CAD gene[20]. Each transformant maintained the donated genes without restriction pattern changes after passage for 90 generations without selection (data not shown).

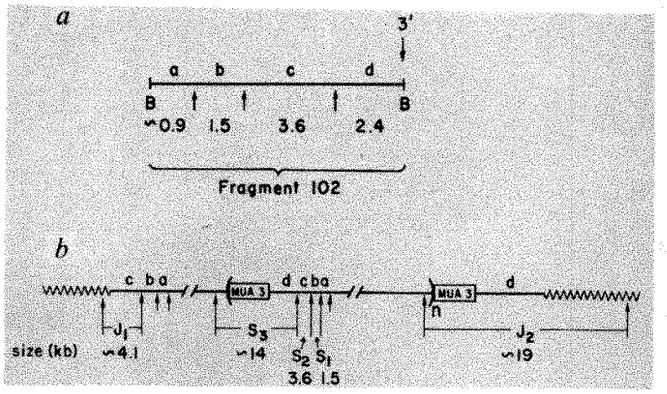
Due to their similar levels of CAD expression, transformants and wild-type CHO K1 cells are sensitive to the same concentrations of PALA (*N*-phosphonacetyl-L-aspartate, see LD_{50} in Table 1), a specific inhibitor of the aspartate transcarbamylase activity of CAD[26]. Since PALA resistance has been found only when the CAD gene is amplified[27,28], we used the frequency of PALA resistance as an indicator of CAD gene amplification frequency. Table 1 shows the number of PALA-resistant ($PALA^r$) colonies that arose in transformants T1, T5, T6 and T7 at the three PALA concentrations tested. Transformant T5 differs from wild-type Syrian hamster[28], from CHO K1 cells and from the other transformants: in T5 the frequency of PALA resistance is at least 100 times greater and T5 resists higher concentrations of PALA in a single step even though the other transformants have equivalent or higher aspartate transcarbamylase specific activities. All of the $PALA^r$ T5, T6 and T7 clones tested maintain their initial levels of PALA resistance when grown for 60 generations in the absence of the drug, indicating that PALA resistance is relatively stable in these lines. The amplified CAD genes in one $PALA^r$ T1 mutant appeared to be much less stable (see below).

To determine whether high frequency PALA resistance in transformant T5 results from amplification of donated CAD sequences, DNA isolated from the parental transformant and from five independently isolated $PALA^r$ mutants of T5 was digested with endonuclease *Sst*I and then analysed by blot hybridization[29,30] using the CAD-specific probe P102[31] (Fig. 1). The bands representing the endogenous CHO CAD genes (labelled CHO_1 and CHO_2) are easily resolved from the three Syrian hamster CAD gene bands (labelled S_1, S_2, S_3). It is clear that the donated sequences are amplified in each of the $PALA^r$

* Present address: The Pasteur Institute, 25 Rue du Dr Roux, Cedex 7595, France.

0028–0836/84/060516—05$01.00

© 1984 Macmillan Journals Ltd



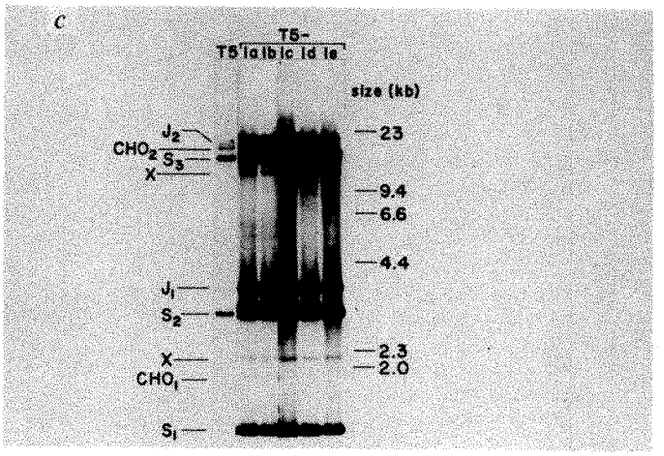


Fig. 1 Analysis of the donated CAD genes in transformant T5 and in PALA^r mutants. ***a***, Map of CAD gene fragment 102. 102 is an 8.6-kb *Bam*HI (B) fragment derived from the 3'-end of the 25–30-kb Syrian hamster CAD gene and contains only unique sequences[31]. The map of fragment 102 published previously had an inversion of fragments b and c[20]. ↑ indicates *Sst*I cleavage sites. ***b***, Simplified map of the inserted sequences in transformant T5. The donated array consists of four to six CAD genes in a head-to-tail tandem array (one indication of this arrangement is the presence of fragment S_3 which has been shown by hybridization analysis to contain sequences at the 5' end of one CAD gene, vector sequences, and the 3' end of another CAD gene). Only the relevant *Sst*I sites (↑) are shown. Not to scale. ***c***, Hybridization of *Sst*I-digested DNA isolated from T5 and PALA^r T5 mutants with fragment 102. The lanes indicate the clones used as the source of the genomic DNA. T5 is the parental transformant, and T5-1a to T5-1e are independent PALA^r mutants isolated from T5 after selection in 250 μM PALA. Each mutant was expanded from a single, well-isolated colony for DNA isolation. Mutant T5-1d is the same as T5-S1-3 shown in Figs 2 and 3. Exposures, which show the endogenous CHO CAD gene fragment CHO_2 more clearly, demonstrate that all lanes contain approximately the same quantity of DNA. CHO_1 and CHO_2 indicate the Chinese hamster CAD gene fragments, S_1, S_2, S_3 are the donated Syrian hamster CAD gene fragments, J_1 and J_2 are the joints between the donated and CHO DNA, and X is a fragment produced by inefficient *Sst*I cleavage which is also seen after cleavage of CAD cosmid DNA. **Methods:** Cellular DNAs (10 μg) were digested with a 5–10-fold excess of *Sst*I, fractionated by electrophoresis through a 0.7% agarose gel, and analysed by the Southern blot hybridization technique[29,30]. The DNA was depurinated *in situ* in 0.25 M HCl for 10 min (instead of 20–30 min as described previously[30]). The filter was hybridized with 3×10^6 c.p.m. per ml of nick-translated[47] p102 (see Fig. 1*a*) in the presence of 10% dextran sulphate for 16 h and washed as described previously[30].

T5 mutants. Three other conclusions derive from this experiment. First, even though each T5 mutant was isolated after a single step of selection, there is an approximately 25–50-fold increase in copy number (determined by densitometry of autoradiographs exposed in the linear range of the film). This compares with a 5–10-fold increase in PALA^r mutants of other transformants (for example, see results below for transformant T1). Second, the molecular structure of the amplified donated sequences is identical in all independently isolated T5 mutant. Third, amplification of the donated CAD genes in T5 does not result in their rearrangement. The bands labelled 'X' were observed after restriction digestion of purified cosmid DNA, and probably represent low efficiency cleavage sites for endonuclease *Sst*I. Fragments 'J_1' and 'J_2' are derived from the junctions between the donated and flanking CHO DNA as described below.

Amplification mediated by flanking sequences

DNA blotting experiments shown above for transformant T5 and below for transformant T1 (data not shown for the other transformants) demonstrate that donated CAD genes can be amplified in each transformant. All of the transformants were derived from the same recipient cell line (Urd-A), and all were transformed with the same CAD cosmid. Thus, the capacity for high frequency amplification in T5 is not related to the DNA used or to some general characteristic of the Urd-A cells following transfection. Since the frequency of dihydrofolate reductase (DHFR) gene amplification is essentially the same in CHO K1, Urd-A and T5 (data not shown), T5 is not a genetic variant capable of amplifying all sequences at a high frequency. Therefore, the high frequency amplification in T5 relates either to the organization of the donated sequences or to an effect of the position in which they are inserted.

The restriction analysis shown in Fig. 1*c* indicates that T5 contains four to six donated genes (for example, compare the intensities of S_1–S_3 with those of CHO_1 and CHO_2). The *in situ* hybridization analysis in Fig. 2 shows that the donated genes in T5 are contained in a single chromosomal location and that amplification occurs near to or at this site without obvious chromosome rearrangements. Hybridization analysis with different probes has shown that fragment S_3 (Figs 1*c*, 3) contains sequences at the 5' end of one CAD gene, the adjacent vector, and the 3' end of the next CAD gene, indicating that the donated genes are in a head-to-tail array (see Fig. 1*b*). From previous studies, we infer that the tandem array arose by homologous recombination either before or after chromosomal integration[22,32]. Since tandem arrays mediate amplification by unequal sister chromatid exchanges in prokaryotes[19] and yeast[33], we tested whether such exchanges occurring within the tandem array in T5 cause the observed high frequency amplification.

Reciprocal unequal exchanges within the tandem array of CAD genes should generate two chromatids with unequal numbers of CAD genes. After mitosis and cell division, only the daughter cell receiving the chromosome with the greater number of CAD genes will survive the PALA selection. Thus, the abundance of joint sequences between donated and CHO DNA should not increase following amplification by unequal exchange since half of the joint sequences are lost in the cell which does not survive the selection. To test this prediction, we identified the joints between the donated and CHO DNA and measured their abundance after amplification in five independently isolated mutants.

Since there is a single insertion site in T5, the joints between the CHO and donated DNA should be contained in two restriction fragments which do not correspond to the known CAD gene restriction map. These fragments should have unique sequence abundance. Fragments J_1 and J_2 (Fig. 1*c*), which hybridize with fragment 102, are the only two *Sst*I restriction fragments which fulfill these criteria. Furthermore, since probes for other regions of the CAD gene do not reveal other novel fragments, the sequence disruption caused by gene insertion is

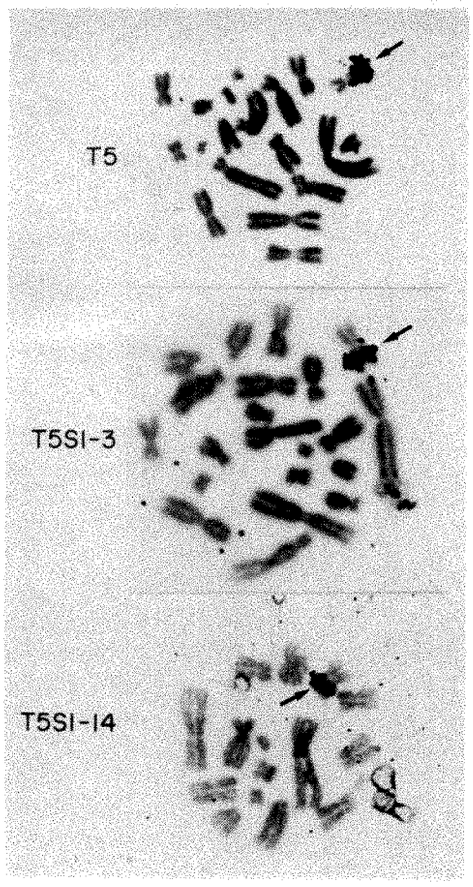


Fig. 2 Localization of the donated CAD genes in T5 and PALA^r T5 mutants by *in situ* hybridization. Cell lines used for the metaphase spreads are indicated in each panel. Hybridization to the chromosomes indicated by the arrows represents the only significant hybridization observed in 10 metaphase spreads from each cell line. The dense packing of grains in these exposures, the unequal hybridization to chromosomes prepared from the different cell lines at different times, and the uneven hybridization seen across the slides prevents these data being used to determine the relative number of CAD sequences in each cell line.
Methods: Methods for spread preparation and *in situ* hybridization have been described in detail previously and were modified only by use of ^{3}H-labelled rather than ^{125}I-labelled p102[5]. The probe was nick-translated p102. Exposures were for 2 weeks at −70 °C.

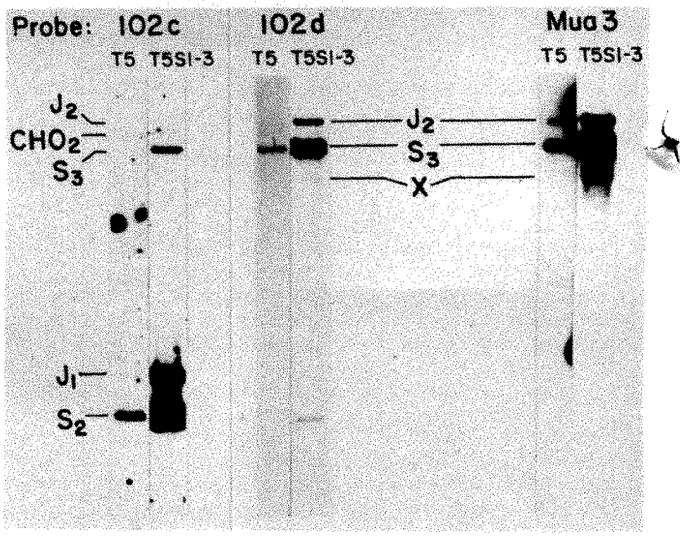


Fig. 3 Analysis of joint fragment amplification in transformant T5 and PALA^r mutant T5-S1-3. The blot used in Fig. 1 was washed to elute bound counts (see below) and then hybridized with the indicated probes. For simplicity, we show the pattern of hybridization to DNA isolated from only one PALA^r T5 mutant since all the hybridization patterns from the five mutants analysed were identical (for example, Fig. 1*c*).
Methods: The blot shown in Fig. 1 was washed with boiling 0.1 × SSPE, 0.1% SDS briefly to remove the bound counts. Subsequent hybridization reactions contained 3–5 × 10^6 c.p.m. per ml indicated probes, and all reactions were performed as described in the legend to Fig. 1. Film exposures: 84 h for 102c, 72 h for 102d; 120 h for probe MUA3. Reduced hybridization signals were seen with probe 102d because it was 2 weeks old at the time it was used.

localized to fragment 102 (see Fig. 1*b*). When subfragments 102C and 102D (Fig. 1*a*) were hybridized to the blot shown in Fig. 1*c*, subfragment 102C hybridized preferentially with J_1 and S_2, and subfragment 102D and vector sequences hybridized with both J_2 and S_3 (Fig. 3). These data agree with the map in Fig. 1*b*. The results show that J_1 and J_2 are the joints between the donated and CHO DNA, and demonstrate that the joint fragments are amplified in the PALA^r T5 mutants to the same extent as the CAD sequences which they flank (see Figs 1*c*, 3). Thus, it is unlikely that amplification of the donated genes in T5 occurs by reciprocal unequal exchanges within the donated sequences. The data indicate that the amplified unit extends beyond the CHO-donated DNA junctions, implying that high frequency amplification in T5 is related to the position of the inserted sequences in the genome.

Chromosome rearrangements

The genetic, molecular and cytogenetic aspects of amplification of the donated genes in transformant T1 contrast with those observed in transformant T5. The frequency of amplification (10^{-5}–10^{-6}), maximal level of amplification (5–10-fold) and resistance to PALA attained in a single step are similar in T1 and wild-type CHO K1 cells (see Table 1). In contrast to transformant T5 (Fig. 2), CAD gene insertion in T1 is accompanied by numerous CAD gene restriction fragment rearrangements (Fig. 4). Since T1 contains an *Sst*I restriction fragment which co-migrates with and has the same hybridization characteristics as fragment S_3 of transformant T5, T1 must also have a core of CAD genes present in a head-to-tail tandem array. The numerous other *Sst*I fragments which hybridize with fragment 102 indicate this tandem array is surrounded by rearranged CAD sequences (two fragments are approximately the same size as CHO_1 and CHO_2 and obscure them). All of these sequences amplify to the same degree without further rearrangements (see Fig. 4, lane T1-1), indicating the amplified unit extends beyond the boundaries of the inserted DNA.

Figure 5 shows the location of the inserted sequences in T1 and the cytogenetic consequences of their amplification. A single insertion site coinciding with the centromeric constriction of a large telocentric chromosome was seen in the 12 metaphase spreads examined. Amplified sequences in independently isolated PALA^r mutants of T1 were found in different chromosomal locations. In mutant T1-1, an entire arm of one medium-sized metacentric chromosome contains most if not all of the amplified sequences. A second chromosome which is very similar to the parental chromosome with regard to the number and location of donated sequences was also found in 14 out of 15 spreads of T1-1. By contrast, the amplified CAD sequences in T1-2 often reside in one or more small chromosomes or chromosome fragments (see Fig. 5, panels T1-2). Preliminary studies of T1-2 show that after passage in the absence of PALA for 40 generations gene copy number decreases by at least half and PALA resistance is diminished. In five out of eight spreads

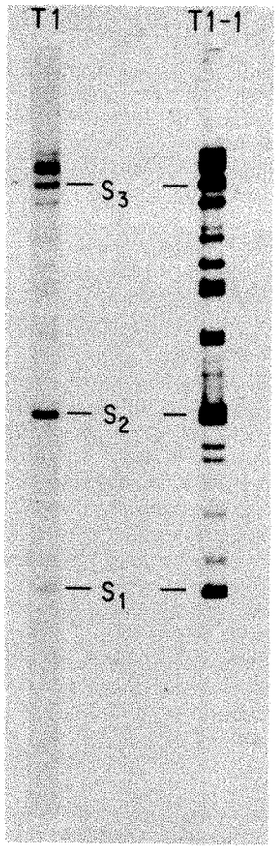


Fig. 4 *Sst*I fragments in T1 and PALA^r mutant T1-1 which hybridize with CAD fragment 102. The lanes indicate the clones used as the source of the DNA. T1 is the parental line and T1-1 is a mutant selected to resist 250 μM PALA. The positions of the normal Syrian hamster fragments are indicated by S_1, S_2 and S_3 (see Fig. 1*b*). The other fragments come from rearranged Syrian hamster CAD fragments. The restriction digestion, blotting and hybridization conditions are described in the legend to Fig. 1. The ethidium bromide staining pattern showed about twice as much T1-1 as T1 DNA. The numerous rearranged Syrian hamster bands obscure hybridization to the CHO CAD gene fragments. Overexposed autoradiograms showed that all CAD sequences amplified in T1-1 are found in T1, and all amplify to the same extent.

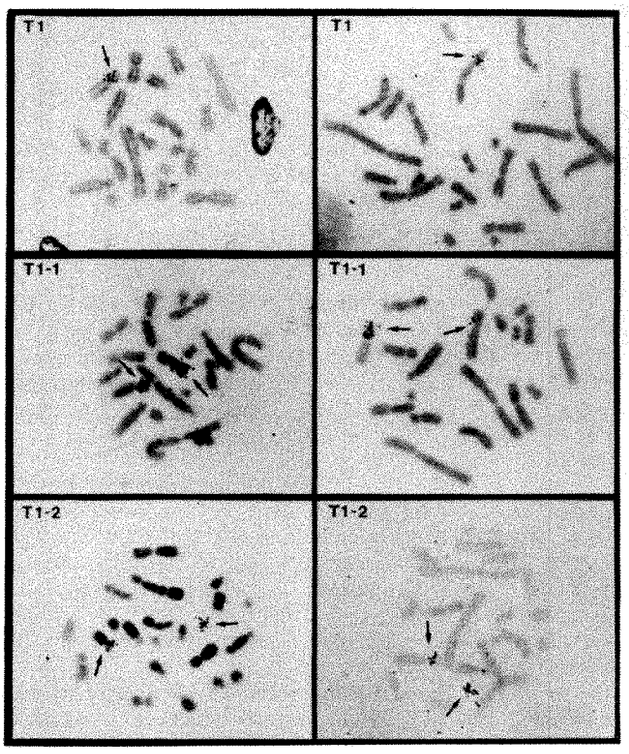


Fig. 5 *In situ* hybridization analysis of T1, T1-1 and T1-2. Representative metaphase chromosome spreads from the indicated cell lines were hybridized with ^{3}H-labelled p102 as described in the legend to Fig. 2.

of T1-2 the donated genes occurred in a chromosome which resembles that found in the parental transformant (T1) and two out of eight metaphase spreads revealed amplified sequences at chromosome telomeres.

Discussion

We used *in vitro* gene transfer to introduce cloned CAD genes into new chromosomal locations in CAD-deficient CHO cells to determine whether position affects the frequency or cytogenetic result of CAD gene amplification. We found that both can be affected significantly by gene position. For example, the donated genes in transformant T5 amplify approximately 100 times as frequently as those in three other transformants or the endogenous CAD genes in several wild-type cell lines. Furthermore, the donated genes in T5 are amplified 25–50-fold after the first selective step in contrast to the 5–10-fold increases seen in the other transformants or several wild-type lines[27,28].

The transfected CAD genes in T5 are in a head-to-tail tandem array 200–300 kb long. Within the array, each CAD gene contains *Alu*-like elements repeated in many introns[34] and the vector sequences are repeated every 40 kb. Such a structure should provide many opportunities for amplification mediated by homologous unequal exchanges. However, we have found no evidence that high frequency amplification in T5 results from reciprocal unequal exchanges within the tandem array. The data agree better with an amplified region which includes the donated sequences and extends into the flanking chromosomal DNA. The amplified region in the three other transformants examined

Table 1 Characterization of transformants T1, T5, T6 and T7

Cell line or transformant	CAD specific activity (μmol per mg protein per 30 min)	LD_{50} (μM)	Frequency of resistance (colonies/3×10^5 cells)		
			100 μM PALA	After selection in 250 μM PALA	1 mM PALA
CHO-k1	0.27	20–30	ND	3	0
T1	0.17	5–10	0	1	0
T5	0.19	5–10	130	152	75
T6	0.33	20–30	1	ND	0
T7	ND	20–30	1	ND	0

CAD-specific activity was determined by measuring the ATCase activity as described previously[20]. The LD_{50} refers to the PALA concentration (μM) required to reduce the plating efficiency of 10^3 cells per 35-mm dish by 50%. The frequency of PALA resistance was determined by seeding 3×10^5 cells per 10-cm dish, allowing attachment to occur overnight, and then challenging the cells in the indicated concentrations of PALA. The number of colonies which developed after 3–4 weeks of selection were then determined by counting the plates after fixing with methanol and staining with Giemsa. ND, not determined.

also extends into CHO DNA. The donated unit in these lines consists of a core of tandem CAD genes flanked by rearranged CAD sequences (Fig. 4). Thus, neither intact nor rearranged CAD sequences can mediate their own amplification by reciprocal unequal exchanges which occur solely within the donated array: flanking CHO DNA participates in the amplification of the transfected genes in each transformant. Hence, if unequal exchanges can cause sequence amplification in mammalian cells, it is likely that factors other than extended regions of sequence homology are required to initiate or mediate the process.

One model for amplification in T5 which agrees with the large single step increase in gene copy number is that unscheduled rounds of synthesis of the donated sequences are followed by recombinational events to generate a stable amplified region[35–37]. Unscheduled DNA synthesis mediates chorion gene amplification in *Drosophila*[38] and has been postulated to lead to DHFR gene amplification in mammalian cells[39], the *in situ* replication of simian virus 40 and polyoma sequences in transformed cells[36,40] and the amplification of adenine phosphoribosyl transferase-thymidine kinase genes transfected into mouse cells[37]. It is tempting to speculate that high frequency and high level amplification in T5 occur because gene insertion disrupts a domain or sequence that controls the replication of an adjacent region, thereby allowing frequent unscheduled synthesis of the donated array.

Gene amplification frequently occurs at sites of chromosome rearrangements[5,41,42]. While obvious cytogenetic changes did not occur in PALA^r T5 mutants, significant chromosomal rearrangements did occur during amplification of the donated genes in T1. Interestingly, the CAD genes were inserted near to a centromere in T1 but in the middle of a chromosome arm in T5. PALA^r T1 mutants retain the chromosome with the donated genes and contain the amplified copies of these genes in another chromosome (in T1-1) or in extrachromosomal elements or small chromosomes (in T1-2). Similar observations to those made in T1 have been reported for the amplification of sequences in IMR-32 neuroblastoma cells[43]. The apparent retention of the parental chromosome and the appearance of amplified sequences in other chromosomes in PALA^r T1 mutants suggests that sequence amplification near or at a centromere is lethal in cells with only one copy of this chromosome. Perhaps an event such as nondisjunction is first required to generate a cell with two copies of the chromosome containing the inserted CAD genes. Amplification in one of these chromosomes could then generate a dicentric chromosome which undergoes a breakage–fusion–bridge cycle[44] to yield chromosome fragments which attach to telomeres of other chromosomes as in T1-1, or remain free and engender an unstable phenotype as in T1-2. A similar model has been proposed recently to explain the frequent telomeric location of amplified DHFR minigenes in CHO cells[42]. On the other hand, the maintenance of sequences in one chromosomal site and their appearance in a second site characterizes transposition[45]. If amplification is associated with transposition of sequences in T1, the transposed sequence must be at least 300 kb, the size of the inserted sequences! Moreover, since *Drosophila* contains elements capable of transposing sequences of this length[46], the involvement of large-scale DNA transposition in gene amplification deserves further consideration.

We thank Judy Meinkoth, Walter Eckhart, Ron Evans, Suzanne Bourgeois and Marguerite Vogt for suggestions, Valerie Jameson for manuscript preparation, and Lee Hartwell and George Stark for valuable discussions leading respectively to the ideas that DNA insertion could create a hyperamplifiable region and that DNA transposition could be involved in gene amplification.

Received 18 May; accepted 14 November 1983.

1. Schimke, R. T. (ed.) *Gene Amplification* (Cold Spring Harbor Laboratory, New York, 1982).
2. Brennand, J., Chinault, A. C., Konecki, D. S., Melton, D. W. & Caskey, C. T. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1950–1954 (1982).
3. Hiscott, J., Murphy, D. & Defendi, V. *Cell* **22,** 535–543 (1980).
4. Cowell, J. K. *A. Rev. Genet.* **16,** 21–59 (1982).
5. Wahl, G. M., Vitto, L., Padgett, R. A. & Stark, G. R. *Molec. cell.* Biol. **2,** 308–319 (1982).
6. Dolnick, B. J. *et al. J. Cell Biol.* **83,** 394–402 (1979).
7. Nunberg, J. H., Kaufman, R. J., Schimke, R. T., Urlaub, G. & Chasin, L. A. *Proc. natn. Acad. Sci. U.S.A.* **75,** 5553–5556 (1978).
8. Biedler, J. L., Melera, P. W. & Spengler, B. A. *Cancer Genet. Cytogenet.* **2,** 47–60 (1980).
9. Milbrandt, J. D., Heintz, N. H., White, W. C., Rothman, S. M. & Hamlin, J. L. *Proc. natn. Acad. Sci. U.S.A.* **78,** 6043–6047 (1981).
10. Bostock, C. J. & Clark, E. M. *Cell* **19,** 709–715 (1980).
11. George, D. & Powers, V. *Cell* **24,** 117–123 (1981).
12. Dalla Favera, R., Wong-Staal, F. & Gallo, R. C. *Nature* **299,** 61–63 (1982).
13. Biedler, J. L. & Spengler, B. A. *Science* **191,** 185–187 (1976).
14. Alitalo, K., Schwab, M., Lin, C. C., Varmus, H. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1707–1711 (1983).
15. Schwab, M., Alitalo, K., Varmus, H., Bishop, J. M. & George, D. *Nature* **303,** 497–501 (1983).
16. Pall, M. L. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2464–2468 (1981).
17. Klein, G. *Nature* **294,** 313–318 (1981).
18. Bishop, J. M. *A. Rev. Biochem.* (in the press).
19. Anderson, R. P. & Roth, J. R. *Proc. natn. Acad. Sci. U.S.A.* **78,** 3113–3117 (1981).
20. Robert de Saint Vincent, B. *et al. Cell* **27,** 267–277 (1981).
21. Robins, D. M., Ripley, S., Henderson, A. S. & Axel, R. *Cell* **23,** 29–39 (1981).
22. Folger, K. R., Wong, E. A., Wahl, G. M. & Capecchi, M. R. *Molec. cell. Biol.* **2,** 1372–1387 (1982).
23. Patterson, D. & Carnright, D. V. *Somatic Cell Genet.* **3,** 483–495 (1977).
24. Graham, F. L. & van der Eb, A. S. *Virology* **52,** 456–467 (1973).
25. Wigler, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **76,** 1373–1376 (1979).
26. Swyryd, E. A., Seaver, S. & Stark, G. R. *J. biol. Chem.* **249,** 6945–6950 (1974).
27. Wahl, G. M., Padgett, R. A. & Stark, G. R. *J. biol. Chem.* **254,** 8679–8689 (1979).
28. Kempe, T. D., Swyryd, E. A., Bruist, M. & Stark, G. R. *Cell* **9,** 541–550 (1976).
29. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
30. Wahl, G. M., Stern, M. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76,** 3683–3687 (1979).
31. Padgett, R. A., Wahl, G. M. & Stark, G. R. *Molec. cell. Biol.* **2,** 293–301 (1982).
32. Robert de Saint Vincent, B. & Wahl, G. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2002–2006 (1983).
33. Szostak, J. W. & Wu, R. *Nature* **284,** 426–430 (1980).
34. Padgett, R. A., Wahl, G. M. & Stark, G. R. *Molec. cell. Biol.* **2,** 302–307 (1982).
35. Tartof, K. D. *Am. Rev. Genet.* **9,** 355–385 (1975).
36. Botchan, M., Topp, W. & Sambrook, J. *Cold Spring Harb. Symp. quant. Biol.* **43,** 709–719 (1979).
37. Roberts, J. M., Buck, L. B. & Axel, R. *Cell* **33,** 53–63 (1983).
38. Spradling, A. C. & Mahowald, A. P. *Cell* **27,** 203–209 (1981).
39. Schimke, R. T. in *Gene Amplification* (ed. Schimke, R. T.) 317–333 (Cold Spring Harbor Laboratory, New York, 1982).
40. Basilico, C. *Proc. natn. Acad. Sci. U.S.A.* **77,** 3850–3854 (1980).
41. Andrulis, I. L., Duff, C., Evans-Blackler, S., Worton, R. & Siminovitch, L. *Molec. cell. Biol.* **3,** 391–398 (1983).
42. Kaufman, R. J., Sharp, P. A. & Latt, S. A. *Molec. cell. Biol.* **3,** 699–711 (1983).
43. Kanda, N. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
44. McClintock, B., *Genetics* **26,** 234–282 (1941).
45. Kleckner, N. *A. Rev. Genet.* **15,** 341–404 (1981).
46. Paro, R., Goldberg, M. L. & Gehring, W. J. *EMBO J.* **2,** 853–860 (1983).
47. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113,** 237–251 (1977).

Close similarity of epidermal growth factor receptor and v-*erb-B* oncogene protein sequences

J. Downward*, Y. Yarden†, E. Mayes*, G. Scrace*, N. Totty*, P. Stockwell*§, A. Ullrich‡, J. Schlessinger† & M. D. Waterfield*

* Protein Chemistry Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK
† Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel
‡Genentech Incorporated, 460 Point San Bruno Boulevard, San Francisco, California 94080, USA

*Each of six peptides derived from the human epidermal growth factor (EGF) receptor very closely matches a part of the deduced sequence of the v-*erb-B *transforming protein of avian erythroblastosis virus (AEV). In all, the peptides contain 83 amino acid residues, 74 of which are shared with v-*erb-B*. The AEV progenitor may have acquired the cellular gene sequences of a truncated EGF receptor (or closely related protein) lacking the external EGF-binding domain but retaining the transmembrane domain and a domain involved in stimulating cell proliferation. Transformation of cells by AEV may result, in part, from the inappropriate acquisition of a truncated EGF receptor from the c-*erb-B *gene.*

REGULATION of the proliferation of cells in culture can be influenced by a number of mitogens including a series of polypeptide growth factors which, acting alone or synergistically with other mitogens, can induce DNA synthesis and proliferation of specific target cells (for recent reviews see ref. 1). Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) are probably the best characterized growth factors: the precise function of these polypeptides *in vivo* is, however, unclear. EGF may have a role in cell proliferation and differentiation since it will induce early eyelid opening and incisor development in new born mice[2]; PDGF on the other hand, which is released from platelets during blood clot formation at wound sites, may have a role in repair processes[3]. These and other growth factors *in vitro* can trigger a variety of morphological and biochemical changes that resemble those characteristic of transformed cells, and have also been implicated in the abnormal regulation of proliferation shown by transformed and tumour-derived cell lines (reviewed in refs 4 and 5). Thus it has been suggested that transformed cells may both synthesise and respond to growth factors and consequently proliferate independently through 'autocrine' secretion[6]. Direct support for such an autocrine role for aberrantly expressed growth factors in the control of abnormal cell proliferation came recently from the discovery that the putative transforming protein ($p28^{sis}$) of simian sarcoma virus (SSV) is structurally related to the growth factor PDGF[7–9] and can also function like PDGF as a growth factor for cells in culture[10]. Other growth factors produced by transformed cells such as insulin-like growth factor (IGF)[11,12], fibroblast-derived growth factor[13,14] and the transforming growth factors (TGFs)[15–20], may also act as autocrine regulators of proliferation. Besides the specificity mediated by regulation of the production of growth factors, cellular specificity could also be controlled at several other levels—the most obvious being by binding of ligand to specific receptors present only on target cells. In addition the binding of one growth factor to its specific receptor can also alter the affinity of another growth factor for its receptor (for example, PDGF and the EGF receptor[21,22]). Conversely two growth factors may, as appears to be the case with αTGFs and EGF, bind to the same receptor[23,24].

It is clear that binding of different growth factors to their specific receptors can induce a cascade of biochemical events including rapid changes in ion movements and intracellular *p*H, stimulation of tyrosine specific protein kinases and several other changes which can culminate in DNA synthesis and proliferation of certain target cells[1,4–6]. It seems likely that at least in the case of the EGF receptor the primary function of EGF may be to induce cross-linking or conformational changes of receptors, and that following such an activation step, all the 'information' necessary for triggering a proliferative response may reside in the receptor itself (see ref. 1). One known function intrinsic to the EGF receptor is its ability to phosphorylate tyrosine residues[25–28], a property shared with five of the putative transforming proteins of the family of retroviruses whose oncogenes are structurally related to *src* but not by two others, the proteins encoded by *mos* and *erb-B*[29]. At present this tyrosine kinase activity provides the only functional activity associated with the oncogenes of this subset of retroviruses.

Here we report amino acid sequence analysis of six distinct peptides from human EGF receptors isolated by monoclonal immunoaffinity purification from A431 cells and placenta, and show that 74 out of 83 of the residues sequenced are identical to those of the transforming protein encoded by the v-*erb-B* oncogene of avian erythroblastosis virus (AEV)[30]. The amino acid sequences of several other peptides purified from the EGF receptor could not be aligned with sequences of the v-*erb-B* protein suggesting that AEV has acquired cellular sequences encoding only a portion of the avian EGF receptor. Several lines of evidence suggest that the v-*erb-B* oncogene encodes only the transmembrane region of the EGF receptor and the domain associated with the tyrosine kinase activity. These results suggest a hypothesis that the *src* related subset of oncogenes, which includes v-*erb-B*, may be derived from cellular sequences which encode growth factor receptors and produce transformation through expression of uncontrolled receptor functions.

Purification of EGF receptor

EGF receptors can be detected in a variety of cells either by measurement of EGF binding (reviewed in ref. 31), by cross-linking of labelled EGF to its receptor (reviewed in ref. 32) or by using monoclonal antibodies[33–38]. In this study the receptor has been purified from two sources: the human epidermoid carcinoma cell line A431, which expresses about 50 times more receptors than the majority of other cells[39,40], and human placenta[41] which is a readily available normal tissue. The recent isolation of monoclonal antibodies which recognize the human EGF receptor[34,38] has made it possible to use immunoaffinity chromatography for receptor purification. Here we compare by peptide mapping EGF receptor protein purified by either immunoaffinity or EGF affinity chromatography[26] and also compare the structures of the A431 and placental receptors.

A radioimmunoassay (RIA) which uses a monoclonal antibody (R1) has been used to quantitate various preparative

§ Present address: Department of Biochemistry, University of Otago, Dunedin, New Zealand.

0028-0836/84/060521—07$01.00

© 1984 Macmillan Journals Ltd

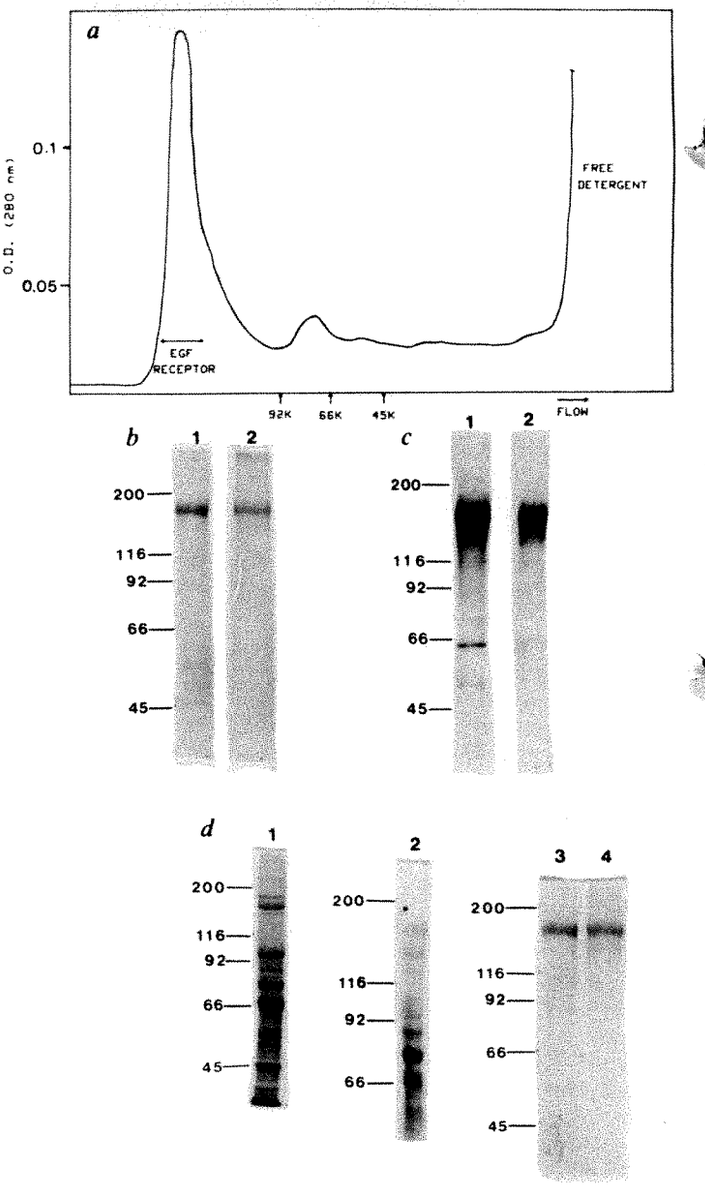


Fig. 1 Immunopurification of EGF receptor from A431 cells and human placenta. ***a***, Purification by gel permeation. Reduced and alkylated receptor was purified on a TSK4000 column (0.7 × 60 cm, LKB) using 0.1 M potassium dihydrogen phosphate buffer *p*H 4.5 containing 6 M guanidine HCl at a flow rate of 0.5 ml min^{-1} (ref. 43). The absorbance of the eluate was monitored at 280 nm (molecular weights of protein standards are indicated) and 0.25 ml fractions were collected. Fractions containing EGF receptor were dialysed against 10 mM ammonium bicarbonate. Panels ***b–d*** show 7% polyacrylamide SDS gels[72] used to monitor purification (MW × 10^{-3} of protein standards are indicated). *b*, R1 purified A431 EGF receptor: track 1, *p*H 3 eluate from R1 immunoaffinity matrix; track 2, eluate from the TSK4000 column. *c*, 29–1purified A431 EGF receptor: track 1, *p*H 3 eluate from 29–1 immunoaffinity matrix; track 2, eluate from SDS preparative gel electrophoresis. *d*, Lectin and R1 purified placental EGF receptor: track 1, placental vesicles; track 2, eluate from lectin affinity matrix; track 3, eluate from R1 immunoaffinity matrix; track 4, eluate from the TSK4000 column.

Methods: Approximately 2×10^9 A431 cells were washed in calcium and magnesium free phosphate-buffered saline (PBS) and solubilized in 400 ml lysis buffer (50 mM Tris-HCl *p*H 7.4, 0.15 M NaCl, 5 mM EGTA, 0.1% bovine serum albumin, 1% NP40, 25 mM benzamidine, 0.2 mM PMSF, 10 μg ml^{-1} leupeptin). After filtration through muslin the lysate was adjusted to *p*H 8.5 and centrifuged at 100,000g_{max} for 30 min. The supernatant was incubated for 2 h at 4 °C with immunoaffinity matrix, which consisted of 15 mg of monoclonal antibody R1[34] coupled to 15 ml of Affi-Gel 10 (BioRad). Unbound lysate was removed by suction through a 0.4 micron filter. The matrix was then washed by gentle agitation and filtration with 500 ml PBS, containing 0.65 M NaCl and 0.1% NP40, followed by 500 ml PBS, containing 0.1% NP40. The EGF receptor was eluted by gentle agitation and filtration of the matrix with 2 × 10 ml aliquots of 50 mM sodium citrate *p*H 3, containing 0.05% NP40 for 10 min each. Eluates were adjusted to *p*H 7. The yield of receptor was approximately 250 μg, measured by Bradford technique[70] or by amino acid analysis after gel permeation HPLC (see below). Alternatively EGF receptor was purified from A431 cells using monoclonal antibody 29-1[38] coupled to CNBr-activated Sepharose (Pharmacia) at 5–10 mg ml^{-1}. The purification procedure used was similar to that described for the R1 immunoaffinity matrix except that the EGF receptor was phosphorylated whilst bound to the matrix with 50 μCi [$\gamma-^{32}$P]ATP (3,000 Ci $mmol^{-1}$, Amersham International) in the presence of 3 mM $MnCl_2$. Eluted receptor was further purified by preparative SDS gel electrophoresis, followed by dialysis against 10% methanol at 4 °C. For purification of placental EGF receptor, vesicles were made from syncytiotrophoblast microvilli by a modification of the method of Smith *et al.*[71], using 2 mM EGTA in all buffers. Vesicles were solubilized by addition of an equal volume of 100 mM HEPES *p*H 7.4, 0.15 M NaCl and 5% Triton X-100. After centrifuging at 100,000g_{max} for 30 min the supernatant was incubated for 1 h at room temperature with 200 mg wheat germ agglutinin coupled to 10 g of Affi-Gel (BioRad). The lectin matrix was washed by filtration through a 0.4 μm filter with 100 ml of PBS, containing 0.1% Triton X-100. Bound protein was eluted by agitation and filtration with 2 × 15 ml aliquots of 0.25 M *N*-acetylglucosamine in 10 mM HEPES *p*H 7.4, 0.1% Triton X-100 for 15 min each. The eluate was incubated with R1 immuno-affinity matrix (15 mg antibody per placenta) for 2 h at 20 °C, followed by extensive washing and elution as described above for receptor purification from A431 cells. The yield of EGF receptor per placenta was 25 μg, measured by Bradford technique[70] and amino acid analysis after gel permeation HPLC (see below). Solutions containing EGF receptor were lyophilized and resuspended in 0.5 M Tris-HCl *p*H 8.5, 6 M guanidine hydrochloride (Schwarz-Mann) at 0.5–1 mg ml^{-1}. After incubation at 37 °C for 16 h with 10 mM dithiothreitol, cysteine residues were alkylated with ^{14}C-iodacetamide (40–60 mCi $mmol^{-1}$, Amersham International) as described previously[44].

techniques[42]. Receptors from A431 cells and placenta were both found to be unstable in detergent-solubilized whole cell or tissue lysates, perhaps as a result of the release of proteases from the cellular lysosomal compartment. This problem was overcome for placenta by the preparation of syncytiotrophoblast microvillus plasma membranes and as a result a 50-fold purification with a 30% yield of receptor was achieved. Unfortunately, with A431 cells the yield of receptor in plasma membrane preparations was impractically low. However, quantitative studies with the receptor RIA showed that rapid adjustment of the lysate *p*H to 8.5 followed by fast immunoaffinity chromatography of whole cell lysates minimised the effects of the proteases.

Placental membranes were solubilized and glycoproteins separated by wheat germ agglutinin (WGA) affinity chromatography to achieve a partial purification. EGF receptor was then purified from the placental glycoprotein fraction or from A431 cell lysates by immunoaffinity chromatography on either monoclonal antibodies R1[34] or 29-1[38] immobilised on agarose or Sepharose respectively. Nonspecifically bound protein was removed by washing the columns with a high salt buffer and the receptor was eluted at *p*H 3. The receptors were further purified either on preparative SDS-polyacrylamide gels or by gel permeation HPLC in guanidine solutions[43]. Details of the methods used and the yields of purified receptors are given in the legend to Fig. 1. Since the EGF binding and protein kinase activity were partially destroyed during purification, receptor was also purified by EGF affinity chromatography[26]. Comparative HPLC tryptic peptide maps were then carried out to establish the purity and structural similarity of receptor prepared by immunoaffinity chromatography from A431 cells and placental tissue. The peptide maps of the receptors (Fig. 2) showed that the elution profiles of the receptor tryptic peptides were very

similar whether receptor was purified by EGF affinity or by immunoaffinity chromatography; from A431 cells or from placental tissue.

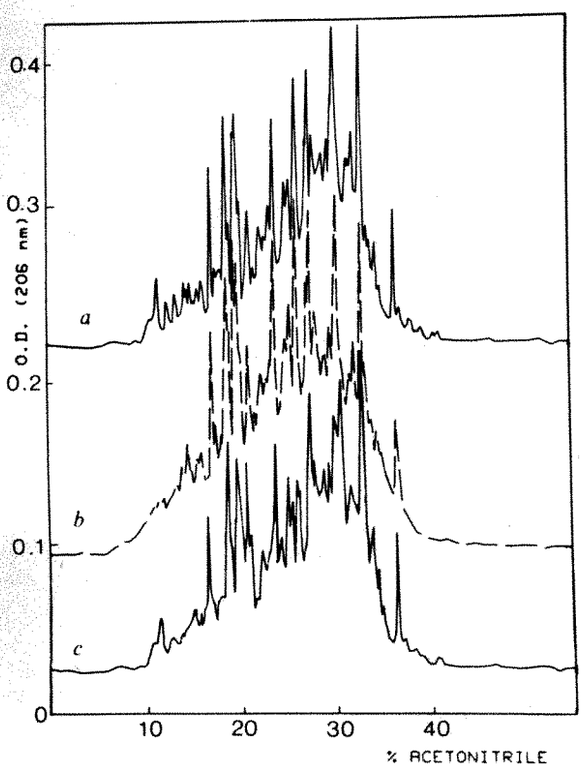


Fig. 2 Reverse phase HPLC analysis of tryptic peptides from EGF receptor purified by three different affinity methods. ***a***, Immunoaffinity purified A431 receptor. EGF receptor was purified from A431 cells using the monoclonal antibody R1 and gel permeation chromatography in guanidine solutions as described in Fig. 1. Pooled fractions containing the 175,000 MW EGF receptor were dialysed against 10 mM ammonium bicarbonate, lyophilized and resuspended in 500 μl of 100 mM ammonium bicarbonate, 10 mM $CaCl_2$. TPCK treated trypsin (Sigma) was then added (100:1, receptor:trypsin, w/w). This mixture was incubated at 37 °C for 12 h, a further identical aliquot of trypsin added and the incubation continued for another 12 h. Peptides were then loaded directly onto a Synchropak RPP C18 reverse phase HPLC column (Synchrom, Linden, Indiana, 4.6×75 mm) equilibrated in 0.1% trifluoroacetic acid (TFA, Rathburn, Scotland) and a gradient from zero to 45% acetonitrile was run over 45 min at 1 ml min^{-1} and 1 ml fractions collected[46]. A Waters HPLC system including two M6000 A pumps, a U6K manual injector and a 660 solvent programmer with 2 LKB 2138 Uvicord S absorbance detectors with filters at 206 nm and 280 nm was used[45]. The figure shows the optical density of the eluate at 206 nm plotted against acetonitrile concentration. ***b***, EGF affinity purified receptor. EGF receptor was purified from A431 cells using wheat germ agglutinin affinity chromatography followed by EGF affinity chromatography[26]. A431 cells were lysed as described in the legend to Fig. 1. Purification of this lysate on the WGA affinity column was identical to that described for the placental preparation (Fig. 1). The eluate from the WGA affinity column was mixed with 5 ml of Affi-Gel 10 (BioRad) having 1 mg of bound EGF. The mixture was tumbled for 4 h at room temperature before washing the immobilized EGF receptor with 100 ml of PBS, 0.1% Triton X-100. Receptor was then eluted with 10 mM ethanolamine, *p*H 9.7, 0.1% Triton X-100. This eluate was further purified on a TSK4000 gel permeation column as described in Fig. 1. Fractions containing EGF receptor were pooled, dialysed, lyophilized and trypsinized as described for *a* above. The resulting tryptic peptides were separated by reverse phase HPLC under identical conditions to those described above. ***c***, Immunoaffinity purified placental receptor. EGF receptor was purified from fresh term human placenta as described in the legend to Fig. 1. The receptor was digested with trypsin and peptides separated by reverse phase HPLC as described above. Peptides in the column breakthrough are not shown.

Amino acid sequence

Receptor was purified by immunoaffinity chromatography followed by either preparative SDS gel electrophoresis or by gel permeation HPLC in guanidine[43] after reduction and alkylation[44] to cleave disulphide bonds (see Fig. 1). Purified receptor was then digested with trypsin or cyanogen bromide (see Figs 2 and 3) and peptides were separated by preparative reverse phase HPLC[45,46] (Fig. 3). Amino acid sequences were determined with a gas phase sequencer constructed and operated as described by Hewick *et al.*[47], using the analytical techniques for the quantitation of phenyl thiohydantoin (PTH) amino acids described by M.D.W. *et al.*[48]. The quantitative data for analysis of six peptides are shown in Fig. 4.

The amino acid sequences of 14 different peptides from the human EGF receptor, three from placenta and 11 from A431 cells, were compared with sequences in an oncogene sequence data base (set up at ICRF using published sequences) by the rapid search techniques of Wilbur and Lipman[49]. A remarkable identity was found between the sequences of six of these peptides and regions of the predicted sequence of the putative transforming protein v-*erb-B* of the AEV-H isolate of avian erythroblastosis virus[30]. Of the 83 amino acid residues from these six sequenced peptides, 74 residues were identical and four showed conservative substitutions when they were aligned with the v-*erb-B* encoded protein sequence, as shown in Fig. 5. Peptide 1 was located near the amino terminus of the v-*erb-B* protein (residues 107–125) and peptide 6 at the carboxy terminus (residues 583–599), with the other four peptides in between. It was not necessary to introduce any deletions or insertions into the sequence to optimize the alignments.

Although the full extent of the similarity between the v-*erb-B* protein and EGF receptor sequences is not revealed by these limited sequence studies, it is likely that the region of the v-*erb-B* protein from residue 107 to the carboxy terminus has extensive homology to the EGF receptor. The degree of identity observed is very high and since the v-*erb-B* sequences of AEV were presumably of avian origin[30], while the EGF receptor sequences were from the human protein, it is likely that the v-*erb-B* sequences were mainly acquired by AEV from those cellular sequences which encode the avian EGF receptor. This suggests that the c-*erb-B* locus encodes the EGF receptor in humans and birds.

The amino acid sequence of 8 of the 14 peptides purified from the EGF receptor (data not shown) could not be aligned with the predicted sequences of the v-*erb-B* protein. Since the polypeptide backbone of the EGF receptor glycoprotein is thought to be about 1,250 amino acids[50] and the predicted v-*erb-B* protein is only 604 amino acids[30] the most likely explanation is that these eight peptides are encoded by a region of c-*erb-B* which has not been acquired by AEV. This could have arisen by a recombination event(s) which resulted in only a part of the EGF receptor coding sequences being acquired by AEV. Although it is possible that DNA rearrangements of receptor coding sequences occur similar to those found with immunoglobulins, it is more likely that differential mRNA splicing would be involved in any such recombination events. It has been shown that avian cells contain two c-*erb-B* related transcripts[51] and studies of the biosynthesis of the EGF receptor in A431 cells suggest that both a normal and a truncated receptor may be synthesized[50]. Alternatively two or more loci encoding polypeptides having very similar amino acid sequences to those of the EGF receptor exist on chromosome 7 (see below). An example of two closely related putative transforming proteins with tyrosine kinase activity has been reported in studies of the avian retroviruses Rous sarcoma virus (RSV) and Yamaguchi Y63[52]. The predicted amino acid sequences of the proteins encoded by the *src* and *yes* oncogenes were shown to have 82% match over a region covering 436 amino acid residues (while the DNA sequences showed only 31% overall match) and presumably the chicken genome contains both *src* and *yes* proto-oncogenes encoding separate proteins sharing extensive regions of sequence. It is not known whether human c-*src* and c-*yes*

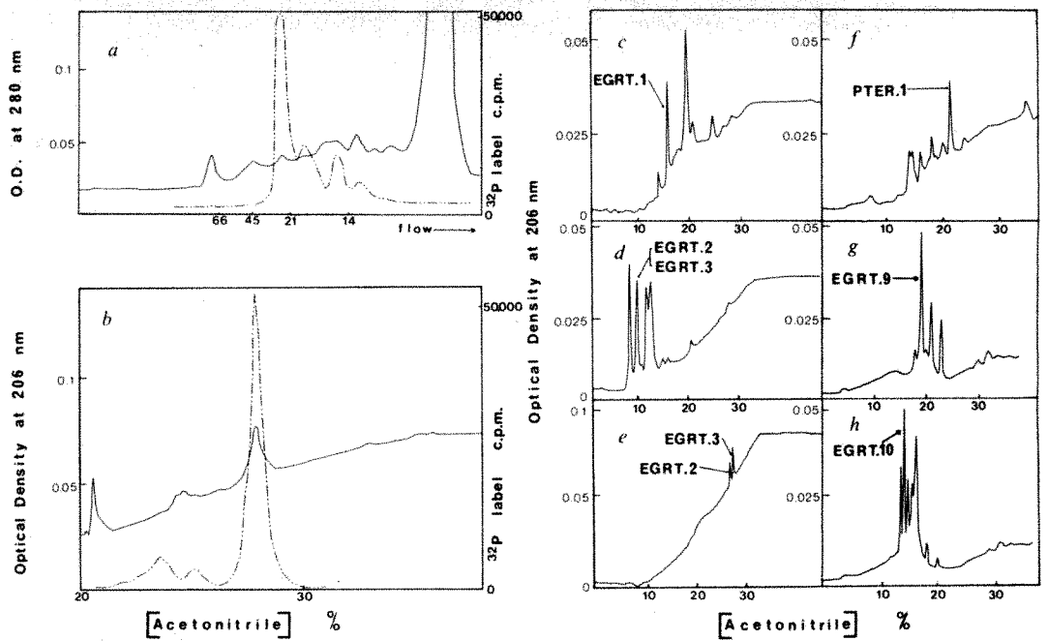


Fig. 3 Purification of peptides from EGF receptor for sequence analysis. ***a***, Cyanogen bromide cleavage and fractionation of peptides by size. ^{32}P-labelled EGF receptor in ammonium bicarbonate solution was lyophilized and resuspended in 70% formic acid. Cyanogen bromide was added under nitrogen, the tube sealed and incubated in the dark at room temperature for 24 h. Formic acid and excess cyanogen bromide were removed by repeated cycles of drying and resuspension in water using a Speed-vac concentrator (Savant). The dry sample was resuspended in 0.1 M potassium dihydrogen phosphate buffer, *p*H 4.5 containing 6 M guanidine HCl and the peptides separated by gel permeation HPLC on a TSK3000 column (0.7×60 cm, LKB) equilibrated in the same buffer at a flow rate of 0.3 ml min^{-1} [43]. The optical density of the eluate was monitored at 280 nm (– – –) and 0.3 ml fractions collected and counted for ^{32}P (– · · –). Molecular weights $\times 10^{-3}$ of protein standards are indicated. ***b***, Subfractionation of cyanogen bromide fragments. The peak from the TSK3000 column containing most of the ^{32}P-label was pooled and dialysed against 10 mM ammonium bicarbonate. After lyophilization, the sample was redissolved in 0.1% TFA and peptides separated by reverse phase HPLC on a Synchropak RPP C18 column (see Fig. 2) equilibrated in 0.1% TFA, 10% acetonitrile[45,46]. A gradient of 10–40% acetonitrile run over 60 min was used to elute peptides, at a flow rate of 1 ml min^{-1}. The optical density of the eluate was monitored at 206 nm (– –) and 1 ml fractions were collected and counted for ^{32}P-label (– · · –). ***c***, The fractions corresponding to 23–24% acetonitrile from the HPLC analysis of A431 EGF receptor tryptic peptides were pooled. Peptides were further purified by reverse-phase HPLC on a Synchropak RPP C18 column equilibrated in 10 mM ammonium acetate buffer *p*H 6.5. A linear gradient of 0–45% acetonitrile was run over 45 min at a flow rate of 1 ml min^{-1}. The optical density of the eluate was monitored at 206 nm and 0.5 ml fractions collected. ***d***, The fractions corresponding to 19–20% acetonitrile from the reverse-phase HPLC purification of A431 EGF receptor tryptic peptides (Fig. 2*a*) were pooled. Peptides were separated as described in *c*. ***e***, The peak fractions arrowed in *d* were pooled and peptides subfractionated by reverse-phase HPLC on a μ Bondapak phenyl column (0.46×25 cm, Waters Assoc.) equilibrated in 0.1% TFA. A linear gradient of 0–45% acetonitrile over 45 min was used to elute peptides, at a flow rate of 1 ml min^{-1}. The optical density of the eluate was monitored at 206 nm and 0.2 ml fractions collected. ***f***, The fractions corresponding to 27–28% acetonitrile concentration from the reverse-phase HPLC analysis of placental EGF receptor tryptic peptides (Fig. 2*c*) were pooled. Peptides were subfractionated as described in *c*. ***g***, The fractions corresponding to 25–26% acetonitrile from the reverse-phase HPLC purification of A431 EGF receptor tryptic peptides (Fig. 2*a*) were pooled. Peptides were subfractionated as described in *c*. ***h***, The fractions corresponding to 21–22% acetonitrile from the reverse-phase HPLC analysis of A431 EGF receptor tryptic peptides (Fig. 2*a*) were pooled. Peptides were subfractionated as described in *c*.

are encoded by closely linked loci. However, analysis of human-mouse somatic cell hybrids has shown that the locus encoding the human EGF receptor is on chromosome 7 (7p13-7q22)[53–55] and that for c-*erb-B* is in the same region of this chromosome (7pter–7q22)[56]. This observation supports the concept that the c-*erb-B* locus encodes the human EGF receptor or is very closely linked to that encoding the EGF receptor.

Analysis of EGF receptor mRNA transcripts found in A431 and avian cells by cDNA cloning is currently in progress which, together with analysis of human and avian genomic clones, should define the relationship of c-*erb-B* and the locus encoding the EGF receptor (A.U. *et al.*, in preparation).

Shared regions of sequence

Several lines of evidence suggest that the EGF receptor protein can be divided into three major domains; an EGF binding domain which lies external to the plasma membrane, a transmembrane domain and a cytoplasmic kinase domain having both the kinase activity and the autophosphorylation sites.

Investigations of receptor biosynthesis show that the A431 receptor is a glycoprotein of apparent molecular weight (MW) 175,000, having ~37,000 MW of oligosaccharide side chains with a polypeptide backbone of ~138,000 MW (~1,250 amino acids). Limited proteolysis of the mature receptor suggests that the domain external to the plasma membrane which contains the oligosaccharide side chains and the antigenic sites for monoclonal R1 has a MW of ~115,000 (~710 amino acids)[50]. Several studies show that the EGF binding site is external to the plasma membrane[25,31,32].

The location of the tyrosine kinase enzymatic activity and the autophosphorylation sites on the cytoplasmic domain is supported by studies made using A431 and placental membrane vesicles (J.D. and M.D.W., in preparation). These show that EGF stimulated tyrosine kinase activity directed towards artificial substrates or towards autophosphorylation sites is significantly activated only after membrane permeabilization. Furthermore, the tyrosine kinase activity can phosphorylate pp36—a protein known to be located at the cytoplasmic side of the membrane[57]. In addition recent studies show that antibodies raised against synthetic peptides from pp60$^{v\text{-}src}$ recognize antigenic sites on the human EGF receptor that are from regions of sequence homologous to the sequence of v-*erb-B* (see below). These sites are only accessible in permeabilized cells (J.S. *et al.*, in preparation).

Autophosphorylation sites are located within peptide 5 (EGRC.1), a 20,000 MW cyanogen bromide fragment which contains 70% of the ^{32}P-label present in the autophosphorylated receptor (see Fig. 3*a*). Although the precise location of the residues phosphorylated has not been determined a consensus tyrosine phosphorylation sequence[58] was found near the amino

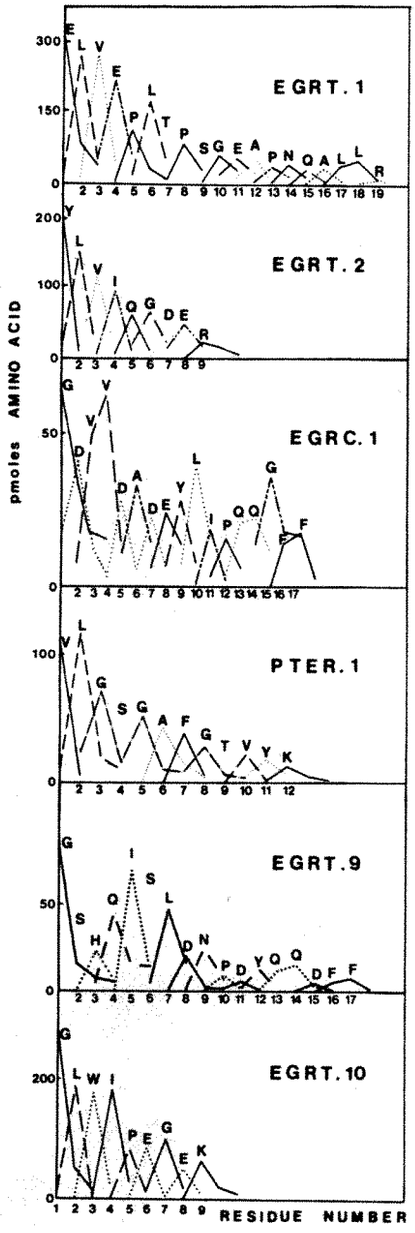


Fig. 4 Sequence analysis of peptides from the EGF receptor. Peptides were purified as described in Fig. 3. Sequence determination of each peptide was carried out using a gas phase sequencer assembled and operated as described by Hewick *et al.*[47]. PTH amino acids were analysed by HPLC using a Zorbax C8 column (4.6×150 mm, Dupont) at 43 °C with a linear gradient over 8 min of acetonitrile from 24% to 38% at a flow rate of 2 ml min^{-1} using 9 mM sodium acetate buffer *p*H 4.1[48]. A Waters HPLC system including two M6000 A pumps, a WISP autoinjector and system controller with a Beckman Model 160 detector was used. The recovery of PTH amino acids at each degradative cycle was measured using an integrative recorder (Waters Data module). The amounts of each peptide *analysed* (pmol) were measured by amino acid analysis (EGRT.1, 380; EGRT.2, 260; EGRC.1, 80; PTER.1, 145; EGRT.9, 100; EGRT.10, 325). The analysis for serine and threonine could not be accurately measured due to the presence of multiple peaks obtained during analysis of the PTH amino acids. The presence of these amino acids is thus indicated without quantitative data; these residues are assigned to the sequence using semi-quantitative recovery data based on peak heights rather than areas. Prior to loading peptides, fibre glass disks were treated with polybrene and glycylglycine and precycled for ten cycles. Each peptide was sequenced twice; on the second run of peptide EGRC.1 filters were treated with polybrene and cysteic acid and precycled ten times to clarify the assignment of an amino terminal glycine residue. However the background glycine at step 1 is still significant and this residue may be incorrect.

terminus of this peptide. Therefore we believe that the tyrosine phosphorylation sites lie within the cytoplasmic domain of the EGF receptor which is contained in the sequence shared with the v-*erb-B* protein.

Preliminary nucleotide sequence analysis (A.U. *et al.*, in preparation) of cDNA clones selected from a placental cDNA library using synthetic oligonucleotide probes synthesized on the basis of the receptor amino acid sequence shows that the predicted carboxyl terminus of the EGF receptor extends 32 amino acids from an amino acid equivalent to residue 601 (see Fig. 5) of the predicted v-*erb-B* protein sequence. This analysis when complete will show the precise size and sequence of the presumptive cytoplasmic domain of the EGF receptor. The approximate molecular weight of this domain would be 60,000 (or 545 amino acids) since that part which is external to the membrane is thought to have a molecular weight of 115,000 (see above)[50]. Thus the cytoplasmic domain would be predicted to be similar in size to that region of the v-*erb-B* protein which is carboxy terminal to a putative transmembrane sequence (see Fig. 5 and ref. 30) at residues 66–88. This carboxy terminal region of v-*erb-B* would have a molecular weight of 56,760 and would contain 516 amino acids.

The putative transmembrane sequence of the v-*erb-B* protein is not preceded by a signal sequence for membrane insertion. Nevertheless, immunofluorescence studies of AEV transformed cells show that the v-*erb-B* protein has antigenic sites external to the plasma membrane[59]. This external region probably corresponds to the 65 residue amino terminal section that precedes the putative transmembrane sequence and contains three asparagine residues which have the oligosaccharide attachment recognition sequence Asn-X-Ser or Thr. Some or all of these residues may be glycosylated since *in vitro* translation studies of mRNA from AEV infected cells show that post translational processing of nascent polypeptides occurs in the presence of membrane vesicles[59,60].

Together these studies suggest that the predicted v-*erb-B* transforming protein closely resembles the transmembrane region of the EGF receptor and the domain which is thought to be cytoplasmic. If the v-*erb-B* sequence was acquired from the gene encoding the EGF receptor then the v-*erb-B* protein represents a truncated receptor which lacks the EGF binding domain. It is particularly interesting that studies of EGF receptor biosynthesis in A431 cells have suggested that a polypeptide equivalent to the external domain of the receptor (of MW 115,000) is synthesized[50] in addition to the normal receptor. Further studies are necessary to understand the origin of this truncated receptor but the results show that defective receptors may be synthesized by this human tumour cell line.

Growth factors and transformation

Recently it has been shown that the transforming protein of simian sarcoma virus has a close structural and functional relationship to the growth factor PDGF[7–10] supporting the hypothesis that autocrine growth factor production may be involved in abnormal growth control and neoplasia. These observations together with those presented here illustrate two distinct but related mechanisms for subversion of normal growth regulation. In the case of SSV the oncogene encodes a growth factor which can act as a mitogen for target cells having PDGF receptors[10]. AEV on the other hand appears to utilize a different mechanism where a part of a growth factor receptor which is thought to be involved in transducing the EGF signal may be expressed in transformed cells. The absence of the EGF binding domain might remove the control generated by ligand binding and the result could be the continuous generation of a signal equivalent to that produced by EGF, causing cells to proliferate rapidly. How this could result in the block in differentiation observed in AEV infected haemopoietic cells[61] is unclear. However, EGF has been shown to promote proliferation while inhibiting terminal differentiation of human keratinocytes[31].

The ES4 strain of AEV has two oncogenes v-*erb-A* and v-*erb-B*, which are thought to encode proteins of MWs 75,000

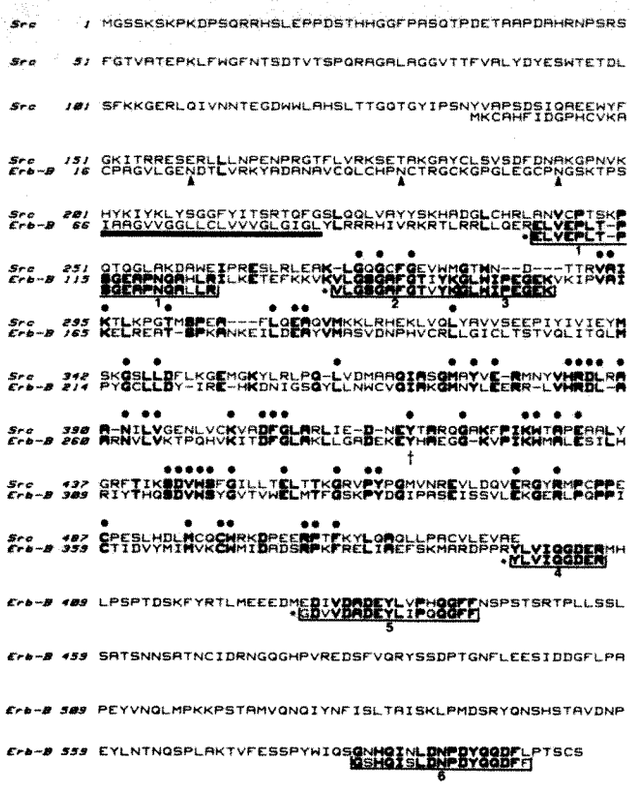


Fig. 5 The relationship between the amino acid sequences of the EGF receptor peptides and the predicted amino acid sequences of the putative transforming proteins of v-*src* and v-*erb-B*. The predicted amino acid sequence of the v-*src* gene product (pp60$^{v\text{-}src}$) is translated from the presumptive initiation codon at nucleotide 7,129 of the Prague C strain of Rous sarcoma virus[73]. The predicted amino acid sequence of the v-*erb-B* gene product is translated from the presumptive initiation codon at nucleotide 155 of the v-*erb-B* gene in AEV-H[30]. The partial amino acid sequences of the six peptides purified from the EGF receptor are shown (underlined): 1, EGRT.1; 2, PTER.1; 3, EGRT.10; 4, EGRT.2; 5, EGRC.1; 6, EGRT.9. Letters in bold type represent residues shared by pp60$^{v\text{-}src}$ and the v-*erb-B* protein. Residues shared by the EGF receptor peptides and v-*erb-B* protein or pp60$^{v\text{-}src}$ are in bold type. ● indicates amino acid residues, which are common to the putative transforming proteins of v-*erb-B*, v-*src*, v-*fes*, v-*fps*, v-*yes* and v-*abl*. †, Phosphoacceptor tyrosine of pp60$^{v\text{-}src}$[74]. ▲, Possible *N*-linked glycosylation sites at the amino terminus of the v-*erb-B* protein. ▬ indicates the putative transmembrane sequence in the v-*erb-B* protein. *, Amino acid residues which would be expected to produce enzymatic or cyanogen bromide cleavages to generate the observed peptides. Numbers to the left of the sequences are residue numbers taking the presumptive initiation methionine as 1 in both cases. Sequences were aligned using a computer program[75] to optimize match.

and 65,000 respectively (for a recent review see ref. 62). Cells transformed by AEV *in vitro* and *in vivo* have the properties of erythroblasts which are late erythroid progenitors, although the target cells themselves may be earlier erythroid precursor cells. AEV can also transform fibroblasts and induce sarcomas. Evidence from deletion mutants[63] and from an isolate (AEV-H[30]) which lacks the v-*erb-A* gene suggests that the v-*erb-B* gene alone can induce transformation. This is supported by studies which show that RAV-1, a leucosis virus which has no oncogene, can activate the c-*erb-B* gene by a promoter insertion mechanism[64] perhaps similar to that presently being unravelled for c-*myc* activation[65–67]. It is possible that RAV-1 could induce expression of a normal receptor or a truncated receptor. EGF receptors have not generally been detected on haemopoietic cells by EGF binding studies but since these studies are limited in scope and sensitivity a more rigorous survey is needed before conclusions about normal receptor expression in different haemopoietic cell types can be made. Although many normal cells express 10–100,000 EGF receptors[31] only very low levels of c-*erb-B* transcripts have been found in normal chicken fibroblasts[51]. However a recent study of normal and neoplastic human lymphocytes suggests that both types of cells contain c-*erb-B* related transcripts. Clearly more detailed studies will be necessary to search for normal or altered c-*erb-B* or v-*erb-B* transcripts in normal, neoplastic or AEV transformed cells.

Previous reports have shown that the predicted amino acid sequences of the putative viral transforming proteins encoded by the oncogenes *erb-B*, *src*, *yes*, *fes*, *fps*, *mos* and *abl* show regions of similarity, implying homology[29,30,69]. In the case of *src*, *yes*, *fes*, *fps* and *abl* the putative transforming proteins have been shown to have tyrosine kinase activity (reviewed in ref. 29) but as yet those encoded by *erb-B*[30] and *mos* have not. Since the receptors for EGF and αTGF, PDGF, insulin and IGF-I also have associated tyrosine kinases, the structural relationship between the v-*erb-B* transforming protein and the EGF receptor observed here, suggests that other oncogenes from this subset of retroviruses could be derived in part from sequences encoding these or other growth factor receptors. Alternatively the other oncogenes in this group may be derived from sequences which encode proteins having a less direct functional relationship to the EGF and other receptors. Further study will clarify the precise function of growth factors, their receptors and *src* related oncogenes in normal and malignant growth.

M.D.W. thanks B. Ozanne and E. Rozengurt for introducing him to the field of study involving growth factors and their receptors. We acknowledge P. Parker, P. Stroobant, H. S. Earp and M. Fried for helpful discussions and advice, W. Gullick for help with receptor quantitation techniques and P. Bennett and R. Philp for technical assistance. We also thank W. J. Wilbur and D. J. Lipman for computer programs and N. Teich and the ICRF Computer Unit for their help. M.D.W. thanks S. J. for continued support. We are indebted to Queen Charlotte's Hospital and to E. J. Owen for help in providing placental tissue. This work was supported in part by grants from the USNIH (CA 25820) (J.S.) and from the US–Israel Binational Science Foundation (J.S.).

Received 18 January; accepted 23 January 1984.

1. Guroff, G. (ed.) *Growth and Maturation Factors* (Wiley, New York, 1983).
2. Cohen, S. *J. biol. Chem.* **237,** 1555–1562 (1962).
3. Ross, R. in *Tissue Growth Factors* (ed. Baserga, R.) 133–159 (Springer, Berlin, 1981).
4. Rozengurt, E. *Molec. Biol. Med.* **1,** 169–181 (1983).
5. James, R. & Bradshaw, R. A. *A. Rev. Biochem.* (in the press).
6. Sporn, M. B., & Todaro, G. J. *New Engl. J. Med.* **303,** 878–880, (1980).
7. Waterfield, M. D. *et al. Nature* **304,** 35–39 (1983).
8. Doolittle, R. F. *et al. Science* **221,** 275–277 (1983).
9. Robbins, K. C., Antoniades, H. N., Devare, S. G., Hunkapiller, M. W. & Aaronson, S. A. *Nature* **305,** 605–608 (1983).
10. Deuel, T. F., Huang, J. S., Huang, S. S., Stroobant, P. & Waterfield, M. D. *Science* **221,** 1348–1350 (1983).
11. Dulak, N. C. & Temin, H. M. *J. cell. Physiol.* **81,** 161–170 (1973).
12. De Larco, J. E. & Todaro, G. J. *Nature* **272,** 356–358 (1978).
13. Burk, R. R. *Expl Cell Res.* **101,** 193–298 (1976).
14. Bourne, H. & Rozengurt, E. *Proc. natn. Acad. Sci. U.S.A.* **73,** 4555–4559 (1976).
15. De Larco, J. E. & Todaro, G. J. *Proc. natn. Acad. Sci. U.S.A.* **75,** 4001–4005 (1978).
16. Roberts, A. B., Frolik, C. A., Anzano, M. A. & Sporn, M. B. *Fedn Proc.* **42,** 2621–2626 (1983).
17. Ozanne, B., Fulton, R. J. & Kaplan, P. L. *J. cell. Physiol.* **105,** 163–180 (1980).
18. Kaplan, P. L., Anderson, M. & Ozanne, B. *Proc. natn. Acad. Sci. U.S.A.* **79,** 485–489 (1982).
19. Kaplan, P. L. & Ozanne, B. *Cell* **33,** 931–938 (1983).
20. Marquardt, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 4684–4688 (1983).
21. Bowen-Pope, D. F., Di Corletto, P. E. & Ross, R. *J. Cell Biol.* **96,** 679–683 (1983).
22. Collins, M. K. L., Sinnett-Smith, J. W. & Rozengurt, E. *J. biol. Chem.* **258,** 11689–11693 (1983).
23. Marquardt, H. & Todaro, G. J. *J. biol. Chem.* **257,** 5220–5225 (1982).
24. Carpenter, G., Stoscheck, C. M., Preston, Y. A. & De Larco, J. E. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5627–5630 (1983).
25. Cohen, S., Ushiro, H., Stoscheck, C. & Chinkers, M. *J. biol. Chem.* **257,** 1523–1531 (1982).
26. Buhrow, S. A., Cohen, S. & Staros, J. V. *J. biol. Chem.* **257,** 4019–4022 (1982).
27. Cohen, S., Fava, R. A. & Sawyer, S. T. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6237–6241 (1982).
28. Buhrow, S. A., Cohen, S., Garbers, D. L. & Staros, J. V. *J. biol. Chem.* **258,** 7824–7827 (1983).
29. Bishop, J. M., *Rev. Biochem.* **52,** 301–354 (1983).
30. Yamamoto, T., Nishida, T., Miyajima, N., Kawai, S., Ooi, T. & Toyoshima, K. *Cell* **35,** 71–78 (1983).
31. Adamson, E. D. & Rees, A. R. *Molec. cell. Biochem.* **34,** 129–152 (1981).
32. Linsley, P. S., Das, M. & Fox, C. F. in *Membrane Receptors* Vol. B11 (eds Jacobs, S. & Cuatrecasas, P.) 87–113 (Chapman & Hall, London, 1981).
33. Schreiber, A. B., Lax, I., Yarden, Y., Eshhar, Z. & Schlessinger, J. *Proc. natn. Acad. Sci. U.S.A.* **78,** 7535–7539 (1981).
34. Waterfield, M. D. *et al. J. Cell Biochem.* **20,** 149–161 (1982).

35. Kawamoto, T. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 1337–1341 (1983).
36. Richert, N. D., Willingham, M. C. & Pastan, I. *J. biol. Chem.* **258,** 8902–8907 (1983).
37. Gregoriou, M. & Rees, A. R. *Cell Biol. Int. Rep.* **7,** 539-540 (1983).
38. Schlessinger, J., Lax, I., Yarden, Y., Kanety, H. & Libermann, T. A. in *Receptors and Recognition: Antibodies against Receptors* (ed. Greaves, M. F.) (Chapman and Hall, London, in the press).
39. Fabricant, R. N., De Larco, J. E. & Todaro, G. J. *Proc. natn. Acad. Sci. U.S.A.* **74,** 565–569 (1977).
40. Wrann, M. M. & Fox, C. F. *J. biol. Chem.* **254,** 8083–8086 (1979).
41. O'Keefe, E., Hollenberg, M. D. & Cuatrecasas, P. *Archs biochem. Biophys.* **164,** 518–526 (1974).
42. Gullick, W., Downward, D. J. H., Marsden, J. J. & Waterfield, M. D. *Analyt. Biochem.* (in the press).
43. Ui, N. *Analyt. Biochem.* **97,** 65–71 (1979).
44. Skehel, J. J. & Waterfield, M. D. *Proc. natn. Acad. Sci. U.S.A.* **72,** 93–97 (1975).
45. Waterfield, M. D. & Scrace, G. T. in *Biological/Biomedical Applications of Liquid Chromatography* Vol. 18 (ed. Hawk, G. L.) 135–157 (Dekker, New York, 1981).
46. Bennett, H. P. J., Browne, C. A. & Solomon, S. J. *Liquid Chromat.* **3,** 1353–1365 (1980).
47. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. *J. biol. Chem.* **256,** 7990–7997 (1981).
48. Waterfield, M. D., Scrace, G. & Totty, N. in *Practical Protein Biochemistry* (eds Darbre, A. & Waterfield, M. D.) (Wiley, New York, in the press).
49. Wilbur, W. J. & Lipman, D. J. *Proc. natn. Acad. Sci. U.S.A.* **80,** 726–730 (1983).
50. Mayes, E. L. V. & Waterfield, M. D. *EMBO J.* (in the press).
51. Vennström, B. & Bishop, J. M. *Cell* **28,** 135-143 (1983).
52. Kitamura, N., Kitamura, A., Toyoshima, K., Hirayama, Y. & Yoshida, M. *Nature* **297,** 205–208 (1982).
53. Goodfellow, P. N., Banting, G., Waterfield, M. D., Ozanne, B. *Cytogenet. cell. Genet.* **32,** 282 (1982).
54. Shimizu, N., Behzadian, M. A. & Shimizu, Y. *Proc. natn. Acad. Sci. U.S.A.* **77,** 3600–3604 (1980).
55. Kondo, I. & Shimizu, N. *Cytogenet. cell. Genet.* **35,** 9–14 (1983).
56. Spurr, N. *et al. EMBO J.* **3,** 159–163 (1984).
57. Greenberg, M. E. & Edelman, G. M. *Cell* **33,** 767–779 (1983).
58. Groffen, J., Heisterkamp, N., Reynolds, F. H. Jr. & Stephenson, J. R. *Nature* **304,** 167–169 (1983).
59. Hayman, M. J., Ramsay, G. M., Savin, K., Kitchener, G., Graf, T. & Beug, H. *Cell* **32,** 579–588 (1983).
60. Privalsky, M. L., Sealy, L., Bishop, J. M., McGrath, J. P. & Levinson, A. D. *Cell* **32,** 1257–1267 (1983).
61. Beug, H., Palmieri, S., Freudenstein, C., Zentgraf, H. & Graf, T. *Cell* **28,** 907–919 (1982).
62. Graf, T. & Beug, H. *Cell* **34,** 7–9 (1983).
63. Frykberg, L. *et al. Cell* **32,** 227–238 (1983).
64. Fung, Y-K. T., Lewis, W. G., Crittenden, L. B. & Kung, H-J. *Cell* **33,** 357–368 (1983).
65. Rabbitts, T. H., Forster, A., Baer, R. & Hamlyn, P. H. *Nature* **306,** 806–809 (1983).
66. Rabbitts, T. H., Hamlyn, P. H. & Baer, R. *Nature* **306,** 760–765 (1983).
67. Gelmann, E. P., Psallidopoulos, M. C., Papas, T. S. & Favera, R. D. *Nature* **306,** 799–803 (1983).
68. Roy-Burman, P., Devi, B. G. & Parker, J. W. *Int. J. Cancer* **32,** 185–191 (1983).
69. Reddy, E. P., Smith, M. J. & Srinivasan, A. *Proc. natn. Acad. Sci. U.S.A.* **80,** 3623–3627 (1983).
70. Bradford, M. M. *Analyt. Biochem.* **72,** 248–254 (1976).
71. Smith, C. M. *et al. Am. J. Obstet. Gynec.* **128,** 190–196 (1977).
72. Laemmli, U. K. *Nature* **227,** 680–685 (1970).
73. Schwartz, D., Tizzard, R. & Gilbert, W. *Cell* **32,** 853–869 (1983).
74. Smart, J. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 6013–6017 (1981).
75. Orr, H. T., Lancet, D., Robb, R., Lopez de Castro, J. A. & Strominger, J. L. *Nature* **282,** 266–270 (1979).

LETTERS TO NATURE

Interstellar scintillation and ultra-low-frequency gravitational wave observations

J. W. Armstrong

Jet Propulsion Laboratory, Mail Stop 238–737,
4800 Oak Grove Drive, Pasadena, California 91109, USA

High-precision pulsar timing measurements have been used[1–4] to set upper limits on the flux of ultra-long period ($T \geq 3$ years) stochastic gravitational waves. The data used to produce the current limits appear to be limited by timing noise[5,6] that is intrinsic to the pulsars. Recently, however, there have been very low noise timing observations[7] of the 1.5-millisecond pulsar[8]. This suggests that significant sensitivity improvements in searches for ultra-low-frequency (ULF) gravitational waves may be possible. Here the effects of a heretofore secondary noise source—phase scintillations caused by interstellar electron density fluctuations—are calculated and the prospects for improved ULF gravitational wave measurements using pulsar timing are discussed.

The observed quantity in these experiments is a time series of the difference between the observed and expected pulse arrival times. The time series contains contributions from intrinsic timing noise, gravitational radiation (buffeting of the pulsar and Earth at different epochs), estimation error due to finite signal to noise ratio, and interstellar phase scintillation (ISS). The ISS contribution arises due to dispersion measure fluctuations as irregular clouds of electrons rearrange and drift across the pulsar–Earth line[9,10]. To estimate the contribution to pulsar timing noise measurements a model of the interstellar turbulence spectrum and the relevant propagation theory are required. Observations[10–12] are consistent with an isotropic three-dimensional electron density power spectrum, $P_{3N}(q)$, of the form $P_{3N}(q) = C_N^2 q^{-11/3}$ where q is the spatial wavenumber. The 'structure constant' C_N^2 is typically 10^{-5} to 10^{-2} m$^{-6.67}$ (ref. 11), but can be estimated for the the line-of-sight to the 1.5-millisecond pulsar from the measured frequency correlation bandwidth in the dynamic spectrum[8] or the measured temporal broadening of pulses[13]. Using the observed correlation bandwidth (≈1 MHz at $\lambda = 0.21$ m observing wavelength) and the observed pulse broadening (73 μs at $\lambda = 0.94$ m), C_N^2 can be estimated using equations 31 and 41 of ref. 10. For homogeneous turbulence along the pulsar–Earth path of 5 kpc (ref. 14), those observations yield identical estimates of $C_N^2 = 8 \times 10^{-5}$ m$^{-6.67}$. Because both decorrelation bandwidth and pulse broadening are diffraction phenomena, those observables are dominated by density fluctuations on scales of the Fresnel zone size (~10^{10} m). However, direct and indirect measurements of P_{3N} on the 10^{11}–10^{13} m scales relevant to ULF gravitational wave measurements are consistent with an extrapolation of the power at smaller scales; the inferred shape and level of the spectrum on these scales is in fairly good agreement with $P_{3N}(q) = (10^{-4}\,\mathrm{m}^{-6.67})\, q^{-11/3}$ (ref. 11).

For gravitational wave searches, noise sources can be characterized either by the power spectrum of fractional frequency fluctuations[2,15] or the Allan variance[16] of the frequency time series[17]. The spectrum of fractional frequency fluctuations caused by ISS, $S_y(f)$, can be calculated in three steps. First, ignoring diffraction at the relatively large scales of interest here, the two-dimensional spatial spectrum of phase on the Earth's surface is given by (ref. 9)

$$P_{2\phi}(\kappa) = 2\pi\lambda^2 r_e^2 z C_N^2 \kappa^{-11/3} \quad (1)$$

where κ is the two-dimensional wavenumber, λ is the observing wavelength, r_e is the classical electron radius, and z is the pulsar–Earth distance. Second, to convert to a temporal spectrum of phase fluctuations it is assumed that the phase scintillation pattern is 'frozen' in a reference frame that moves with a uniform velocity v transverse to the pulsar–Earth line. Integrating over the wavenumber component associated with the direction perpendicular to the velocity vector then gives the temporal phase spectrum. Finally, noting that frequency is the temporal derivative of phase, the 'derivative theorem' for Fourier transforms[18] can be applied to obtain $S_y(f)$. The result is:

$$S_y(f) = \pi^{-1/6} 2^{-2/3} v^{5/3} \lambda^4 c^{-2} z C_N^2 r_e^2 [\Gamma(4/3)/\Gamma(11/6)]\, f^{-2/3} \quad (2)$$

where c is the speed of light, v is the velocity of the pattern, Γ is the gamma function and f is the temporal frequency.

Equation (2) can be used to predict ISS noise for ULF gravitational wave observations. Figure 1 shows the current observational limits to a ULF gravitational wave background[2–4], the envelope of stochastic gravitational wave spectra having closure density[19], and plots of equation (2) evaluated with the following parameters: $\lambda = 0.21$ m and 0.06 m, $z = 5$ kpc, $C_N^2 = 8 \times 10^{-5}$ m$^{-6.67}$, $v = 100$ km sec^{-1}. Also shown in Fig. 1 is the $S_y(f)$ that would be observed for daily observations of a pulsar having white intrinsic timing noise with spectral density 9×10^4 μs^2 Hz^{-1}. That is, the normalization is set by 1 μs (r.m.s.) white timing noise distributed over the Nyquist band[18] 0–0.5 cycles d^{-1} set by the daily sampling interval.

© 1984 Macmillan Journals Ltd

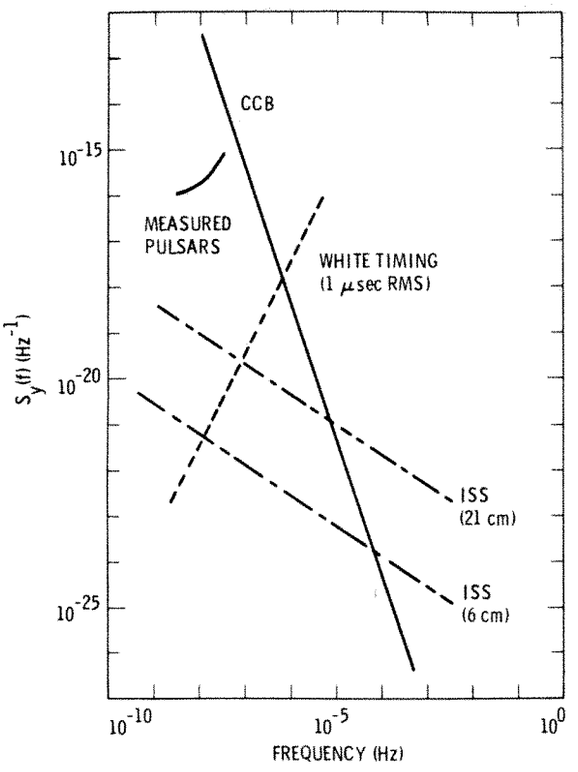


Fig. 1 Plot of power spectrum of fractional frequency fluctuations, $S_y(f)$, as a function of fluctuation frequency, f. Curve labelled 'CCB' is envelope of cosmic gravitational wave background spectra having closure density[19]; curve labelled 'Measured Pulsars' is observed limit to S_y from Hellings and Downs[2] and Romani and Taylor[3]; curves labelled 'ISS' are spectra expected due to interstellar scintillations (parameters: $\lambda = 0.21$ m and 0.06 m, $C_N^2 = 8\times10^{-5}\,\mathrm{m}^{-6.67}$, $z = 5$ kpc, $v = 100\,\mathrm{km\,s^{-1}}$); curve labelled 'White Timing' is for white intrinsic pulsar timing noise with r.m.s. value of 1 μs in the band 0–0.5 cycles d^{-1}.

From Fig. 1, the ISS limit is about three orders of magnitude below the current observational limit to S_y at $f = 10^{-8}$ Hz. However, as the intrinsic timing noise becomes lower ISS becomes increasingly important; white 1 μs r.m.s. timing noise becomes ISS-noise dominated at periods ~4 months for 21 cm observations. Expressed as a fractional frequency stability, the 21 cm ISS-curve in Fig. 1 corresponds to an Allan variance of $\cong 1.5\times10^{-14}\,(\tau/1\,\mathrm{yr})^{-1/6}$. (It should be noted that plasma phase scintillations are also an important noise source for gravitational wave observations in the 10^{-1} to 10^{-4} Hz frequency range[15]. Here fluctuations in the interplanetary plasma cause perturbations in the radio tracking record of a distant spacecraft[17]. The sensitivities achievable with an X-band tracking link are expected to be $\sim5\times10^{-15}$ at $\tau = 1{,}000$ s integration times for the raw data. Since the spacecraft coherently transponds the signal transmitted from the earth, a gravitational wave produces a characteristic three-pulse response[15] in the tracking time series. Thus correlation analysis should allow further improvement in the sensitivity in the spacecraft tracking case.)

What are the prospects for reducing the ISS noise? Since S_y is proportional to λ^4, observations at shorter wavelengths rapidly reduce ISS noise. However, pulsar signal-to-noise ratio considerations may make high frequency observations impractical. Dual frequency observations could be used to estimate and remove these dispersion measure fluctuations (ref. 20 for example). Although only changes in the dispersion measure are relevant here, the complicating factors of frequency-dependent pulse shape and frequency-dependent pulse broadening due to ISS diffraction will still make observations difficult at the level of precision required for a good ISS calibration. Without higher frequency observations or high-precision dispersion measure monitoring, the ISS-noise in Fig. 1 will constitute a limit to the sensitivity of ULF gravitational radiation observations even using timing data from an intrinsically noiseless pulsar.

I thank D. C. Backer, F. B. Estabrook, R. W. Hellings, and J. M. Rotenberry for discussions. This research was supported by the NASA Astronomy/Relativity branch and was carried out by the Jet Propulsion Laboratory, California Institute of Technology, under contract with NASA.

Received 12 August; accepted 10 November 1983.

1. Detweiler, S. *Astrophys. J.* **234,** 1100–1104 (1979).
2. Hellings, R. W. & Downs, G. S. *Astrophys. J.* **265,** L39–L42 (1983).
3. Romani, R. W. & Taylor, J. H. *Astrophys J.* **265,** L35-L37 (1983).
4. Bertotti, B., Carr, B. J. & Rees, M. J. *Mon. Not. R. astr. Soc.* **203,** 945–954 (1983).
5. Helfand, D. H., Taylor, J. H., Backus, P. R., & Cordes, J. M. *Astrophys. J.* **237,** 206–215 (1980).
6. Cordes, J. M. & Helfand, D. J. *Astrophys. J.* **239,** 640–650 (1980).
7. Backer, D. C., Kulkarni, SD. R. & Taylor, J. H. *Nature* **301,** 314–315 (1983).
8. Backer, D. C. *et al. Nature* **300,** 615–618 (1982).
9. Lovelace, R. V. E. thesis, Cornell Univ. (1970).
10. Rickett, B. J. *A. Rev. astr. Astrophys.* **15,** 479–504 (1977).
11. Armstrong, J. W., Cordes, J. M., & Rickett, B. J. *Nature* **291,** 561–564 (1981).
12. Armstrong, J. W. & Rickett, B. J. *Mon. Not. R. astr. Soc.* **194,** 623–638 (1981).
13. Stinebring, D. R. & Cordes, J. M. *Bull. Am. astr. Soc.* **15,** 674 (1983).
14. Heiles, C. *et al. Astrophys. J. Lett.* (in the press, 1983).
15. Estabrook, F. B. & Wahlquist, H. D. *Gen. rel. Gravit.* **6,** 439–447 (1975).
16. Barnes, J. A. *et al. IEEE Trans.* IM-**20,** 105 (1971).
17. Wahlquist, H. D., Anderson, J. D., Estabrook, F. B., & Thorne, K. S. *Atti dei Conv. Lincei* **34,** 335–350 (1977).
18. Bracewell, R. *The Fourier Transform and Its Applications* (McGraw Hill, New York, 1965).
19. Zimmerman, R. L. & Hellings, R. W. *Astrophys. J.* **241,** 475–485 (1980).
20. Cordes, J. M. & Stinebring, D. R. NAIC preprint 184 (1983).

Surface albedo and the Sahel drought

M. F. Courel*, R. S. Kandel† & S. I. Rasool*‡

* Centre Scientifique, IBM-France, 36 avenue Raymond-Poincaré, F-75116 Paris, France
† Service d'Aéronomie du CNRS, F-91370 Verrières-le-Buisson, France
‡ Laboratoire de Météorologie Dynamique du CNRS, Ecole Normale Supérieure, F-75231 Paris Cedex 05, France

The persistence of the Sahel drought, which reached a peak in 1973, appears to be typical for such dry episodes over past decades and centuries[1–3]. Such strong persistence can be understood if a strong positive feedback mechanism is operating, partly driven by changes in surface properties[4,5]. The key factors in the mechanisms thus far studied are the surface albedo and the soil moisture, both of which affect the radiation balance at the surface, the first directly, the second indirectly through its influence on the latent heat flux. We have now studied the evolution of the albedo of this region since 1972. We find that dry season albedo in the Sahel (notably Ferlo and Gondo regions, see Fig. 1*b*) declined from a maximum close to 0.30 in 1973 to values close to 0.20 in 1979. This decline is consistent with changes in plant cover determined by analysis of spectral changes in the Landsat multispectral scanner (MSS) data and field studies[6,7].

The effect of drought, reducing soil moisture and thus evaporation and cloud cover, and increasing surface albedo as plant cover is destroyed, is generally to increase ground and near-surface air temperatures while reducing the surface radiation balance and exacerbating the deficit in the radiation balance of the local surface–atmosphere system. This entails increased atmospheric subsidence and consequently further reduced precipitation. The biogeophysical feedback mechanism involving albedo was first proposed by Charney[8], who noted that to the extent that man's agricultural and pastoral activities increased the stress on plant cover, they might be a factor in the increase of surface albedo, thus contributing to the persistence of the Sahel drought. Various numerical studies have confirmed that very large increases in surface albedo should indeed lead to substantial reduction in precipitation[9–12], but the question remains whether these assumed changes in surface albedo bear

0028-0836/84/060528—04$01.00

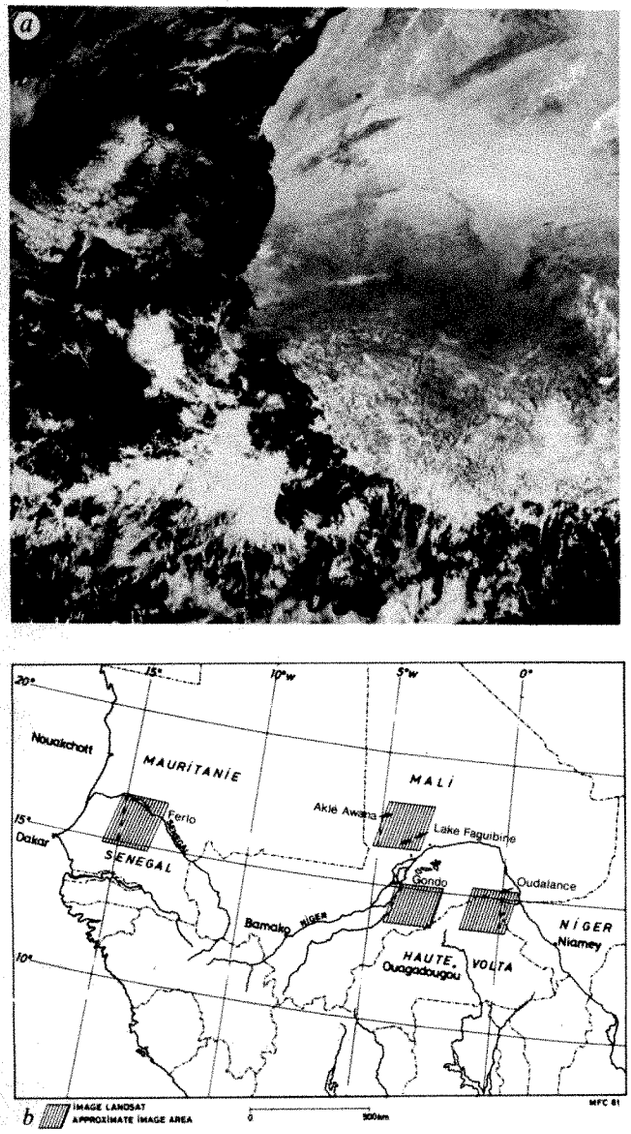


Fig. 1 West Africa: Saharo-Sahelian area. *a*, Portion of the Meteosat visible channel midday image of 2 August 1979. *b*, Map showing areas observed with Landsat; from west to east these are: Ferlo (Senegal), Aklé Awana with Lake Faguibine (Mali), Gondo (Mali), Oudalance (Haute-Volta).

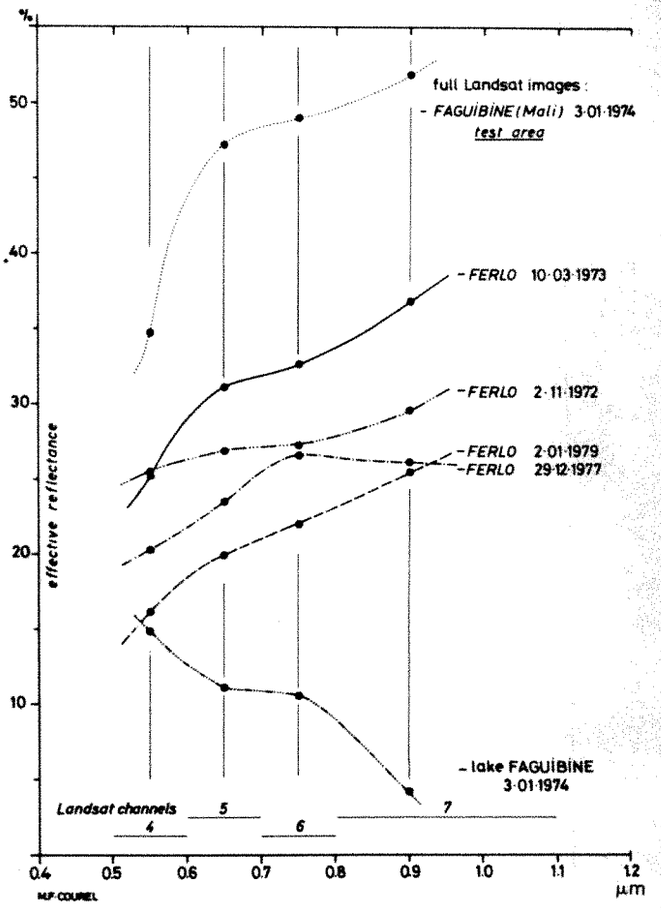


Fig. 2 Spectral reflectance based on Landsat MSS data. The two extreme curves are for the water surface of Lake Faguibine (lowest values) and for the full Landsat image including the lake and made up mostly of sand dunes (Aklé Awana), yielding the highest values.

any relation to those which have occurred in reality. For the period 1967–74 when the drought was intensifying, Norton and co-workers[13] found moderate increase in surface albedo, strong during the wet season months. The limitation that we found to the extension of the albedo, despite the continued relatively dry conditions, may result from accumulation of plant debris[14] (in regions such as Ferlo protected from overgrazing) and/or rapid recovery of grass cover even when the total amount of living vegetation continues to decrease. Thus the Charney albedo mechanism may not have a major role in the long-term evolution of the Sahel climate.

Our analysis considers the western part, from the Atlantic coast to longitude 3° E, of the Saharo-Sahelian zone of Africa, between latitudes 14 and 20° N. Norton and co-workers studied the changes in this region in the period 1967–74 using green sensor data from the Applications Technology Satellite (ATS-3), presenting results for areas 2° square[13]. We have studied the evolution of the region since 1972, using two sets of satellite data: (1) on the larger scale, directly comparable with the ATS-3 data, we use hourly visible channel data from Meteosat-1, for several periods of 1–6 days distributed through the year from November 1978 to November 1979. The spatial resolution of 2.5×5 km^2 (somewhat better than ATS-3) has been degraded to (40 km)^2 in preparing albedo maps of the entire region, and then to the 2° squares of Norton *et al.* (2) On a much finer scale, we use digital Landsat MSS data (spatial resolution 79 m) for several areas of (185 km)^2 (see Fig. 1*b*), obtained on a series of dates from 1972 to the present, at about 9.30 LT in each instance.

To estimate the planetary albedo from satellite measurements of emergent radiances, one must consider not only the sensor calibration, but also possible bias associated with limited spectral, angular and temporal sampling[15]. To estimate the surface albedo, one must also consider atmospheric effects.

The calibrated relatively-narrow bands of Landsat (Fig. 2) provide quasi-monochromatic radiance values[16,17] from which we compute the spectral reflectances and the isotropic estimate α_i of the albedo, integrated over the solar spectrum. Results obtained for the water surface of Lake Faguibine (Fig. 2) agree with theoretical computations of the spectral reflectance[18]. On the same Landsat images, the spectral albedo of the bright sand dune area of Aklé Awana is found to be quite high (from 0.35 in channel 4 to 0.52 in channel 7), in agreement with the values of 0.45–0.50 found for the solar spectrum-weighted albedo from the Meteosat images. This bright area was also used as a normalization point (with green channel albedo 0.41) in the earlier ATS-3 study.

For the Meteosat study, we have used Koepke's calibration[19] relating raw visible count to the radiance filtered by the Meteosat visible channel. These values are corrected using a 'filter factor' which is determined using spectral reflectance curves deduced from the Landsat MSS data and from laboratory studies of soil and plant samples from the area of interest[6,7]. This yields a good

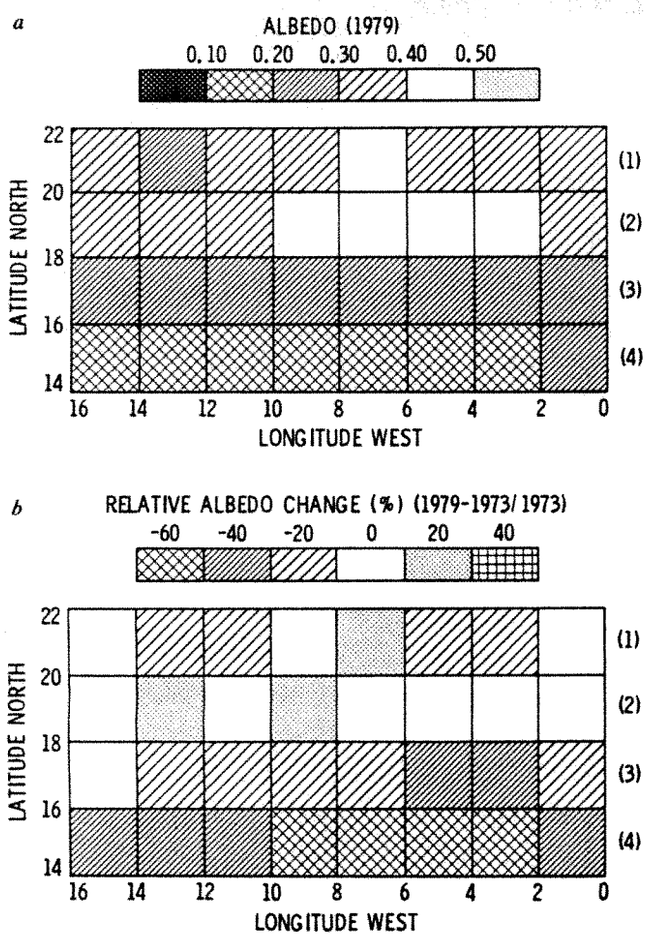


Fig. 3 *a*, Total solar spectrum-weighted surface albedo of the western Sahel, estimated from Meteosat-1 VIS data for 3–5 November 1979, with spatial resolution degraded to 2°. *b*, Relative albedo change $100\,\Delta\alpha/\alpha_{1973}$ observed between 19 October 1973 (ATS-3 data) and November 1979.

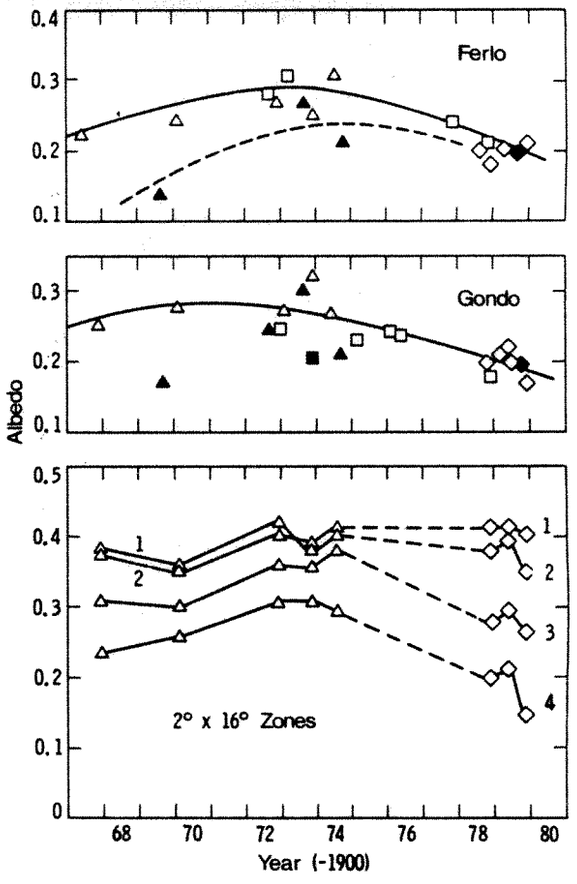


Fig. 4 Albedo history of the western Sahara and Sahel between 1967 and 1979: triangles, estimates based on the 2° maps of Norton *et al.*[13]; squares, Landsat data (Ferlo, Gondo); lozenges, Meteosat-1 data. The filled symbols correspond to observations in the rainy season. Curves 1–4 are integrated albedo values for the four strips 2° wide in latitude, extending from longitude 16° to 0° W, illustrated in Fig. 3.

estimate of the total solar spectrum-weighted reflectance. Also, corrections for anistropy are small (<0.02 in the instantaneous value of the albedo) because both Meteosat and Landsat are close to the zenith of the areas observed, so that the outgoing direction of greatest weight in determining the albedo is sampled. With the Meteosat data, temporal sampling (solar incidence sampling) is complete: we obtain several albedo estimates per day and find them approximately constant to solar zenith angle 60° over Sahelian and Saharan land surfaces. This suggests that the bias associated with the fixed 9.30 LT of the Landsat observation is fairly small, and even if such bias affects Landsat determinations of an annual albedo cycle, it disappears when comparing observations of different years made at about the same date.

Thus we believe that the various sampling biases in our data are small, or can be accounted for, as far as the planetary albedo values are concerned. The Landsat images used are cloud-free, and judging from reflectance of water surface, turbidity is low. The periods studied with Meteosat were chosen to be relatively cloud-free over the western Sahel, on the basis of visual inspection of both visible and IR images. Days with apparent high turbidity were rejected, and for each 2–3-day period studied, a minimum reflectance was used to estimate cloud-free albedo. Corrections for atmospheric effects (Rayleigh scattering, water vapour and ozone absorption, aerosol scattering and absorption) have been made following the procedure of Otterman and Fraser, and they are small (<5%) in the albedo range of interest[20–22]. Results are shown in Figs 3 and 4 and discussed further below.

Both the Landsat and the Meteosat data show that at its brightest spots (for example, Aklé Awana), the Sahara has an albedo close to 0.50. The mean albedo for the region from latitudes 18° to 23° N, and from longitude 10°W to the Greenwich meridian, is 0.42 ± 0.04, higher than the value of 0.35 used in Charney's model experiment[8,10], higher also than the values which often appear in surface albedo compilations[23]. The high value is consistent with the results of Otterman and Fraser[20–22], who studied a variety of desert sites using both Landsat MSS and laboratory data; it also agrees with some on-site measurements of bright sand in the Empty Quarter of Arabia[24]. It comes about essentially because of the near-IR rise in reflectance of the surface, observed in channels 6 and 7 of Landsat (Fig. 2).

In the western Sahel, the 1979 Meteosat data indicate effective surface albedos ranging from 0.15 to 0.25 (Fig. 3*a*). The values for the Ferlo and Gondo regions are in agreement with the Landsat data for the same period (Fig. 4). The earlier Landsat data for these same regions in 1972–73 also agree with values estimated from the ATS-3 2° × 2° albedo maps. Thus the Landsat data effectively bridge the gap between ATS-3 and Meteosat. For both the Ferlo and Gondo regions, dry season albedo declined from a maximum close to 0.30 in 1973 to values close to 0.20 in 1979 (Fig. 4), following the increase observed between 1967 and 1973. For the western Sahel as a whole, relative decreases of 20–60% were observed between 1973 and 1979 (Figs 3*b*, 4). This change is noteworthy in comparison with the relative stability (±15%) observed in the Saharan zone (Fig. 3*b* and curves 1 and 2 of Fig. 4) over the same period, indicating that the two sets of data are comparable.

Only a small fraction of existing satellite data has been examined, and uncertainties in establishing an albedo history are large. We cannot at present evaluate the 'normal' surface albedo of the Sahel, if indeed such a normal exists. However, even the smallest values obtained for the western Sahel (0.20 ± 0.05) and Sahara (0.42 ± 0.04) are larger than the 'climatological means' used in model calculations. Our study also shows that although drought conditions still persist in the Sahel, the surface albedo declined significantly between 1973 and 1979, in contradiction to the expectations of the Charney albedo feedback mechanism.

We thank E. H. Cissé for valuable assistance, Ph. Chamard and J. Otterman for stimulating comments and discussion, the Club du Sahel of the OECD for its support of this project, as well as the Centre National d'Etudes Spatiales and the ASP (DGRST/CNRS) Evolution des Climats, for partial support at the Service d'Aéronomie.

Received 22 April; accepted 24 November 1983.

1. Lamb, P. J. *Nature* **299**, 46–48 (1982).
2. Nicholson, S. *Mon. Weath. Rev.* **111**, 1646–1654 (1983).
3. Lamb, P. J. *Mon. Weath. Rev.* **106**, 482–491 (1978).
4. Rasool, S. I. in *The Global Climate* (ed. Houghton, J.) (Cambridge University Press, 1983).
5. Nicholson, S. *Mon. Weath. Rev.* **109**, 2191–2208 (1981).
6. Courel, M. F. & Habif, M. *Adv. Space Res.* **2**, No. 8, 37–44 (COSPAR/Pergamon, Oxford, 1982).
7. Courel, M. F. *IBM Scientific Center Rep.* F-061 (1983).
8. Charney, J. G. *Q. Jl R. met. Soc.* **101**, 193–202 (1975).
9. Elsaesser, H. W., MacCracken, M. C., Potter, G. L., & Luther, F. M. *Q. Jl R. met. Soc.* **102**, 655 (1976).
10. Charney, J. G., Quirk, W. J., Chow, S. H. & Kornfield, J. *J. atmos. Sci.* **34**, 1366–1385 (1977).
11. Walker, J. & Rowntree, P. R. *Q. Jl R. met. Soc.* **103**, 29–46 (1977).
12. Shukla, J. & Mintz, Y. *Science* **215**, 1498–1500 (1982).
13. Norton, C. C., Mosher, F. R. & Hinton, B. *J. appl. Met.* **18**, 1252–1262 (1979).
14. Otterman, J. *Science* **186**, 531–533 (1974).
15. Kandel, R. *Beitr. Phys. Atmos.* **56**, 322–340 (1983).
16. NASA, *ERTS Data Users' Handbook*, Doc. 71SD4249 (Gen. Electric Space Div., Valley Forge, Pennsylvania, 1972).
17. Duggin, M. J. *Appl. Opt.* **20**, 3815–3816 (1981).
18. Cox, C. & Munk, W. *J. mar. Res.* **14**, 63–78 (1955).
19. Koepke, P. *Appl. Opt.* **21**, 2845–2854 (1982).
20. Otterman, J. & Fraser, R. S. *Remote Sensing Envir.* **5**, 247–266 (1976).
21. Otterman, J. *Geophys. Res. Lett.* **4**, 441–444 (1977).
22. Otterman, J. *Tellus.* **33**, 68–77 (1981).
23. Hummel, J. R. & Reck, R. A. *J. appl. Met.* **18**, 239–253 (1979).
24. Smith, E. A. & Vonder Haar, T. H. *Rep. NSF Grant ATM-8200808* (Colorado State University, 1983).

Predictability of north-east Brazil droughts

Stefan Hastenrath

Department of Meteorology, University of Wisconsin, 1225 West Dayton Street, Madison, Wisconsin 53706, USA

The droughts of north-east Brazil are unusually well defined in the departures of the quasi-permanent circulation systems in the tropical Atlantic sector. Anomaly patterns in the large-scale atmospheric and oceanic fields evolve in the course of the half-year preceding the March/April rainy season. Based on these diagnostic results, dynamically plausible predictor candidates are selected and entered into a stepwise multiple regression scheme. The circulation in the Atlantic sector proves to be essential for the prediction of rainfall anomalies, while predictor candidates from other parts of the globe are of subordinate importance. The regression equations derived from the 1921–56 record are used to produce rainfall forecasts for the years 1958–72. Forecast and observed values are strongly correlated. In particular, the severe 1958 drought was eminently predictable from this method.

The Sêcas or droughts of Brazil's 'Nordeste', which constitutes the eastern-most corner of the continet (Fig. 1), have recurrently led to starvation and mass exodus, and their eventual prediction has remained a central concern[1]. Earlier diagnostic studies[2–7] have elucidated the general circulation mechanisms of the Sêcas. Drawing on this diagnostic research and combining dynamical and statistical approaches, a method has been developed for the prediction of seasonal rainfall from the antecedent circulation departures. An early report of this work is made here, while full details will be presented elsewhere in due course[8].

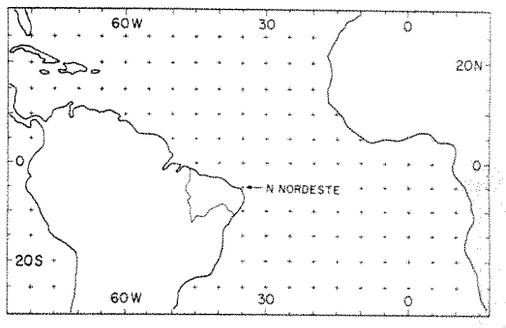


Fig. 1 Orientation map showing the northern Nordeste and 5° square areas for which ship observations were compiled.

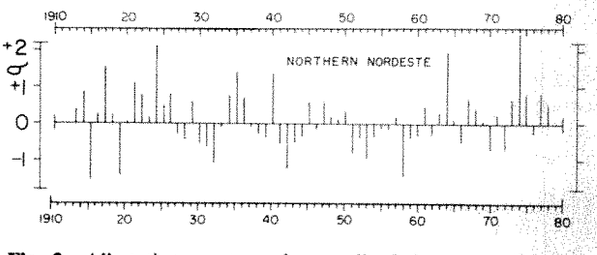


Fig. 2 All-station average of normalized departure of annual rainfall for the northern Nordeste.

The surface circulation in the tropical Atlantic is documented by ship observations stemming from the TDF-11 deck of the National Climatic Center at Asheville, North Carolina, compiled into 5° square values[4] (Fig. 1). The record of 1921–72 is used here, and elements of interest include sea level pressure (SLP), zonal (u) and meridional (v) components of resultant surface wind, sea surface temperature (SST), and total cloudiness. Rainfall observations for north-east Brazil are in part published[9]. Also used are selected observation series from other parts of the globe.

The march of the seasons in north-east Brazil is dominated by the latitudinal migration of the near-equatorial trough of low pressure and associated convergence zone over the western North Atlantic, sandwiched as they are between the subtropical highs of either hemisphere. During the Northern Hemisphere winter the trough and convergence zone shift southward, reaching a southernmost position in March and April. This is the peak of the rainy season in the drought-prone northern Nordeste, on which the present study is focused.

A collective of stations in the northern Nordeste (Fig. 1) are combined into rainfall index series of all-station normalized departure[3]. This precedure safeguards against the notorious space and time variability of tropical rainfall, so that the resulting index series are representative of the hydrometeorological conditions of large homogeneous areas. The October–September index ascribed to the calendar year containing the March–April rainy season peak, is plotted in Fig. 2. Such indices were also compiled for various portions of the hydrological year. Figure 2 illustrates the pronounced interannual variability of rainfall in the northern Nordeste, including the severe drought year 1958, remembered for its particularly serious social and economic consequences[1,3,7].

Analyses of large-scale circulation patterns[3] show that the Sêcas of the northern Nordeste are characterized by an equatorward expansion of the South Atlantic, and a poleward retraction of the North Atlantic high, associated with a northward displacement of the enclosed near-equatorial trough of low pressure. Concurrently, the zonally oriented bands of maximum cloudiness and precipitation stay farther north, the North Atlantic

0028-0836/84/060531—03$01.00
© 1984 Macmillan Journals Ltd

Table 1 Percentage of explained variance, period 1921–40 plus 1946–56, for regression on March–April and March–September rainfall index

	O	N	D	J	F	N
Regression on						
March–April	18	55	25	63	56	71
March–September	34	61	48	66	71	—

trades weaken, and the South Atlantic trades become stronger than in the long-term mean. The SST pattern shows positive departures in a broad band across the North Atlantic and anomalously cold waters in most of the South and equatorial Atlantic. The following three factors apparent in the surface circulation seem conducive to drought: (1) the near-equatorial convergence band stays unusually far to the north of the northern Nordeste; (2) the anomalously cold south equatorial waters hamper the moisture content and instability of boundary layer flow upstream from the Nordeste; (3) the perennial contrast between warm north equatorial waters and cold waters to the south of the equator is enhanced. It has been hypothesized[7] that such a juxtaposition of SST anomalies would induce a meridional circulation cell with subsidence over the Nordeste.

During abundant rainy seasons in the northern Nordeste, departure patterns are approximately inverse to those typical of drought years. Anomaly configurations in the large-scale atmospheric and oceanic fields evolve gradually in the course of the preceding months[3].

The aforementioned diagnostic research suggests dynamically plausible predictors of north-east Brazil rainfall anomalies. Thus droughts may be heralded by: anomalously low/high pressure on the equatorward side of the North/South Atlantic subtropical high, enhanced easterly wind component over the low-latitude Atlantic, weakened northerly over the North Atlantic and enhanced southerly wind component over the equatorial and South Atlantic, positive SST departures in a zonally oriented band across the North Atlantic and anomalously cold surface waters in the equatorial South Atlantic, as well as an anomalously far northerly position of the near-equatorial belts of maximum surface convergence and cloudiness. Inasmuch as the pressure distribution over the Atlantic—which is instrumental in establishing the circulation patterns characteristic of the north-east Brazil rain-fall anomalies—appears related to large-scale mass redistributions of the Southern Oscillation type[3], various indications of the Southern Oscillation and parameters from more remote parts of the globe are of interest.

While the rainfall index series plotted in Fig. 2 extends from 1913–78, our ship data bank covers 1911–72. However, because of the scarcity of observations in the first decade of this period, only the record since 1921 is used here. With a view towards identifying suitable predictor candidates, isocorrelate maps were constructed of the October–September north-east Brazil rainfall index with SLP, SST, u and v wind components, and cloudiness over the Atlantic. The highest correlations plausibly occur in the areas described above as being meteorologically significant for the various atmospheric and oceanic fields. The regions of correlation exceeding 0.3 in absolute value were identified and for the combination of adjacent 5° blocks with correlation of same sign and exceeding 0.3, time series were compiled to serve as input to a regression model. This screening is intended to safeguard against noise. Other predictor candidates considered include pre-season rainfall in the northern Nordeste itself, rainfall at Georgetown and Paramaribo in the Guyanas, pressure at Port Stanley in the South Atlantic, temperature in the southeastern United States, India rainfall[10], and Wright's[11,12] Southern Oscillation index.

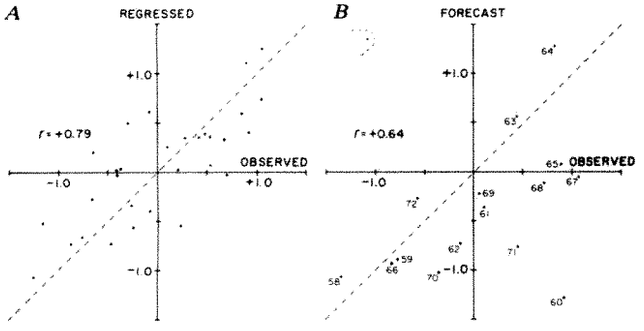


Fig. 3 Scatter diagrams of March/April rainfall index based on observations that would exist by the end of January. *A*, Regression, period 1921–40 plus 1946–56, correlation coefficient $r = +0.79$. *B*, Forecast, period 1958–72, with numbers indicating the years, $r = +0.64^{**}$. (Two asterisks denote significance of correlation coefficient at the 1% level.) Broken lines denote 45° angle. Quenouille's[17] method is used to account for the reduction of effective number of degrees of freedom due to persistence.

Of 17 candidate series, 11 possess a correlation with north-east Brazil rainfall exceeding 0.3 in absolute value, and were input to a stepwise multiple regression scheme with a 5% significance margin[13,14]. This identified as most important predictors SLP, SST, and u and v wind components over the tropical Atlantic. Of further use was the pre-season rainfall in northern north-east Brazil itself, and the May–June–July Southern Oscillation index value[11,12].

Regression models were constructed from the record 1921–40 plus 1946–56, for all months from October through March for two 'predictands', namely the March–April and the March–September rainfall indices. Part *A* of Fig. 3 is a scatter diagram for January of 'regressed' versus 'observed' values of the March–April rainfall index; the other models are not depicted here. Table 1 lists the variance explained by the regression models for the various months. The variance explained generally increases as one approaches the peak rainy season, no hypothesis being offered for the remarkably weaker relation in December. The peak March–April and March–September rainfalls are specified to a very high degree by the departure ensemble of the general circulation in February and March. However, February leaves no lead time for the practical use of the forecast, while November accounts for a smaller percentage of the rainfall variability. Accordingly, the January models merit particular attention.

The regression models developed from the 1921–40 plus 1946–56 record were used to predict the rainfall anomalies of the 15 years 1958–1972. Part *B* of Fig. 3 is a scatter diagram of the January model predicting the March–April rainfall index. No details are presented here for the other January model (March–September rainfall) and the two November models. Information such as part *A* of Fig. 3 serves as a background reference for rainfall index forecasts for years beyond the base period of the regression models, as presented in part *B* of Fig. 3.

As a basis for the appraisal of forecast performance, various forms of evidence are considered. (1) The scatter diagram in part *B* of Fig. 3 shows a positive relation between forecast and observed values, although the scatter is larger than for the regression model plotted in part *A*. (2) Correlation coefficients between forecast and observed values such as given in part *B* of Fig. 3 are high. (3) In addition, skill scores[15] and stochastic joint probabilities[16] were calculated. These also indicate appreciable forecast skill. Details are not discussed here, but the pertinent information is contained in Fig. 3. In context, the various measures of prediction quality all bear out a remarkable forecast performance. The forecasts are particularly good for the extreme drought year 1958.

A wide range of dynamically plausible predictor candidates was examined, but it is found that the large-scale atmospheric and oceanic fields in the tropical Atlantic are most essential for the prediction of extreme hydrometeorological regions in the northern Nordeste. For operational applications, such parameters are required on a timely basis. Remote sensing by satellite appears an obvious choice, of particular promise being lower-tropospheric cloud motion vectors, SST, and cloudiness or its proxies. It must be realized, however, that time series of inter-

nally consistent parameters extending over at least ten years are needed, absolute calibration not being essential. Such a satellite-derived reference climatology has not yet been created. The development of an operational monitoring and prediction system entails a substantial material commitment. This is something that deserves to be considered seriously in the light of the demonstrated predictability of northeast Brazil droughts and their social and economic impact.

This research was supported by NSF Grant ATM82-00511. M. C. Wu and P. S. Chu participated in the regression study.

Received 28 July; accepted 16 November 1983.

1. *Workshop on drought forecasting for Northeast Brazil, INPE, February* 1980 (Conselho Nacional de Pesquisas, São José dos Campos, 1980).
2. Namias, J. *Tellus* **24**, 336–343 (1972).
3. Hastenrath, S. & Heller, L. *Q. Jl Roy. met. Soc.*, **103**, 77–92 (1977).
4. Hastenrath, S. & Lamb, P. J. *Climatic Atlas of the Tropical Atlantic and Eastern Pacific Oceans* (University of Wisconsin Press, Madison, 1977).
5. Covey, D. C. & Hastenrath, S. *Mon. Weath. Rev.* **106**, 1280–1287 (1978).
6. Markham, C. G. & McLain, D. R. *Nature* **265**, 320–323 (1977).
7. Moura, A. D. & Shukla, J. *J. atmos. Sci.* **38**, 2653–2675 (1981).
8. Hastenrath, S., Wu, M. C. & Chu, P. S. *Q. Jl R. met. Soc.* (submitted).
9. *Observacões pluviométricas no Nordeste do Brazil* (Departamento Nacional de Obras contra as Secas, Brazil, 1969).
10. Hastenrath, S. & Rosen, A. *Tellus*, **35A**, 324–331 (1983).
11. Wright, P. B. *An Index of the Southern Oscillation* (Climatic Research Unit, University of East Anglia, Res. Publ. 4, 1975).
12. Wright, P. B. *The Southern Oscillation—Patterns, and Mechanisms of the Teleconnections and the Persistence* HIG-77-13, 107 (Hawaii Institute of Geophysics, 1977).
13. Draper, N. R. & Smith, H. *Applied Regression Analysis* (Wiley, New York, 1981).
14. *STATJOB Summary* (Academic Computing Center, University of Wisconsin, Madison, 1980).
15. Panofsky, H. A.. & Brier, G. W. *Some Applications of Statistics to Meteorology* 191–208 (Pennsylvania State University, 1968).
16. Preisendorfer, R. W. & Mombley, C. D. *Climate Forecast Verifications, U.S. Mainland, 1974–82* (NOAA Tech. Mem. ERL PMEL-36, Seattle, 1982).
17. Quenouille, M. H. *Associated Measurements* (Butterworths, London, 1952).

Evidence against surface state limitations on efficiency of p-Si/CH_3CN junctions

Charles M. Lieber, Chris M. Gronet & Nathan S. Lewis*

Department of Chemistry, Stanford University, Stanford, California 94305, USA

We report here the first efficient p-type Si-based semiconductor–liquid junction system. p-Type Si photocathodes have been previously reported to yield open circuit photovoltages, V_{oc}, of 0.38–0.40 V with several redox systems[1–8]. Photocurrent–voltage studies of p-Si cathodes in CH_3CN solvent have demonstrated 2.5% efficiency for conversion of 632.8-nm light to electricity with the *N,N'*-dimethyl-4,4'-bipyridinium$^{2+/+}$ redox system[1], and 0.5–2.4% with other macrocyclic complexes[7,8]. Such modest energy conversion efficiencies for small band gap semiconductors have been attributed to surface states which pin the semiconductor Fermi level[6–9] and promote recombination processes[10]. However, we find that p-type Si/CH_3CN interfaces can yield solar conversion efficiencies in excess of 10%, and can display open circuit photovoltages within 0.08 V of the theoretical limit for an ideal junction.

We have chosen the cobaltocene$^{+/0}$ ($CoCp_2^{+/0}$)/1.0 M $[(C_2H_5)_4N]^+[BF_4]^-/CH_3CN$ redox system to probe the interface behaviour of p-Si in CH_3CN solvent. $(CoCp_2)^+(PF_6)^-$ is sufficiently soluble in CH_3CN to be used in concentrations which minimize mass transport polarization losses at the working electrode. In addition, the electrochemically reversible[11,12] $CoCp_2^{+/0}$ system possesses an $E^{0\prime}$ (−0.95 V/SCE) sufficiently negative to effect a large degree of band bending at the semiconductor–liquid interface. Uncompensated ohmic resistance losses can be minimized with proper cell design in this highly conductive ($\rho = 18\ \Omega$-cm) electrolyte; thus, all current–voltage parameters have been measured under potentiostatic control versus a Pt reference electrode in a 0.2-mm outer diameter Luggin capillary. Only when all of the above criteria are met might one conclude that any inefficiencies are due to surface states at the interface.

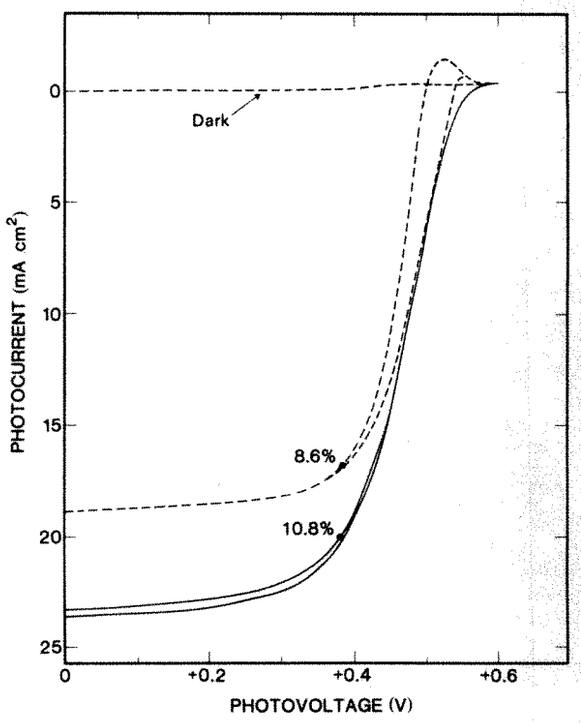


Fig. 1 Current–voltage properties of a single crystal p-type Si photocathode in a stirred CH_3CN/1.0 M $[(C_2H_5)_4N]^+[BF_4]^-$/0.2 M cobaltocenium/0.5 mM cobaltocene solution. Light intensity is 70 mW cm^{-2} from an ELH-type tungsten–halogen solar simulator, and photocurrent–voltage curves are uncorrected for any losses due to optical reflection or solution absorption. ---, Shiny Si surface obtained from a 10-s etch in 48% aqueous HF, followed by a 10-s rinse with H_2O, then a 10-s rinse with CH_3CN. —, Matte-textured surface[13] in identical illumination conditions. Both current–voltage curves were measured potentiostatically (50 mV s^{-1}) versus a Pt reference electrode (−0.82 V/SCE) through a Luggin capillary, and represent photoelectrode efficiencies of the semiconductor cathode.

Use of a mirror-finished p-type Si electrode (HF-etched, 1.5 Ω-cm resistivity, (100) oriented) in the optimized 1.0 M $[(C_2H_5)_4N]^+[BF_4]^-$/0.2 M $CoCp_2^+$/0.5 mM $CoCp_2/CH_3CN$ system with 70 mW cm^{-2} of ELH-type tungsten–halogen irradiation[13–15] yields V_{oc} of 0.48–0.52 V, short circuit photocurrent densities, J_{sc}, of 20–21 mA cm^{-2}, fill factors of 0.52–0.55, and photoelectrode efficiencies for conversion of incident light to electricity of 8.5 ± 0.8% (Fig. 1). Texturing of the photoelectrode surface to expose (111) planes and produce a matte black surface yields decreased reflectivity[13] and produces a system with $V_{oc} = 0.49$–0.53 V, $J_{sc} = 23$–26 mA cm^{-2}, and efficiencies of 10.5 ± 1.0%. Actual direct sunlight yields similar behaviour, and at 70 mW cm^{-2} of insolation on matte-etched surfaces, we observe $V_{oc} = 0.49$–0.53 V, $J_{sc} = 23$–26 mA cm^{-2}, and efficiencies of 10.5 ± 1.0%.

The photocurrents depicted in Fig. 1 are certainly not due to cathode corrosion. With suitable precautions to exclude air and water from the cell, we have maintained stable (±10%) short circuit currents at AM2 photocurrent densities (>20 mA cm^{-2}) for passage of $>2 \times 10^3$ C cm^{-2} through the interface. Longer term stability of this system has not been investigated because of the documented photodecomposition processes of $CoCp_2$ (ref. 11); however, other soluble, outer-sphere redox couples should be available which would yield similar photocurrent–

* To whom reprint requests should be addressed.

© 1984 Macmillan Journals Ltd

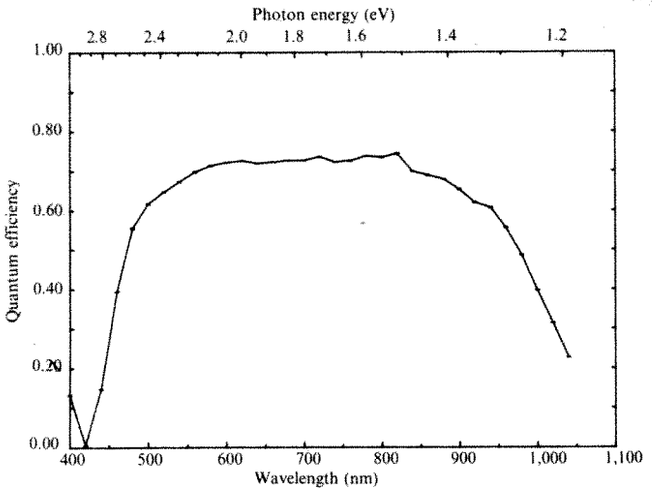


Fig. 2 Spectral response of the short circuit quantum yield plotted against wavelength of incident photons for the p-Si/CH_3CN/cobaltocene$^{+/0}$ system. Average incident light intensity is 1 mW cm^{-2}, and quantum efficiencies (±10%) are uncorrected for losses due to optical reflection or solution absorption of light. Shiny p-Si cathodes yield 20–30% less current than a matte-textured surface, but both electrodes exhibit excellent response over a broad spectral range.

voltage parameters. Our stability and efficiency measurements represent photoelectrode efficiencies of the working electrode only, and do not represent actual operating two-electrode solar cells. However, the system certainly illustrates that it is possible to observe excellent junction behaviour for p-Si photocathodes in the proper conditions.

The photocurrent response versus incident photon energy for mirror-finished p-Si cathodes is depicted in Fig. 2. The onset of photocurrent is in accord with the 1.1-eV absorption edge of Si, and the high-energy cutoff is attributable to solution absorption of light. An integration of the spectral response properties in Fig. 2 with the solar spectral input yields short circuit photocurrent densities which are in excellent agreement with experimental J_{sc} values. The observation of significant photocurrent near the Si absorption edge implies that unthermalized hot carriers do not provide the sole source of photocurrent flow at these p-Si/CH_3CN junctions. In addition, the high absolute value of the short circuit quantum efficiency, both at low light intensities and solar irradiance levels, yields little evidence for surface recombination sites at the p-Si/CH_3CN interface.

We view these results as consistent with observations in our laboratory[13,17] and by others[18,19] that the chemistry of the Si/SiO_2 interface will preclude substantial carrier recombination by surface states. With a minority carrier diffusion length of 300 μm and a dopant density of 2×10^{15} carriers cm^{-3}, photocurrent densities of 20 mA cm^{-2} imply a bulk diffusion-limited open circuit voltage of 0.59 V (refs 20–22). Thus, the V_{oc} of 0.48–0.52 V at 20–21 mA cm^{-2} for the p-Si/CH_3CN system is within 0.08 V of the maximum attainable value for any junction fabricated with our semiconductor samples. Furthermore, the V_{oc} of p-Si/CH_3CN junctions substantially exceed V_{oc} values typically found for direct p-Si/Au or p-Si/Pt Schottky barriers ($V_{oc}<0.20$–0.25 V)[23]. In fact, the V_{oc} values at high light intensity (>1 W cm^{-2}) for the p-Si/CH_3CN system (>0.55 V) exceed reported values of barrier heights for p-Si Schottky barriers with metals such as Ag, Au, Cr, Cu, Mo, Ni, Pb, Pd, Pt and W (ref. 20). Clearly, intrinsic surface states do not dominate the chemistry at both the semiconductor/liquid and semiconductor/metal interfaces.

Our data on the p-Si/CH_3CN interface yield no evidence for surface state limitations which would prevent the rational design and study of efficient semiconductor–liquid junctions. Investigations of the p-Si system are thus consistent with recent studies of other common, small band gap semiconductors (n-Si (ref. 13), n-GaAs (ref. 24), n-$GaAs_{1-x}P_x$ (ref. 25)) which suggest that control of bulk electrode properties and of solution overpotential losses can eliminate most inefficiencies previously ascribed to surface states at the semiconductor–liquid interface. Extension of the $CoCp_2^{+/0}$ redox system, and similar metallocenes, to other photocathode materials is underway.

We thank G. Cogan and J. Gibbons for helpful discussions, and for access to spectral response and metal evaporation facilities at SERA Solar Corporation. We acknowledge a Cottrell Grant from the Research Corporation for partial support of this work.

Received 9 September; accepted 17 October 1983.

1. Bocarsly, A. B., Bookbinder, D. C., Dominey, R. N., Lewis, N. S. & Wrighton, M. S. *J. Am. chem. Soc.* **102,** 3683–3688 (1980).
2. Bruce, J. A., Murahashi, T. & Wrighton, M. S. *J. phys. Chem.* **86,** 1552–1563 (1982).
3. Dominey, R. N., Lewis, N. S., Bookbinder, D. C., Bruce, J. A. & Wrighton, M. S. *J. Am. chem. Soc.* **104,** 467–482 (1982).
4. Abruna, H. D. & Bard, A. J. *J. Am. chem. Soc.* **103,** 6898–6901 (1981).
5. Byker, H. J., Wood, V. E. & Austin, A. E. *J. electrochem. Soc.* **129,** 1983–1987 (1982).
6. Bard, A. J., Bocarsly, A. B., Fan, F.-R. F., Walton, E. G. & Wrighton, M. S. *J. Am. chem. Soc.* **102,** 3671–3677 (1980).
7. Bradley, M. G. & Tysak, T. *J. electroanalyt. Chem.* **135,** 153–157 (1982).
8. Bradley, M. G., Tysak, T., Graves, D. J. & Vlachopoulos, N. A. *JCS Chem. Commun.* 349–350 (1983).
9. Bard, A. J., Fan, F.-R. F., Gioda, A. S., Nagasubramanian, G. & White, H. S. *Disc. Faraday Soc.* **70,** 19–31 (1980).
10. Fan, F.-R. F. & Bard, A. J. *J. Am. chem. Soc.* **102,** 3677–3683 (1980).
11. Gubin, S. P., Smirnova, S. A. & Denisovich, L. I. *J. organometall. Chem.* **30,** 257–265 (1971).
12. Geiger, W. E. Jr *J. Am. chem. Soc.* **96,** 2632–2634 (1974).
13. Gronet, C. M., Lewis, N. S., Cogan, G. & Gibbons, J. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1152–1156 (1983).
14. Seaman, C. H., Anspaugh, B. E., Downing, R. G. & Estey, R. S. *Proc. 14th IEEE Photo. Spec. Conf.* 494–499 (1980).
15. Godfrey, R. B. & Green, M. A. *Appl. Phys. Lett.* **34,** 790–793 (1979).
16. Borrell, P. & Henderson, E. *Inorg. chim. Acta* **12,** 215–218 (1975).
17. Lewis, N. S. *et al.* in *Physics and Chemistry of Electrocatalysis* (ed. McIntyre, J. M.) (The Electrochemical Society, Princeton, in the press).
18. Heller, A., Lewerenz, H. J. & Miller, B. *J. Am. chem. Soc.* **103,** 200–201 (1981).
19. Morrison, S. R., Frese, K. W. Jr & Madou, M. J. *Adv. Chem. Ser.* 179–187 (1981).
20. Sze, S. M. *Physics of Semiconductor Devices* 2nd edn, 270–293 (Wiley, New York, 1981).
21. Fonash, S. J. *Solar Cell Device Physics* (Academic, New York, 1981).
22. Lewis, N. S. *J. Electrochem. Soc.* (in the press).
23. Fahrenbruch, A. L. & Bube, R. H. *Fundamentals of Solar Cells*, 465–471 (Academic, New York, 1983).
24. Gronet, C. M. & Lewis, N. S. *Appl. Phys. Lett.* **43,** 115 (1983).
25. Gronet, C. M. & Lewis, N. S. *Nature* **300,** 733–735 (1983).

Photochemical dehydrogenation of ethanol in dilute aqueous solution

John Handman, Anthony Harriman & George Porter

Davy Faraday Research Laboratory, The Royal Institution, 21 Albemarle Street, London W1X 4BS, UK

The need for alternative sources of energy has stimulated research into the storage of sunlight as chemical potential. Many systems have been investigated but photogeneration of H_2 seems to be the most practical. The cyclic photodissociation of water has yet to be realized in homogeneous solution using visible light, although heterogeneous photosystems are known[1–3]. Efficient photogeneration of H_2 in homogeneous conditions can be achieved[4–7] at the expense of an added electron donor but such systems have little application in practical devices. Replacement of the sacrificial donor with waste material, for example sulphide[8], offers a route for improving these systems and, here we describe efficient H_2 photogeneration from a system using aqueous ethanol, as available from low grade fermentation, as donor. To couple the photoproduction of H_2 to the oxidation of ethanol we have used NADH/alcohol dehydrogenase as a relay.

Dichloro tin(IV) *meso*-tetrakis (*N*-methyl-4-pyridyl)porphine ($SnTMPyP^{4+}$) dissolves readily in water throughout the entire *p*H range and such solutions absorb strongly all light

0028–0836/84/060534—02$01.00

© 1984 Macmillan

below 620 nm and are therefore capable of collecting some 40% of the energy available in sunlight. Upon excitation with visible light ($\lambda < 620$ nm), the triplet excited state of the metalloporphyrin is formed in high yield ($\phi_T \sim 0.95$) and it is quite long-lived ($\tau_T = 0.9$ ms) in the absence of oxygen. The triplet state is a powerful oxidizing agent and is quenched by NADH at the diffusion controlled bimolecular rate limit due to transfer of a single electron, as shown by flash photolysis studies.

$$^*SnTMPyP^{4+} + NADH \rightarrow SnTMPyP^{3+\cdot} + NADH^{+} \quad (1)$$

$$NADH^{+} \rightleftharpoons NAD^{\cdot} + H^{+} \quad (2)$$

$$pK = -4$$

The one-electron reduction product of the metalloporphyrin is the π-radical anion ($SnTMPyP^{3+\cdot}$) which can be identified by its characteristic absorption spectrum[9]. The π-radical anion is unstable with respect to disproportionation at $pH < 12$, although it persists for many minutes in alkaline solution, and it is also reduced rapidly by the oxidized form of the donor.

$$2\,SnTMPyP^{3+\cdot} + H^{+} \rightleftharpoons SnTMPyP^{4+} + SnTMPyPH^{3+} \quad (3)$$

$$SnTMPyP^{3+\cdot} + NAD^{\cdot} + H^{+} \rightarrow SnTMPyPH^{3+} + NAD^{+} \quad (4)$$

Both processes result in formation of the metallophlorin ($SnTMPyPH^{3+}$), which is a protonated π-dianion and has a characteristic absorption maximum centred at 830 nm (refs 10, 11). The phlorin is stable in outgassed aqueous solution over the pH range 4–14, although it undergoes acid/base equilibration and is quantitatively reoxidized to the original metalloporphyrin on aeration. For light of λ550 nm, the quantum yield for formation of the phlorin was 0.60 ± 0.05 for concentrations of NADH of 2×10^{-3} mol dm^{-3} and $SnTMPyP^{4+}$ of 1×10^{-5} mol dm^{-3} at pH 7.5. Since the oxidized form of the donor can also reduce ground state metalloporphyrin

$$SnTMPyP^{4+} + NAD^{\cdot} \rightarrow SnTMPyP^{3+\cdot} + NAD^{+} \quad (5)$$

the overall process requires one photon to drive a two-electron reduction of the porphyrin

$$SnTMPyP^{4+} + NADH \xrightarrow{h\nu} SnTMPyPH^{3+} + NAD^{+} \quad (6)$$

It should be emphasized that the porphyrin/phlorin cycle is exceptionally clean for $SnTMPyP^{4+}$ at $pH > 4$ and, unlike most metalloporphyrins studied to date[9,10], irreversible formation of the chlorin is unimportant.

When colloidal Pt (ref. 12) is present, irradiation ($\lambda < 620$ nm) of the solution results in formation of H_2 and the phlorin is no longer observed as a reaction product. Depending on pH, the phlorin, the π-radical anion and NAD$^{\cdot}$ are all capable of reducing protons to H_2 on the surface of a Pt catalyst. With a Pt concentration of 10^{-4} mol dm^{-3} and irradiation with λ550 ± 10 nm, the quantum yield for formation of H_2 was found to be 0.43 ± 0.05 at pH 7.5. Thus, conversion of the reducing equivalents into H_2 is almost quantitative and H_2 formation is not restricted to neutral solution but occurs throughout the range $5 < pH < 10$. It was found that Pt catalysed formation of the metallochlorin, albeit ineffectively, so that at high concentrations of Pt the cycle with respect to the porphyrin was no longer entirely clean. Although very efficient, the above cycle is sacrificial in that the evolved H_2 is at the expense of oxidation of NADH to NAD^{+} and there is a concomitant increase in pH.

$$H_2O + NADH \overset{h\nu}{\rightleftharpoons} NAD^{+} + H_2 + OH^{-} \quad (7)$$

In our conditions, there is no experimental evidence that Pt catalyses reduction of NAD^{+} by H_2 although this reaction is expected to occur on standing in the dark.

Regeneration of NADH from NAD^{+} can be achieved readily using ethanol and an enzyme catalyst such as yeast alcohol dehydrogenase (YADH).

$$C_2H_5OH + NAD^{+} \xrightarrow{YADH} CH_3CHO + NADH + H^{+} \quad (8)$$

Thus, irradiation of $SnTMPyP^{4+}$ (10^{-5} mol dm^{-3}) with green light in N_2-purged aqueous solution at pH 7.5 containing NADH (2×10^{-3} mol dm^{-3}), colloidal Pt (10^{-4} mol dm^{-3}), YADH (25 mg dm^{-3}) and ethanol (2% v/v) resulted in sustained generation of H_2. In a typical experiment, the rate of H_2 formation was approximately 440 cm^3 per dm^3 of solution per h at an incident light intensity of about 10^{20} photons min^{-1}. On prolonged irradiation, turnover numbers of 86 and 131,000 respectively have been observed for NADH and YADH, although that for NADH is not exhaustive. Only a small fraction of the metalloporphyrin is lost (via chlorin formation) in these conditions whilst the ethanol can be converted quantitatively into acetaldehyde and H_2 and the quantum yield for formation of H_2 remained at 0.43 ± 0.05. The solution pH remains constant during the overall reaction and neither acetaldehyde nor its solvate inhibit H_2 evolution. The rate of H_2 formation shows no significant decrease until the YADH or ethanol has been consumed. The rate and quantum yield of H_2 formation compare favourably with any previously reported sacrificial H_2 evolving photosystem while the longevity of the present system greatly exceeds that of any previous system.

Overall, the photoreaction may be summarized by the following scheme:

$$\begin{matrix} H^{+} \\ Pt \\ H_2 \end{matrix} \Big) \Big(\begin{matrix} SnTMPyPH^{3+} \\ \\ SnTMPyP^{4+} \end{matrix} \Big) \overset{h\nu}{\Big)} \Big(\begin{matrix} NAD^{+} \\ \\ NADH \end{matrix} \Big) \Big(\begin{matrix} C_2H_5OH \\ YADH \\ CH_3CHO + H^{+} \end{matrix} \quad (9)$$

Dehydrogenation of ethanol

$$C_2H_5OH_{(L)} \rightarrow CH_3CHO_{(L)} + H_{2(G)} \quad (10)$$

$$\Delta G^0 = 42\ kJ\ mol^{-1}$$

is thermodynamically uphill so that energy is stored during the reaction. The overall efficiency for storage of light energy (λ = 620 nm) is $(0.43 \times 42/193)$ 9.4%. The photoreaction might offer a useful alternative to distillation of ethanol from low grade fermentation processes. However, the idea of using an enzyme to regenerate the electron donor is not restricted to ethanol but D-glucose or lactate could be used instead. Finally, we note that the phlorin is extremely stable in the absence of air so that the photoreaction could be used in a flow cell enabling the H_2 to be liberated at a specific site.

We thank the SERC, the EEC and General Electric (Schenactady) for financial support. Further details regarding the reaction mechanism and intermediates will be reported elsewhere.

Received 30 August; accepted 24 November 1983.

1. Lehn, J. M., Sauvage, J. P. & Ziessel, R. *Nouv. J. Chim.* **4**, 623–627 (1980).
2. Borgarello, E., Kiwi, J., Pellizetti, E., Visca, M. & Grätzel, M. *J. Am. chem. Soc.* **103**, 6324–6329 (1981).
3. Kalyanasundaram, K., Borgarello, E., Duonghong, D. & Grätzel, M. *Angew. Chem.* **93**, 1012–1013 (1981).
4. Koryakin, B. V., Dzhabiev, T. S. & Shilov, A. E. *Dokl. Akad. Nauk SSSR* **233**, 620–613 (1977).
5. Kirch, M., Lehn, J. M. & Sauvage, J. P. *Helv. chim. Acta* **62**, 1345–1384 (1979).
6. Kalyanasundaram, K., Kiwi, J. & Grätzel, M. *Helv. chim. Acta* **61**, 2720–2730 (1978).
7. Darwent, J., Douglas, P., Harriman, A., Porter, G. & Richoux, M. C. *Coord. Chem. Rev.* **44**, 83–126 (1982).
8. Okura, I., Aono, S., Yamada, M., Kita, T. & Kusunoki, S. *Inorg. chim. Acta* **76**, 91–92 (1983).
9. Harriman, A., Richoux, M. C. & Neta, P. *J. phys. Chem.* **87**, 4957–4965 (1983).
10. Baral, S., Neta, P. & Hambright, P. *J. phys. Chem.* (in the press).
11. Closs, G. L. & Closs, L. E. *J. Am. chem. Soc.* **85**, 818–819 (1963).
12. Harriman, A., Porter, G. & Richoux, M. C. *JCS Faraday Trans.* **78**, 1955–1970 (1982).

Large mantle heterogeneity beneath French Polynesia

Ph. Vidal*, C. Chauvel† & R. Brousse‡

* Laboratoire de Géochronologie, Université de Clermont-Ferrand II, 5 Rue Kessler, 63038 Clermont-Ferrand Cedex, France
† Max-Planck-Institut für Chemie, Saarstrasse 23, Postfach 3060, 6500 Mainz, FRG
‡ Laboratoire de Pétrologie-Volcanologie, Université de Paris XI, 91405 Orsay, France

New strontium, lead and neodymium isotope compositions of volcanics from two volcanic island chains of French Polynesia (Marquesas, Australes) are presented. The data from Marquesas fall in the well-established oceanic islands field and are very similar to those of the Society Islands of French Polynesia. In contrast, Tubuaii Island basalts from the Australes chain exhibit very low $^{87}Sr/^{86}Sr$ ratios (~0.7028) and the highest $^{206}Pb/^{204}Pb$ (~21.1) and $^{208}Pb/^{204}Pb$ (~40.4) ratios ever reported for an oceanic island. Their ε_{Nd} value is ~+4.9. These data are similar to those of St Helena, although even more extreme. This convergence indicates that a comparable and as yet undefined process took place in the mantle beneath the Atlantic and Pacific oceans. The isotopic data for the Polynesian volcanics encompass almost the entire range of Sr and Nd isotopic ratios found in oceanic islands ($^{87}Sr/^{86}Sr = 0.7028–0.7083$; $^{143}Nd/^{144}Nd = 0.51257–0.51297$) and more than half of the Pb isotope range ($^{206}Pb/^{204}Pb = 18.9–21.1$; $^{208}Pb/^{204}Pb = 38.9–40.4$).

Mid-ocean ridge basalt (MORB) and oceanic island volcanism are known to originate from totally distinct mantle sources and to have been separated for at least 1,000 Myr. Despite the number of models[1–9], mostly based on isotopic data, the following points are still matters of discussion: (1) What is the depth of the ocean island basalts source regions? Are they located in the upper or in the lower mantle? (2) Are the large variations in isotopic ratios observed in volcanic rocks at the present time the result of primary heterogeneity of the U/Pb, Rb/Sr and Sm/Nd ratios at the time of the formation of the mantle or of later processes? (3) In the case of the lower mantle hypothesis, what is the extent of the interaction with the core? (4) Does altered and subducted oceanic crust or continental material contribute to the oceanic island isotopic pattern? (5) To what degree have oceanic island magmas been chemically and isotopically contaminated as they rise through the depleted upper mantle?

Although a number of oceanic islands are now well documented regarding isotopes there are only a few high-precision data on Pb, Sr and Nd isotopes on the same samples. In addition, a strong emphasis has been put on Atlantic islands and few data are available on the Indian and Pacific oceans. A more precise answer to these questions will be given when the whole picture of isotopic pattern (Pb, Sr, Nd, Hf, rare gases) on all oceans becomes available.

We report here some new measurements on French Polynesia which due to its large surface area (2,200,000 km^2; 8–18 lat. south and 135–155 long. west) allows us to study the mantle heterogeneity on a larger scale than was possible with the other oceanic islands.

In the central Pacific Ocean, four volcanic chains are aligned parallel to the Hawaiian chain: the Marquesas Islands, the Gambier–Tuamoutou Islands, the Society Islands and the Australes Islands (formally called Tubuaii Islands) (Fig. 1). Each of these chains can be interpreted as being the result of a hot spot[10] which is located at the south-east end of each chain.

In the Marquesas Islands, volcanic activity ranges in age from 6.3 ± 0.2 Myr (in Eiao Island) to 1.30 ± 0.02 Myr (in Fatu Hiva Island)[11,12]. In the Australes Islands, volcanism extends from 18 Myr (at the north-west end of the chain[13–15]) to the present as indicated by active volcanism on MacDonald seamount at the south-east end of the chain[16,17].

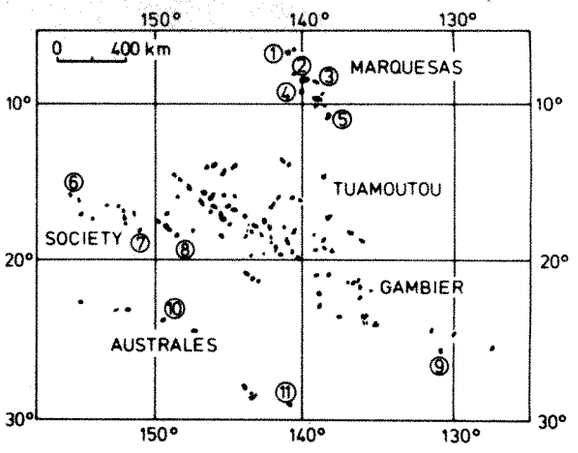


Fig. 1 Location of the Polynesian islands in the Central Pacific. Numbers refer to the islands: 1, Eiao; 2, Nuku Hiva; 3, Ua Huka; 4, Ua Pou; 5, Fatu Hiva; 6, Maupiti; 7, Tahiti; 8, Mehetia; 9, Pitcairn; 10, Tubuaii; 11, MacDonald. Ranges in lava compositions are as follows: Ua Pou—a potassic series, picrite to K-phonolite[21]; Ua Huka—undifferentiated basalt[20]; Nuka Hiva—alkali-olivine basalt to alkali-rich quartz-trachyte[22]; Tubuaii—five distinct series are observed[23]: (i) basal series of the main volcano (12–10.5 Myr) composed of undifferentiated transitional basalts (5436); (ii) medium series of the main volcano (9.5–6.8 Myr) composed of undifferentiated alkali basalts (5434); (iii) top series of the main volcano ranging in composition from differentiated basanites (5433) to phonolites; (iv) a group of later intrusions (≅5.7 Myr) composed of analcimites (5435); (v) the succession of the secondary volcano located on the west part of the island (10.5–8 Myr) ranging in composition from murites (5437) to phonolites. As noted above, one sample has been selected in each series, each of these samples representing the more primitive liquid of the series.

In both the Marquesas and Australes Islands, the volcanic products are of alkalic affinity. Each island is formed by the succession of at least two different volcanic sequences[18], the alkalic character increasing from the beginning to the end of the sequence[19].

We analysed samples collected in three different islands of the Marquesas chain: Ua Pou, Ua Huka and Nuku Hiva, as well as samples from Tubuaii Island in the Australes chain, and the results are presented in Table 1 and in the legend to Fig. 1.

Lead chemistry and mass spectrometry have been performed in Rennes using the method described by Vidal and Clauer[24]. Part of the Sr data was obtained in Rennes using the technique described by Chauvel[25]. The other part of the Sr data as well as the Nd data were obtained in Mainz using the analytical procedures reported by White and Patchett[26].

Sr, Pb and Nd isotopic ratios are reported in Table 1. Due to their low ages (2–10 Myr) and their low Rb/Sr ratios, the *in situ* correction for the increase of $^{87}Sr/^{86}Sr$ by radioactive decay is for most of the samples within the analytical uncertainties. However, for a few samples the correction is on the fourth decimal place. The measured Nd isotopic ratios can be considered as initial ratios because the rocks are very young. For Pb isotopes no correction has been made due to the lack of data regarding Th and U contents. Only $^{206}Pb/^{204}Pb$ ratios are actually sensitive to this correction, although they are also generally considered as initial ratios. In fact, in the present study, the very close grouping of $^{206}Pb/^{204}Pb$ ratios within one single island whatever the petrographic type enables us to consider the observed ratios as closely approaching the initial values.

The results are plotted on four diagrams: $^{207}Pb/^{204}Pb$ against $^{206}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ against $^{206}Pb/^{204}Pb$ (Fig. 2*a*, *b*), $^{143}Nd/^{144}Nd$ against $^{87}Sr/^{86}Sr$ (*c*) and $^{87}Sr/^{86}Sr$ against $^{206}Pb/^{204}Pb$ (*d*).

In all diagrams, the data from Marquesas plot in the middle of the field defined by the other oceanic islands. They are particularly comparable to the data of Society Islands (refs 27,

0028-0836/84/060536—03$01.00

© 1984 Macmillan Journals Ltd

Table 1 Isotopic results for the Polynesian samples

Sample	Type or rock	Rb	Sr	Pb	$^{87}Sr/^{86}Sr$ (measured)	$^{87}Sr/^{86}Sr$ (initial)	$^{206}Pb/^{204}Pb^*$	$^{207}Pb/^{204}Pb^*$	$^{208}Pb/^{204}Pb^*$	$^{143}Nd/^{144}Nd$	ε_{Nd}
Marquesas											
Ua Pou											
5424 (73F)	K-Na Basalt	42	531	3.3	†0.705131 ± 21	0.70512	19.23	15.62	39.23	0.512710 ± 14	+1.4
5425 (74P)	K-phonolite	212	418	18	0.705419 ± 78	0.70536	—	—	—	—	
5426 (60Y)	K-Na phonolite	229	126	20	0.704866 ± 86	0.70466	19.24	15.63	39.26	—	
5427 (73R)	K-benmoreite	117	1,032	10	†0.704743 ± 16	0.70473	19.22	15.63	39.12	0.512750 ± 11	+2.2
5428 (74h)	K-basalt	106	1,300	5.4	0.705225 ± 72	0.70522	—	—	—	—	
Ua Huka											
5429 (67H)	Basanite	12	617	2.6	0.704584 ± 54	—	18.94	15.58	38.92	—	
5430 ($68F_2$)	Basalt	43	450	4.5	†0.705614 ± 22	—	19.15	15.65	39.29	0.512741 ± 34	+2.0
5430 ($68F_2$)	Leached sample	—	—	—	†0.705688 ± 25	—	—	—	—	—	
Nuku Hiva											
5438 (70X)	K-trachyte	147	365	9.7	†0.704487 ± 17	0.70443	19.02	15.61	38.98	0.512765 ± 12	+2.5
5439 (78A)	Basalt	21	415	11	0.703792 ± 30	0.70378	19.11	15.57	38.88	0.512889 ± 28	+4.9
5440 (77t)	Basalt	25	538	2.8	0.704231 ± 68	0.70422	19.08	15.58	39.01	—	
5442 (10E)	Basalt	7	412	1.8	†0.703040 ± 21	0.70304	19.13	15.58	39.13	0.512971 ± 17	+6.5
5443 (76Z)	Na-trachyte	137	635	8.4	0.704361 ± 72	0.70433	19.05	15.62	39.16	—	
5444 (76F)	Benmoreite	174	457	—	0.705134 ± 64	0.70507	—	—	—	—	
Australes											
Tubuaii											
5433 (TU 23)	Basalt	27	831	2.8	*0.702800 ± 12	0.70279	21.14	15.76	40.29	0.512886 ± 16	+4.8
5434 (TU 21)	Basalt	20	509	2.5	*0.702779 ± 20	0.70276	21.07	15.76	40.33	0.512882 ± 14	+4.8
5435 (TU 31)	Basalt	51	1,028	8.6	*0.702793 ± 21	0.70277	21.14	15.78	40.44	0.512887 ± 19	+4.9
5436 (TU 29)	Basalt	16	562	1.0	*0.702793 ± 18	0.70278	21.06	15.78	40.30	0.512884 ± 11	+4.8
5437 (TU 4)	Basalt	59	1,301	4.4	*0.702788 ± 21	0.70277	21.11	15.77	40.41	0.512895 ± 17	+5.0

$^{87}Sr/^{86}Sr$ ratios are normalized to $^{86}Sr/^{88}Sr = 0.1194$ and $^{143}Nd/^{144}Nd$ ratios to $^{144}Nd/^{146}Nd = 0.7219$. Values for E & A standard and La Jolla standard measured at the Max-Planck-Institute are $^{87}Sr/^{86}Sr = 0.707998 \pm 40$ (2σ pop) and $^{143}Nd/^{144}Nd = 0.511847 \pm 21$ (2σ pop) respectively. Value in Rennes for NBS 987 standard is $^{87}Sr/^{86}Sr = 0.71020 \pm 4$ (2σ). Concentrations in Rb, Sr and Pb have been measured by the XRF method. Precision is 2% for Rb and Sr contents and 10% for Pb content.
* As estimated from replicates of NBS 981 analysis, maximum errors on $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ ratios are 0.15% and on $^{207}Pb/^{206}Pb$, 0.07%.
† Sr data obtained in Max-Planck-Institute, Mainz.

28 and W. M. White, unpublished data). This suggests that the Marquesas and the Society Islands may originate from similar sources. In contrast, Tubuaii Island field is very different from the other oceanic islands, with the exception of St Helena.

The strontium data for the Marquesas Islands, presented here, are in excellent agreement with those of Duncan and Compston[29]. Within one single island the spread in $^{87}Sr/^{86}Sr$ ratios can be very large. In contrast, the lead isotopic ratios are very homogeneous for each island. The spread in strontium isotopic ratios might be related to some interaction with seawater (which has an $^{87}Sr/^{86}Sr$ ratio of 0.7091) at the level of the magma chamber. However, in this case, a correlation between $^{87}Sr/^{86}Sr$ ratio and Sr content should be observed, which is obviously not the case (Table 1). In addition, Nd isotopes correlate negatively with Sr isotopes (Fig. 2*c*) and lie on the mantle array. As seawater contamination affects only Sr isotopes and in consequence shifts the points to the right of the mantle array, the present results can be considered indicative of large strontium and neodymium mantle heterogeneity beneath these islands. These data also have a bearing on the genesis of the volcanism within each of the islands: on each, the rocks have isotopic compositions indicating that they cannot have originated from a single primary basaltic melt by a fractional crystallization; either they originate from distinct mantle sources separated for more than 1,000 Myr or they result from the mixing in different proportions of a MORB-type source and of an enriched source. In either case, fractional crystallization occurring in the magma chambers may modify elemental concentrations.

Although each belongs to a separate magmatic suite, the five analysed samples from Tubuaii have remarkably similar Sr, Pb and Nd isotopic compositions. The $^{87}Sr/^{86}Sr$ ratios of 0.70278 (a value comparable to those of Ascension, St Helena and Galapagos[27,30–32] are among the lowest reported for an oceanic island. In contrast, the $^{206}Pb/^{204}Pb$ of 21.10 and the $^{208}Pb/^{204}Pb$ of 40.35 are the highest reported for an oceanic island. Of published data, St Helena has the most similar composition, with $^{206}Pb/^{204}Pb$ ratios ranging between 20.70 and 20.91 and $^{208}Pb/^{204}Pb$ from 40.00 to 40.16 [33].

Both in Nd/Sr and Sr/Pb diagrams (Fig. 2*c*, *d*) the Tubuaii data plot very close to the St Helena data. This shows that the St Helena mantle source is not an exception and that beneath both the South Atlantic and South Pacific oceans, the same kind of peculiar mantle region exists. Zindler *et al.*[34] propose that the St Helena data indicate a mixture of depleted mantle and recycled oceanic crust, the latter having had a low Rb/Sr ratio, relatively high Sm/Nd and high U/Pb ratio for a long period of time. Low Rb/Sr and $^{87}Sr/^{86}Sr$ for the recycled oceanic crust seem unlikely since Hart and Staudigel[35] consider that alteration increases both the Rb content and the Sr isotopic composition of the oceanic crust. However, Michard *et al.*[36] present data on the East Pacific Rise black smokers which shows that the hydrothermal fluid circulation within the oceanic crust may drastically reduce the Rb content of the oceanic crust and increase its U content. If the effects of the hydrothermal alteration are large enough to counteract the increase of the Rb/Sr ratio due to the addition of sediments and to the superficial seawater alteration, then the Rb/Sr ratio of the altered oceanic crust could decrease to below its original value.

If such a decrease of the global Rb/Sr ratio of the altered crust is realistic, data such as those obtained in St Helena and Tubuaii can be explained by melting of a recycled oceanic crust. An alternative explanation for having a mantle source with long time-integrated low Rb/Sr ratio as well as high U/Pb ratio, has already been proposed by Vidal and Dosso[3]. These authors proposed that part of the mantle has been depleted in Pb and Rb by extraction of those elements as sulphide phases from the lower mantle to the core. This could be the end-member close to the St Helena and Tubuaii data in the mantle plane.

The data now available for Marquesas and Tubuaii have two general implications regarding the mantle evolution. First, the particular mantle process which produced the St Helena source and which is still not well understood also took place beneath a portion of the Pacific Ocean, and second, the mantle is extremely heterogeneous beneath French Polynesia. Samples from a single island (Nuku Hiva) cover half the observed Sr-Nd isotopic variation in the oceanic islands, and in the three Marquesas islands studied here, almost the entire range is observed. On the scale of the whole of French Polynesia, more than half of the Pb isotope field is observed. These observations suggest that the large number of Polynesian islands scattered over a very large area offer a unique opportunity to study the scales

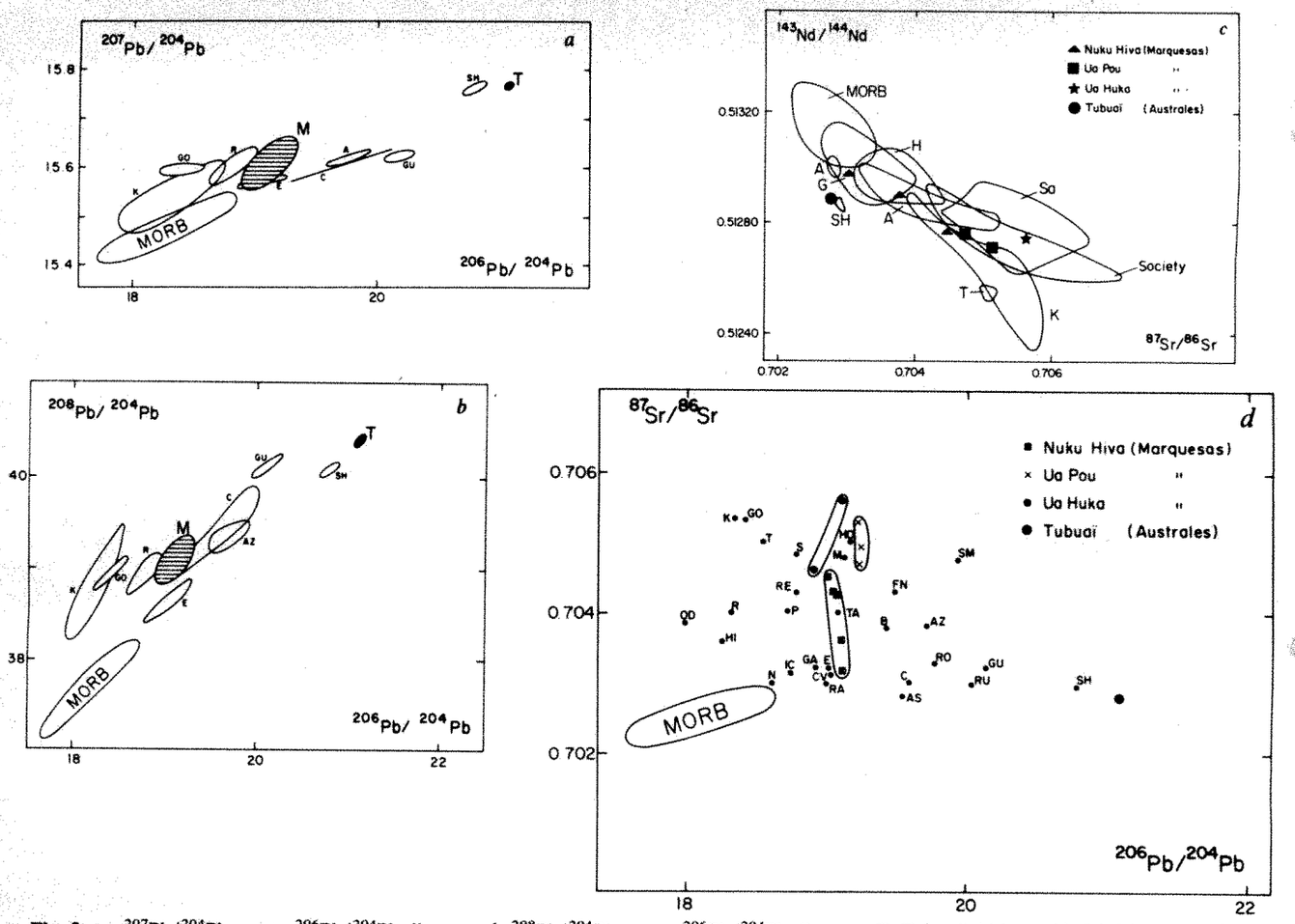


Fig. 2 *a*, $^{207}Pb/^{204}Pb$ versus $^{206}Pb/^{204}Pb$ diagram; *b*, $^{208}Pb/^{204}Pb$ versus $^{206}Pb/^{204}Pb$ diagram. T, Tubuaii (this work); M, Marquesas (this work); other data are from the literature. SH, St Helena; GU, Guadalupe; AZ, Azores; C, Canary; E, Easter; R, Reunion; GO, Gough; K, Kerguelen. *c*, $^{143}Nd/^{144}Nd$ versus $^{87}Sr/^{86}Sr$ diagram. Data from this work are represented by symbols; other data[27,32,37–42] are only shown by fields. *d*, $^{87}Sr/^{86}Sr$ versus $^{206}Pb/^{204}Pb$ diagram. In order to simplify the figure, an average of all data available for each island has been done as already suggested by Zindler *et al.*[34]. Data are from the compilation made by Sun[33] with the exception of Fernando de Noronha (F.N.), which are unpublished data of Ph.V. and G. Cornen. Abbreviations as follows: K, Kerguelen; GO, Gough; T, Tristan de Cunha; S, Samoa; HO; Hiva Oa; M, Moorea; SM, Sao Miguel; RE, Reunion; P, Penhu; TA, Tahiti; B, Bouvet; AZ, Azores; GA, Galapagos; SH, St Helena; GU, Guadalupe; RU, Rurutu (Australes); RO, Ross; C, Canary; AS, Ascension; RA, Rapa (Australes); CV, Cape Verde; E, Easter; N, Nunivak; IC, Iceland; OD, Oki Dogo; R, Rarotonga; HI, Hawaii.

of mantle heterogeneities. In particular it is striking that each of the three Marquesas islands studied has a unique Pb composition, whereas Sr and Nd vary greatly.

We conclude from our observation first that the volume of the mantle involved in the generation of the lavas observed in one island might, in the case of an idealized cubic volume, have a side smaller than a few tens of kilometres, and second that large Sr and Nd heterogeneities might have persisted on this rather restricted scale over thousands of millions of years. In fact, the actual heterogeneities might be smaller, since the spread defined by the data in the Sr against Nd diagram can be due to a mixing between two sources: the first being time-integrated enriched in incompatible elements, and the second being a MORB-type source.

We thank F. Albarède and A. W. Hofmann for help in preparing the manuscript and Bill White for his review. Part of this work was supported by CNRS ATP Transferts (Contract 15–14).

Received 27 June; accepted 24 November 1983.

1. Sun, S.-S. & Hanson, G. N. *Geology* **3,** 297–302 (1975).
2. Tatsumoto, M. *Earth planet. Sci. Lett.* **38,** 63–87 (1978).
3. Vidal, Ph. & Dosso, L. *Geophys. Res. Lett.* **5,** 169–171 (1978).
4. Anderson, D. L. *Earth planet. Sci. Lett.* **57,** 1–12 (1982).
5. Anderson, D. L. *Earth planet. Sci. Lett.* **57,** 13–24 (1982).
6. DePaolo, D. J. & Wasserburg, G. J. *Geophys. Res. Lett.* **3,** 743–746 (1976).
7. Allègre, C. J. *Tectonophysics* **81,** 109–132 (1982).
8. Chase, C. G. *Earth planet. Sci. Lett.* **52,** 277–284 (1981).
9. Hofmann, A. W. & White, W. M. *Earth planet. Sci. Lett.* **57,** 421–436 (1982).
10. Morgan, W. J. *Bull. Am. Ass. Petrol. Geol.* **56,** 203–213 (1972).
11. Brousse, R. & Bellon, H. *C. r. hebd. Séanc. Acad. Sci. Paris* **278,** 827–830 (1974).
12. Duncan, R. A. & MacDougall, I. *Earth planet. Sci. Lett.* **21,** 414–420 (1974).
13. Dalrymple, G. B., Jarrard, R. D. & Clague, D. A. *Bull. geol. Soc. Am.* **86,** 1463–1467 (1975).
14. Duncan, R. A. & MacDougall, I. *J. Volcanol. Geotherm. Res.* **1,** 197–227 (1976).
15. Bellon, H., Brousse, R. & Pantaloni, A. *Cah. de l'Indo-Pacif.* **2,** 219–240 (1980).
16. Brousse, R. & Richer de Forges, B. *C. r. hebd. Séanc. Acad. Sci., Paris* **290,** 1055–1057 (1980).
17. Talandier, J. & Okal, E. *C. r. hebd. Séanc., Acad. Sci. Paris* **295,** 195–200 (1982).
18. Brousse, R. & Guille, G. *R. Ann. Sc. Terre* **80** (1975).
19. Brousse, R. *Cah. du Pacif.* **21,** 107–144 (1978).
20. Brousse, R. & Sevin, M. A. *Cah. du Pacif.* **21,** 204–214 (1978).
21. Brousse, R. & Maury, R. C. *Bull. Soc. fr. Minér, Cristallogr.* **101,** 3–10 (1978).
22. Maury, R. C. & Brousse, R. *Cah. du Pacif.* **21,** 145–188 (1978).
23. Brousse, R. & Maury, R. C. *Cah. de l'Indo-Pacif.* **2,** 131–193 (1980).
24. Vidal, Ph. & Clauer, N. *Earth planet. Sci. Lett.* **55,** 237–246 (1981).
25. Chauvel, C. thesis, Univ. Rennes, 1982.
26. White, W. M. & Patchett, P. J. *Earth planet. Sci. Lett.* (in the press).
27. White, W. M. & Hofmann, A. W. *Nature* **296,** 821–825 (1982).
28. White, W. M., Todt, W., Patchett, P. J. & Hofmann, A. W. *Ged. Soc. Am. Meet. Abstr. with Programs* **14,** 646 (1982).
29. Duncan, R. A. & Compston, W. *Geology* **4,** 728–732 (1976).
30. Harris, C., Bell, J. D. & Atkins, F. B. *Earth planet. Sci. Lett.* **60,** 79–85 (1982).
31. Cohen, R. S. & O'Nions, R. K. *J. Petrology* **23,** 299–324 (1982).
32. White, W. M. & Hofmann, A. W. *Yb. Carnegie Instn Wash.* **77,** 596–606 (1978).
33. Sun, S.-S. *Phil. Trans. R. Soc.* **297,** 409–445 (1980).
34. Zindler, A., Jagoutz, E. & Golstein, S. *Nature* **298,** 519–523 (1982).
35. Hart, S. R. & Staudigel, H. *Earth planet. Sci. Lett.* **58,** 202–212 (1982).
36. Michard, A., Albarède, F., Michard, G., Minster, J. F. & Charlou, J. L. *Nature* **303,** 795–797 (1983).
37. DePaolo, D. J. & Wasserburg, G. J. *Geophys. Res. Lett.* **4,** 465–468 (1977).
38. O'Nions, R. K., Hamilton, P. J. & Evensen, N. M. *Earth planet. Sci. Lett.* **34,** 13–22 (1977).
39. Zindler, A., Hart, S. R., Frey, F. A. & Jakobsson, S. P. *Earth planet. Sci. Lett.* **45,** 249–262 (1979).
40. Dosso, L. & Murphy, V. R. *Earth planet. Sci. Lett.* **48,** 268–276 (1980).
41. O'Nions, R. K. & Pankhurst, R. J. *J. Petrology* **15,** 603–634 (1974).
42. Cohen, R. S., Evensen, N. M., Hamilton, P. J. & O'Nions, R. K. *Nature* **283,** 149–153 (1980).

Metabolic activity and bioluminescence of oceanic faecal pellets and sediment trap particles

Christine C. Andrews*, David M. Karl*, Lawrence F. Small† & Scott W. Fowler‡

* Department of Oceanography, University of Hawaii, 1000 Pope Road, Honolulu, Hawaii 96822, USA
† School of Oceanography, Oregon State University, Corvallis, Oregon 97331, USA
‡ International Laboratory of Marine Radioactivity, Musée Oceanographique, 98000 Monaco

Zooplankton faecal pellets are important in the vertical transport and exchange of elements in the oceanic ecosystem[1,2]. Although microbial, and in particular bacterial, processes have been implicated in the decomposition of aquatic faecal pellets[3–7], few quantitative data exist on the metabolic activities and growth characteristics of the associated microorganisms. As part of the Vertex (Vertical Transport and Exchange) programme, we studied these aspects using field-collected faecal pellets of known source and age. Our results, reported here, show that microbial biomass and metabolic activity are highest at the time of egestion, and decline with subsequent incubation. An independent observation from these experiments was the detection of bioluminescence in freshly excreted faecal pellets and in materials collected from sediment traps. These field data support an existing model for the ecological role of bacterial light emission in the mesopelagic zone of the ocean.

In the present study, freshly produced copepod faecal pellets were incubated in sterile filtered seawater in temperature-controlled conditions. Changes in ATP biomass, RNA and DNA synthesis rates (as measures of total microbial production and cell division) and bioluminescence were monitored with time. The data (Fig. 1) show that rates of RNA and DNA synthesis were highest at the time of egestion and decreased with subsequent ageing. Since nucleic acid synthesis rates are positively correlated with cellular biosynthesis[8–10], it can be concluded that both growth and cell division decreased with time. After 48 h, the RNA synthesis rate was 22% of the initial rate and the DNA synthesis rate had decreased to less than 3% of the original value. Two additional experiments, performed over 96 h, confirmed that microbial rate processes decreased with faecal pellet age. Total microbial biomass (as determined from faecal pellet ATP concentrations) was more variable among experiments. However, in no case was there evidence for an overall increase in the total microbial population during the incubation periods (up to 120 h). ATP values for fresh copepod faecal pellets in these experiments ranged from 70 to 1,156 ng per mg dry weight.

Although low temperatures have been related to decreases in metabolic activity and rates of faecal pellet degradation[5,11], there was no apparent response to temperature changes in our experiments. Pellets, aged at a constant temperature of 19° C, showed reductions in rates of RNA and DNA synthesis similar to those of faecal pellets aged as described in Fig. 1 legend.

The decreases we observed in the above experiments were unexpected as the present scenario for faecal pellet breakdown requires that the associated microbial populations exhibit rapid growth and division. Indeed, Honjo and Roman[4] observed that degradation of the surface membrane of laboratory-produced copepod pellets began within 3 h and was complete after 24 h. The implication that faecal pellets are colonized and degraded by external microbial populations is derived from time-course electron microscopic studies of pellet surfaces[3–5]. In our study, however, the high biomass, metabolic activity and luminescence (see below) associated with field-collected faecal pellets at the time of egestion indicated the presence and importance of gut-derived microflora. Furthermore, we were unable to detect any major differences between faecal pellets incubated in sterile filtered seawater and in unfiltered seawater. Recent transmission electron microscopic observations of thin sections from copepod faecal pellet interiors[7] also suggest that internal microbial communities have a major role in faecal pellet breakdown.

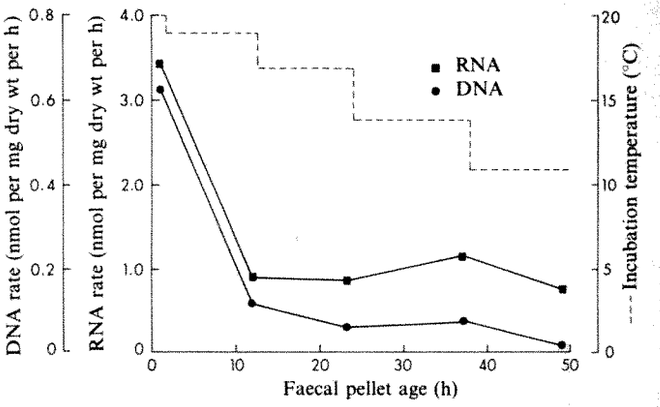


Fig. 1 Changes in rates of RNA and DNA synthesis as a function of faecal pellet age. Fresh faecal pellets from net-collected copepods were obtained by the method of La Rosa[29] as modified by Small *et al.*[13]. Known volumes of pellets, retained on a 68 μm Nitex net, were rinsed with distilled water, dried and weighed using a Cahn electrobalance. Faecal pellet material was also diluted 20-fold with 64 μm Nitex-filtered seawater and replicate 1-ml and 0.1-ml aliquots were incubated in small glass vials. The glass vials were moved through a temperature gradient with time (20–11 °C) to simulate the *in situ* conditions that sinking pellets would encounter. The rate at which the vials were rotated was determined by combining pellet sinking rate data[13] with the temperature regime of the water column. At selected times during the experiment, a 1-ml vial was incubated with 20 μCi ml^{-1} (final concentration) of [2-^{3}H]adenine (15.5 Ci $mmol^{-1}$; NEN) for 1 h, after which determinations of rates of RNA and DNA synthesis[30,31] and ATP[32] were made. Vials containing the 0.1-ml aliquots were filtered onto a combusted glass fibre filter and analysed for organic carbon, using a Perkin-Elmer CN analyser, throughout the incubation period. Faecal pellets were also aged in 0.22-μm Millipore-filtered seawater to assess contamination from planktonic bacteria. No differences in nucleic acid synthesis rates or ATP concentrations were observed between pellets incubated in 68 μm and in 0.22 μm-filtered seawater.

Oceanic zooplankton, grown in conditions of food limitation, are known to have higher assimilation efficiencies than coastal plankton or laboratory cultures maintained with high food concentrations[12]. Major differences between faecal pellets produced by the two groups have been noted[13]. Microbiological activity may similarly depend on the quality, compaction and composition of the egested pellets. Carbon analyses of our source-term pellets revealed that between 12 and 27% of the faecal pellet mass was organic carbon, which is in agreement with published data[4,5,14]. However, 50–100% of this organic carbon was living carbon as estimated from our ATP data (using an average C:ATP ratio of 250 (refs 15, 16)). It seems that reduced carbon sources may be insufficient to support the active growth and division of chemoheterotrophic microbial populations and that these organisms substantially reduce their metabolic activity soon after the pellets are voided. This is consistent with the previous observation that faecal pellets produced by euphausiids feeding on natural food sources can remain intact for weeks at temperatures as high as 18 °C (ref. 17).

In vivo bioluminescence, detected with a shipboard photometer, occurred in 70% of all samples surveyed including sediment trap-collected particulate matter, freshly collected zooplankton faecal pellets, crustacean molts, live animals (such as euphausiids, copepods, amphipods, etc.), and particulate detritus collected with plankton nets and water samplers. Long-term kinetics (s to min) revealed two distinct patterns of light emission, possibly reflecting various sources of luminescence. Erratic, luminous flashes predominated in the live animal and

0028-0836/84/060539—03$01.00
© 1984 Macmillan Journals Ltd

Table 1 Vertical distribution of bioluminescence and ATP from particle interceptor traps (VERTEX II)

Depth (m)	Total sediment trap bioluminescence (10^5 photons cm^{-2} s^{-1})	Total sediment-trap ATP (μg)	Specific bioluminescence (photons cm^{-2} s^{-1} per pg ATP)
30	17.2	1.30 (0.296)	1.32
120	3.39 (0.024)	2.09 (0.172)	0.167
200	2.96 (0.041)	2.52 (0.235)	0.117
400	1.21 (0.022)	1.04 (0.094)	0.116
700	1.30 (0.023)	0.742 (0.161)	0.175
800	1.11 (0.009)	0.833 (0.067)	0.133
900	1.36 (0.020)	0.083 (0.025)	1.64
1,400	1.33 (0.027)	0.068 (0.002)	19.6
1,900	1.52 (0.008)	0.008 (0.010)	18.6

Free-floating MULTITRAP design particle traps[26] were deployed approximately 100 km off the western coast of Mexico (17°8′ N, 109°0′ W) from 27 October to 17 November 1981. The light emission (integrated over 1 min) from a known volume of sediment-trap material, was recorded using an SAI model 2000 ATP photometer set at maximum sensitivity. Mean values ($n = 3$) scaled to total trap volume are reported (±1 s.d.). The phototube detector was calibrated using a standard light source (Optronic Laboratories, Inc. lamp 245 C) and a US National Bureau of Standards traceable photodiode (United Detector Technology silicon photodiode, PIN 10DSB). The spectrum of light from the lamp was regulated by a Melles Griot wide-band filter (03F1B003) with peak transmission at 440 nm and full-width half maximum of 85 nm. The specific bioluminescence measured from a luminescent bacterial culture, *Photobacterium leiognathi* strain 721 (ref. 27), at maximum light intensity ($A_{660} = 0.54$) was 2.22×10^3 photons per cm^2 per s per pg ATP. Total sediment-trap ATP biomass was measured according to the methods of Karl and Knauer[28].

net plankton samples, whereas sediment-trap particles and faecal pellets characteristically showed a continuous and steady light output. The former response has been reported for certain species of dinoflagellates, copepods, euphausiids and planktonic larvae. The duration of these individual flash events is generally of the order of tens (or hundreds) of milliseconds[18–20]. On the other hand, light emission from luminescent bacteria is continuous, even in single cells, and exhibits neither flashes nor oscillations[21]. Our observations suggest that enteric marine luminous bacteria are responsible for the light emission from faecal pellets, and possibly from materials collected in the sediment traps also. Recent data from a subsequent Vertex field experiment, near the same site, confirm this hypothesis. Luminescent bacteria have been isolated from sediment-trap materials and source-term faecal pellets and the *in situ* light emission has been shown experimentally to be of bacterial origin (C.C.A. and D.M.K., in preparation).

The bioluminescence of particulate matter collected in sediment traps was greatest at 30 m, diminished rapidly to less than one-third of this value by 120 m, and gradually decreased to a constant level with depth (Table 1). Interestingly, when light emission per unit biomass is examined (that is, 'biomass-specific' luminescence), the depth profile exhibits a distinct increase below 800 m (Table 1); this may have been a direct effect of the endogenous control of *in vivo* bioluminescence resulting from specific changes in environmental conditions such as oxygen or nutrient concentrations[22,23]. The increase may also have been caused by an overall shift in the species composition favouring the succession of luminous bacteria at depth. A more likely explanation, however, is a direct relationship to the production and downward vertical flux of particulate matter. The observed increase in biomass-specific luminescence below 800 m correlates with the zone of enhanced biological activity and new particle production found just beneath the O_2 minimum (0.1–0.25 ml O_2 per litre). At 800 m, microbial rate processes associated with sediment trap-collected particles were maximal (D.M.K., unpublished) and within the water column, there was a substantial increase in the standing stock of nauplii and in total plankton dry weight (G. Knauer and M. Tuel, personal communication). Furthermore, stimulated *in situ* bioluminescence measured in the water column at the same station also showed increasing (but sporadic) activity below 700 m (ref. 24). These supplementary observations suggest that freshly produced pellets, at 800 m, may account for the greater particle bioluminescence at 900 m and below. It is conceivable that *in situ* measurements of bioluminescence, especially those which are associated with sinking particles, may be used for detecting the input of fresh organic detritus at intermediate oceanic depths.

The fact that copepod faecal pellets, as well as other particulate materials, are luminescent suggests an important relationship between feeding and light in the trophic structure of the mesopelagic realm. It also provides field support for the hypothesis that the functional importance of luminescence in marine bacteria[22,25] is related to the dispersion and propagation of bacterial species. If faecal pellet microbial populations are generally inactive once the pellets are voided into the water, as suggested by this work, then bacterial decomposition would be insufficient for remineralization to occur during descent of the pellets. Slow decomposition combined with rapid settling rates would result in the loss of large quantities of carbon and energy-rich particles to the benthos. Direct feeding on these sinking bioluminescent particles by organisms from higher trophic levels may ensure against such loss and facilitate the propagation of bacterial species.

We thank G. Knauer and M. Tuel for providing their unpublished Vertex zooplankton data, S. Moore for faecal pellet carbon analyses, J. La Rosa for technical assistance, and the scientists and crew of the RV Wecoma (Vertex II) for their assistance in sample collection. D. O'Connor kindly provided the instrumentation and expertise for the calibration of our photometer. This research was supported by NSF grants OCE80-05180 (to D.M.K.) and OCE79-2321 (to L.F.S.). The International Laboratory of Marine Radioactivity is operated under a tripartite agreement between the International Atomic Energy Agency, the Government of Monaco and the Oceanographic Institute at Monaco.

Received 11 May; accepted 24 November 1983.

1. Turner, J. T. & Ferrante, J. G. *Bioscience* **29,** 670–677 (1979).
2. Urrere, M. A. & Knauer, G. A. *J. Plankton Res.* **3,** 369–387 (1981).
3. Ferrante, J. G. & Parker, J. I. *Limnol. Oceanogr.* **22,** 92–98 (1977).
4. Honjo, S. & Roman, M. R. *J. mar. Res.* **36,** 45–57 (1978).
5. Turner, J. T. *Trans. Am. microsc. Soc.* **98,** 131–135 (1979).
6. Azam, F., Cooper, N. & Jacobsen, T. *EOS, Trans. Am. geophys. Un.* **63,** 107 (1982).
7. Gowing, M. M. & Silver, M. W. *Mar. Biol.* **73,** 7–16 (1982).
8. Maaløe, O. & Kjeldgaard, N. O. *Control of Macromolecular Synthesis* (Benjamin, New York, 1966).
9. Pritchard, R. H., Barth, P. T. & Collins, J. in *19th Symp. Soc. gen. Microbiol.* 263–297 (Cambridge University Press, 1969)
10. Nierlich, D. P. *A. Rev. Microbiol.* **32,** 393–432 (1978).
11. Eppley, R. W. *Fish. Bull. (U.S.A.)* **70,** 1063–1085 (1972).
12. Gaudy, R. *Mar. Biol.* **25,** 125–141 (1974).
13. Small, L. F., Fowler, S. W. & Unlu, M. Y. *Mar. Biol.* **51,** 233–241 (1979).
14. Johannes, R. E. & Satomi, M. *Limnol. Oceanogr.* **11,** 191–197 (1966).
15. Holm-Hansen, O. & Booth, C. R. *Limnol. Oceanogr.* **11,** 510–519 (1966).
16. Karl, D. M. *Microbiol. Rev.* **44,** 739–796 (1980).
17. Small, L. F. & Fowler, S. W. *Mar. Biol.* **18,** 284–290 (1973).
18 Harvey, E. N. *Bioluminescence* (Academic, New York, 1952).
19. Eckert, R. in *Bioluminescence in Progress* (eds Johnson, F. H. & Haneda, Y.) 269–300 (Princeton University Press, Princeton, New Jersey, 1966).
20. LaPota, D. & Losee, J. R. *EOS, Trans. Am. geophys. Un.* **63,** 944 (1982).
21. Haas, E. *Biophys. J.* **31,** 301–312 (1980).
22. Hastings, J. W. & Nealson, K. H. *A. Rev. Microbiol.* **31,** 549–595 (1977).

23. Nealson, K. H. *Archs Microbiol.* **112,** 73–79 (1977).
24. Broenkow, W. W., Lewitus, A. J., Yarbrough, M. A. & Krenz, R. T. *Nature* **302,** 329–331 (1983).
25. Robison, B. H., Ruby, E. G. & Morin, J. G. *Abstr. A. Meet. West. Soc. Nat.* **55** (1977).
26. Knauer, G. A., Martin, J. H. & Bruland, K. W. *Deep-Sea Res.* **26,** 97–108 (1979).
27. Reichelt, J. L. & Baumann, P. *Int. J. syst. Bact.* **25,** 208–209 (1975).
28. Karl, D. M. & Knauer, G. A. *Deep-Sea Res.* (in the press).
29. La Rosa, J. *Deep-Sea Res.* **23,** 995–997 (1976).
30. Karl, D. M. *Appl. envir. Microbiol.* **42,** 802–810 (1981).
31. Karl, D. M., Winn, C. W. & Wong, D. C. L. *Mar. Biol.* **64,** 1–12 (1981).
32. Karl, D. M. & Craven, D. B. *Appl. envir. Microbiol.* **40,** 549–561 (1980).

Positional determination of supernumerary blast cell death in the leech embryo

M. Shankland

Department of Molecular Biology, University of California, Berkeley, California 94720, USA

Segmented animals are divided into a longitudinal array of developmentally homologous subunits known as metameres. The embryonic origin of the segmental body plan has been studied in a variety of organisms, with particular emphasis on the mechanisms underlying the delineation of the individual metameres[1–3] and their secondary diversification[4]. I have examined the embryonic events which determine the total number of segments in the glossiphoniid leech *Helobdella triserialis*. The germinal band of the leech consists of chains of segmental founder cells, called blast cells, and is normally reduced to 32 segments by the degeneration of supernumerary blast cells located at its caudal end[5,6]. By using a novel technique for selective cell ablation, the segmental register of the four ectodermal cell lines can be altered so that lineally identifiable blast cells take part in the formation of ectopic segments. I show here that the survival or death of ectodermal blast cells is determined by position independent of their cell lineage identities, implying that the final number of segments is imposed on the ectoderm by interactions with the other embryonic tissues.

The egg of the glossiphoniid leech develops directly into the adult form through a stereotyped sequence of cell divisions[7,8], with the metameric portion of the ectoderm and mesoderm deriving from five bilateral pairs of blastomeres known as the M, N, O, P and Q teloblasts (Fig. 1). Each teloblast undergoes several dozen asymmetric divisions to generate a germinal bandlet—a chain of much smaller primary blast cells which will be designated here by the lower case letter corresponding to their teloblast of origin (Fig. 2*a*). On either side of the embryo the five bandlets come together in parallel to form a unified germinal band, with the firstborn blast cells lying at the future rostral end. By injecting the various teloblasts with vital cell lineage tracers and following the fate of the labelled clones[9,10], it has been shown that each teloblast gives rise to a specific, hemilateral set of segmentally iterated tissues in the mature leech[8,11]. The present study focuses on the ectodermal bandlets o and p in which a single primary blast cell gives rise to one segmental complement of the tissues descended from its teloblast (D. A. Weisblat, in preparation).

The leech's segmental body plan can be envisioned as a two-dimensional array of blast cells in which one dimension—teloblast of origin—is related to the cellular composition of the descendent clone, while the second dimension—birth rank within the bandlet—dictates the rostrocaudal position, that is, the segmental fate of the clone. This strict correlation between a cell's genealogy and its fate suggests that individual blast cells follow distinct developmental pathways as a direct result of their cell lineage identities. The developmental importance of the teloblast of origin has been established by studies in which particular teloblasts were ablated by injecting them with toxic enzymes[12,13], although an instance of positional determination has also been found[14]. To discover whether birth rank is responsible for segment-specific differences in blast cell development, I devised an experimental procedure involving the selective ablation of small groups of blast cells which cause cells in the remainder of the bandlet to take part in the formation of segments for which they are not normally destined.

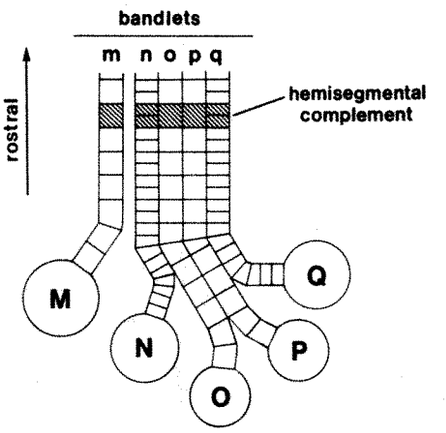


Fig. 1 Schematic diagram of germinal band formation in the leech embryo, taken from D. A. Weisblat (in preparation). The segmental tissues consist of five cell lines descended from the M, N, O, P and Q teloblasts. Each teloblast generates a bandlet of much smaller primary blast cells, and the five bandlets come together in parallel to form a unified germinal band. For the purposes of this illustration the mesodermal m bandlet has been displaced from its normal location beneath the four ectodermal bandlets. Each hemisegmental complement of the mature body wall and nervous system is founded by a homologous ensemble of seven primary blast cells.

Being too small for the reliable injection of toxic agents, blast cells were ablated by means of a photosensitizing lineage tracer inherited from their parental teloblast. This tracer, FDX, consists of fluorescein isothiocyanate covalently linked to a 10,000-molecular weight dextran carrier molecule which prevents passage across gap junctions. FDX is thus confined to the descendants of the injected teloblast and, in normal lighting conditions, serves as a vital cell lineage tracer[10]. However, FDX-labelled cells are dramatically sensitized to radiant energy within the fluorescein absorption spectrum[15], and can be selectively ablated under an epifluorescence microscope using a ×40 water immersion objective and a 50 W mercury arc with narrow band exciter filter (peak transmittance 485 nm). Labelled cells were safely viewed while orienting the embryos by shielding the light source to 1% maximal intensity. The iris was then closed so as to illuminate a circular spot of 15 μm diameter, encompassing two primary blast cells, and the beam raised to maximal intensity for 20–60 s. This exposure bleaches a substantial fraction of the FDX fluorescence (Fig. 2*b*), and causes rapid degeneration of the labelled cells. The residual, unbleached tracer dissipates within 2–10 h after illumination (Fig. 2*c*), suggesting that the dying cells have lost their membrane integrity. In contrast, blast cells labelled with tracers which do not absorb at this wavelength can be illuminated for 300 s and still give rise to their normal descendent clones.

Photoablation of a patch of contiguous blast cells sunders the labelled bandlet into rostral and caudal fragments. If an o or p bandlet is lesioned just past the point where it merges into the germinal band, subsequent observations with low levels of illumination reveal that the caudal or trailing fragment is temporarily retarded from entering the band with a consequent widening of the gap produced by the lesion (Fig. 3*a*). The retarded bandlet slips out of its normal segmental register as the neighbouring bandlets slide past, and the constituent blast cells come to occupy segments for which they are not normally destined.

To demonstrate that blast cells are slipping into ectopic segments, the caudal fragment of the lesioned bandlet was com-

0028/84/060541–03$01.00
© 1984 Macmillan Journals Ltd

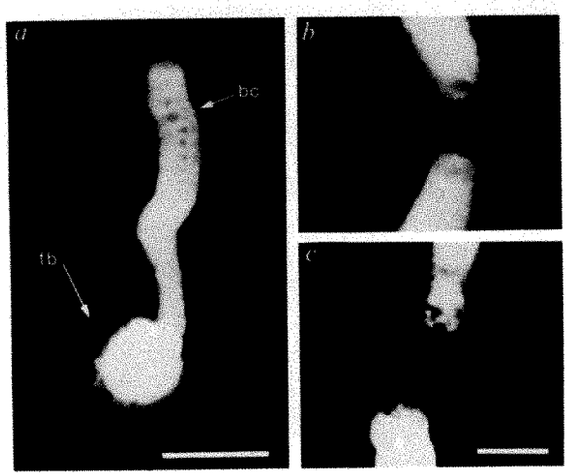


Fig. 2 Fluorescence micrographs of FDX-labelled germinal bandlets. *a*, The P teloblast (tb) has produced 20 labelled blast cell progeny (bc) 25 h after the injection of FDX. This tracer is excluded from the outer, yolky region of the teloblast cytoplasm, and from the blast cell nuclei. Scale bar, 50 μm. *b*, Localized photolesioning of an FDX-labelled bandlet after 60 s exposure of two primary blast cells to intense 485-nm illumination. The target cells can be recognized by the pronounced photobleaching of their FDX fluorescence, and have already begun to display an abnormal, rounded morphology within minutes of illumination. *c*, The dying blast cells, seen here in a different embryo 5 h after illumination, lose their residual, unbleached tracer, suggestive of a breakdown in membrane integrity. Scale bar, 10 μm.

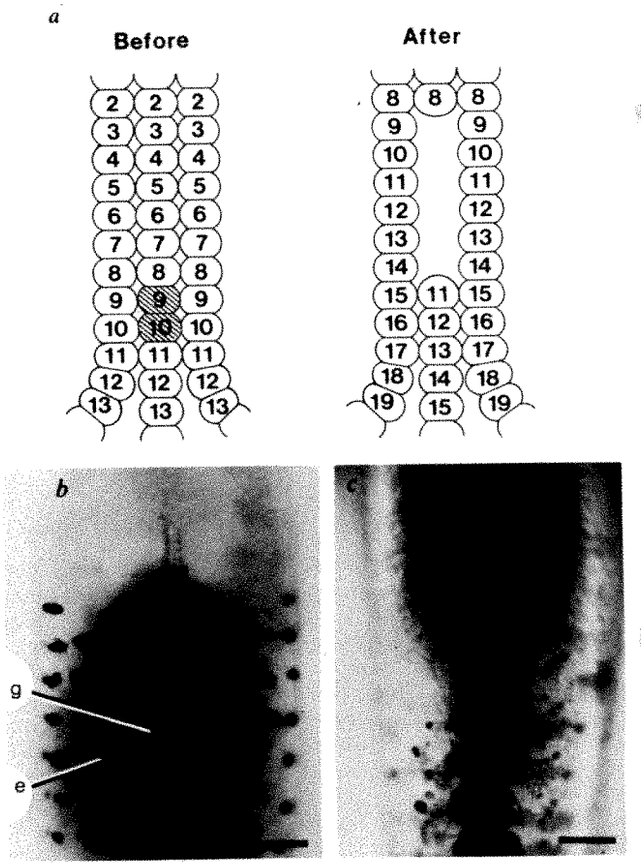


Fig. 3 Photolesioned ectodermal bandlets slip out of their normal segmental register. *a*, Schematic diagram showing the point where the germinal bandlets merge into the germinal band. Numbers represent blast cell birth ranks and thus correspond to their segments of normal destiny. If one of the bandlets is lesioned (cross-hatching) near its point of entry, the trailing portion of the bandlet is temporarily retarded and thereby slips posteriorly with respect to the other, unlesioned bandlets. *b*, Stage 10 embryo in which the right and left O teloblasts were simultaneously injected with HRP during stage 7, midway through blast cell production. Anterior is at the top. Because homologous teloblasts produce their blast cell progeny in virtual synchrony, there are an equal number of labelled segments on the two sides. Note the segmentally iterated pattern of labelled tissues, including patches of body wall epithelium (e) and clusters of neurones within the segmental ganglia (g). *c*, In this embryo the right and left O teloblasts were simultaneously injected with HRP after the o bandlet on the left had been photolesioned near its rostral end, that is, well in advance of the first HRP-labelled cells. The disparity in the anterior boundary of the HRP-labelled region on the two sides demonstrates that the lesioned bandlet has slipped four segments posteriorly. Despite the change in longitudinal register the slipped blast cells have given rise to the normal pattern of descendant tissues within their ectopic segments. Scale bar, 50 μm.

pared with its unlesioned contralateral homologue by means of secondary tracer injections. In a normal embryo the simultaneous injection of bilaterally homologous teloblasts with the lineage tracer horseradish peroxidase (HRP) gives rise to an equal number of labelled segments on the two sides of the mature leech (Fig. 3*b*). If the right and left O or P teloblasts are injected with HRP after having produced 10–25 primary blast cells, the anterior boundaries of the right and left labelled regions fall within the same segment in the majority of cases (82%, 28/34) and never lie more than one segment apart. In a series of 24 experimental embryos the O or P teloblasts were initially injected with FDX, then simultaneously reinjected with HRP after the formation of a dozen or so singly labelled blast cells. On the right side of the embryo the first 2–3 FDX-labelled blast cells were ablated as they entered the germinal band. When these embryos were examined later in development, it was found that there was a sequence of several midbody segments containing both FDX and HRP on the control side, but only FDX on the lesioned side. On the average the anterior boundary of the HRP-labelled region was located six segments (range 0–12) more posteriorly on the lesioned side (Fig. 3*c*), demonstrating that blast cells in the caudal fragment of the lesioned bandlet had failed to reach their normal segmental destinations.

If segmental differences arise from autonomous properties of the individual blast cells, then slipped blast cells should develop in accordance with their birth rank despite their ectopic location. For the present study attention was focused on the process of supernumerary blast cell death. Each teloblast gives rise to 5–15 more blast cells than are necessary to populate the 32 body segments[5,6], and the excess blast cells degenerate—as judged by the dissipation of intracellular lineage tracers—during the construction of the germinal band. This pattern of development could be determined solely on the basis of cell lineage if each teloblast first generates the appropriate number of viable blast cells, and thereafter only produces supernumerary blast cells which differ in a way that brings about their premature destruction. This hypothesis was tested by examining blast cell death at the caudal end of slipped o or p bandlets (Fig. 4*a*). In 17 cases it was found that a bandlet which had slipped 3–10 segments posteriorly began to degenerate at the same point (±1 primary blast cell clone) as the other unlesioned bandlets (Fig. 4*b*). Similar results were obtained following slippage of lesioned n bandlets, and the combined slippage of ipsilateral o and p bandlets. Assuming that the lesioned bandlet(s) slips uniformly—as suggested by the segmental disposition of its descendent tissues (Fig. 3*c*)—then those blast cells which would normally have inhabited the tail segments have degenerated like supernumeraries on slipping past the posterior end of the germinal band.

These results suggest that ectodermal blast cells will degenerate, regardless of their birth rank, if they fail to become incorporated into the first 32 segments. Due to the unidirectionality of slippage it is unknown whether blast cells whose birth rank

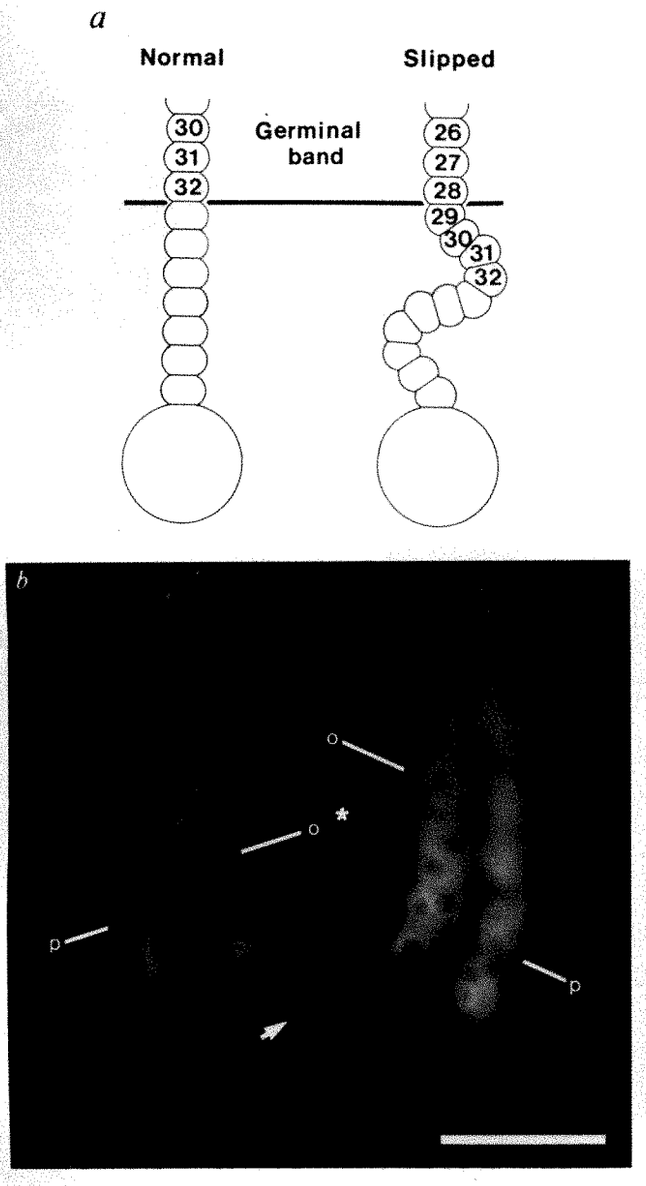


Fig. 4 Each teloblast produces several supernumerary blast cells which do not enter the germinal band and degenerate without giving rise to segmental tissue. *a*, If a lesioned bandlet slips posteriorly, those blast cells which would, on the basis of birth rank, normally inhabit the last of the 32 body segments will fail to enter the germinal band. *b*, Fluorescence micrograph of FDX-labelled o and p bandlets at the posterior end of the two germinal bands. In this stage 8 embryo the supernumerary blast cells have degenerated, leaving bits of fluorescent debris (arrow), and the incorporated blast cells have undergone secondary divisions to produce protosegmental clusters of daughter cells. The o bandlet on the left (labelled with an asterisk) was photolesioned near its rostral end and has slipped five segments posteriorly as measured by secondary tracer injection (not shown). This slipped bandlet terminates in register with the other, unlesioned bandlets, implying that position, not blast cell birth ranks, marks the point of transition between the surviving and dying blast cells. Scale bar, 50 μm.

normally renders them supernumerary would survive and proliferate if they entered the germinal band. Still it would seem that blast cell death can be determined solely by the cell's rostrocaudal position with respect to the germinal band as a whole. Thus, supernumerary blast cell death is probably brought about by interactions with the cellular environment, as has previously been shown for the 'programmed death' of certain lineally identified cells in the nematode[16,17]. Preliminary observations suggest that slipped ectodermal blast cells also exhibit host segment specificity in other aspects of their differentiation, for example the fusion of the seven most caudal neuromeres to form a compound tail ganglion.

It is not yet known how the supernumerary blast cells detect their position relative to the end of the germinal band. For the hirudinid leech embryo it has been proposed that the point of ectodermal bandlet termination is determined by the underlying mesoderm[6]. However, ectodermal blast cells can survive past the stage of supernumerary blast cell death in mesoderm-deficient germinal bands[12]. The role of the mesoderm was not examined in the present study because there was no apparent slippage following lesions of the m bandlet. Nonetheless, the present findings are consistent with the idea that the final length of the germinal band is fixed by some embryonic tissue or landmark other than the ectoderm, then subsequently imposed on the ectoderm by positionally determined death of the supernumerary blast cells.

I thank Gunther Stent, David Weisblat and David Bentley for critical reading of the manuscript. This work was supported by US PHS postdoctoral award 5 F32 NSO6814-02 and a grant from the Rowland Foundation.

Received 22 September; accepted 21 November 1983.

1. Lawrence, P. A. *J. Embryol. exp. Morph.* **30,** 681–699 (1973).
2. Hamilton, L. *J. Embryol. exp. Morph.* **22,** 253–264 (1969).
3. Bellairs, R. *J. Embryol. exp. Morph.* **51,** 227–243 (1979).
4. Struhl, G. *Nature* **293,** 36–41 (1981).
5. Zackson, S. L. *Cell* **31,** 761–770 (1982).
6. Fernandez, J. & Stent, G. S. *J. Embryol. exp. Morph.* **72,** 71–96 (1982).
7. Fernandez, J. *Devl Biol.* **76,** 245–262 (1980).
8. Stent, G. S., Weisblat, D. A., Blair, S. S. & Zackson, S. L. in *Neuronal Development* (ed. Spitzer, N. C.) 1–44 (Plenum, New York, 1982).
9. Weisblat, D. A., Sawyer, R. T. & Stent, G. S. *Science* **202,** 1295–1298 (1978).
10. Weisblat, D. A., Zackson, S. L., Blair, S. S. & Young, J. D. *Science* **209,** 1528–1530 (1980).
11. Weisblat, D. A., Harper, G., Stent, G. S. & Sawyer, R. T. *Devl Biol.* **76,** 58–78 (1980).
12. Blair, S. S. *Devl Biol.* **89,** 389–396 (1982).
13. Blair, S. S. *Devl Biol.* **95,** 65–72 (1983).
14. Weisblat, D. A. & Blair, S. S. *Devl Biol.* (in the press).
15. Spikes, J. D. in *Photophysiology* Vol. 3 (ed. Giese, A. C.) 33–64 (Academic, New York, 1968).
16. Sulston, J. E., Albertson, D. G. & Thomson, J. N. *Devl Biol.* **78,** 542–576 (1980).
17. Sulston, J. E. & White, J. G. *Devl Biol.* **78,** 577–597 (1980).

Inverse relationship between neurotensin receptors and neurotensin-like immunoreactivity in cat striatum

M. Goedert, P. W. Mantyh, P. C. Emson & S. P. Hunt

MRC Neurochemical Pharmacology Unit, Medical Research Council Centre, Medical School, Hills Road, Cambridge CB2 2QH, UK

The adult corpus striatum in mammals is divided into distinct histochemical compartments[1]. If the cat caudate nucleus is stained for acetylcholinesterase a number of macroscopically visible zones appear that have lower acetylcholinesterase activity than the surrounding tissue[2]. These patches, called 'striosomes'[1], correspond to regions of high [Met]-enkephalin-like immunoreactivity[3] and dense opiate receptor binding[4] and are related to the uneven distribution of striatal efferent neurones and cortical afferent terminations[5,6]. One of the highest concentrations of neurotensin-like immunoreactivity is in the striatum and the immunoreactive material co-elutes with synthetic neurotensin on gel chromatography[7]. Recently, we have found that neurotensin-like immunoreactivity in the cat caudate nucleus coincides with the striosomes[8]. We have now localized neurotensin receptors in the cat caudate nucleus by autoradiography[9] and found low density in the neurotensin-rich striosomes and a high density in the neurotensin-poor surrounding tissue.

Adult male cats received a lethal injection of pentobarbitone; their brains were removed and immersed in ice-cold saline for 10 min. Blocks containing the corpus striatum were frozen and sectioned in the transverse plane at 20 μm using a cryostat. Adjacent sections were incubated with either 2 nM ^{3}H-

0028-0836/84/060543-04$01.00

© 1984 Macmillan Journals Ltd

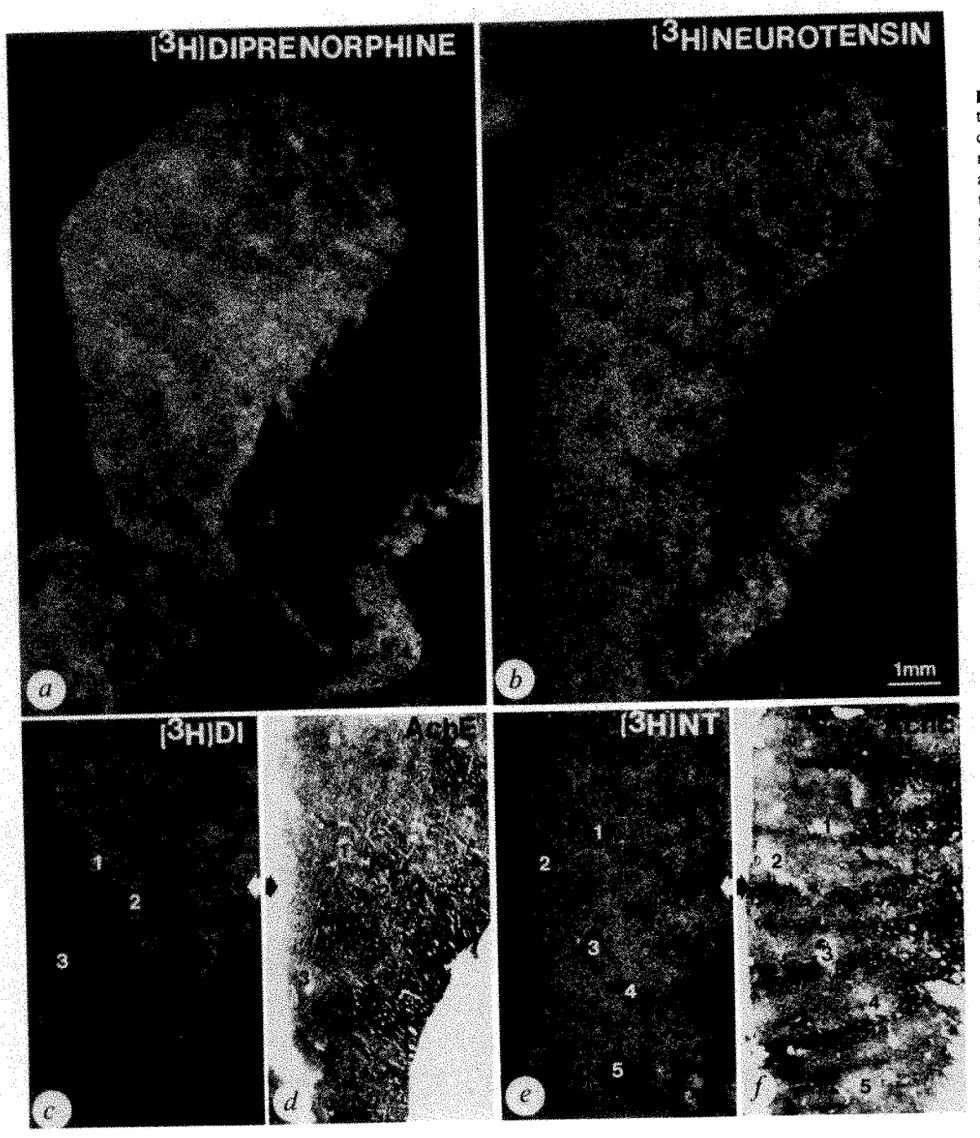


Fig. 1 Autoradiography of ^{3}H-neu tensin and ^{3}H-diprenorphine in cat caudate nucleus. *a*, *b*, The caud nucleus labelled with ^{3}H-diprenorph and ^{3}H-neurotensin respectively; *c*, *d*, s tion labelled with ^{3}H-diprenorphine stained for acetylcholinesterase; *e*, *f*, s tion labelled with ^{3}H-neurotensin stained for acetylcholinesterase. numbers in *c*–*f* refer to the ace cholinesterase-poor striosomes. Note correspondence between striosomes dense ^{3}H-diprenorphine labelling and inverse relationship between striosor and ^{3}H-neurotensin binding sites.
Methods: Sections (20 μm) containing cat striatum were pressed onto gela coated slides, melted in place, dried ov night at 4 °C and stored at −20 °C 24 h. Control experiments had establis that there was no detectable neuroten or [Met]enkephalin-like sections. thawed sections were incubated for 10 at 25 °C in 2 nM ^{3}H-neurotensin (I England Nuclear) in 50 mM Tris-H *p*H 7.40, containing 0.1% bovine se albumin, 40 mg l^{-1} bacitracin and 1 EDTA. Adjacent sections were incub for 40 min at 25 °C in 4 nM ^{3}H-dipre phine (Amersham), as described viously[12]. Non-specific binding was mated in parallel incubations in the p ence of 1 μM neurotensin (Cambr Research Biochemicals) or 1 naloxone (Endo). Following the inc tion the sections were washed twic ice-cold buffer (2 min each time), di into ice-cold water and dried und stream of cold air. They were arranged in X-ray cassettes, covered i dark-room with a tritium-sensitive (^{3}H-Ultrofilm, LKB) and kept at −2 for 6 weeks. Film was developed u safe-light conditions using Kodak D developer. Tissue sections were staine acetylcholinesterase[10].

neurotensin or 4 nM ^{3}H-diprenorphine. Nonspecific binding was defined as binding in the presence of 1 μM neurotensin or 1 μM naloxone. The sections were apposed to a tritium-sensitive film and kept at −20 °C for 6 weeks. The film was then developed and the tissue sections stained for acetylcholinesterase[10]. Test-tube binding of ^{3}H-neurotensin was performed using washed membranes from the cat caudate nucleus.

When sections of the striatum were incubated with ^{3}H-neurotensin an irregular distribution of silver grains was noted: whereas most of the caudate nucleus was labelled, local patches of sparse labelling occurred (Fig. 1*b*). No labelling was observed in the presence of 1 μM neurotensin. When the same sections were stained for acetylcholinesterase the regions rich in neurotensin binding sites (Fig. 1*e*) corresponded to acetylcholinesterase-rich regions (Fig. 1*f*). The neurotensin binding sites were further characterized using a test-tube binding assay for ^{3}H-neurotensin performed on washed membranes of the cat caudate nucleus. The binding was saturable (Fig. 2*a*) and Scatchard analysis indicated a single population of binding sites with a dissociation constant (K_D) of 1.54 nM and a maximum number of binding sites (B_{max}) of 68 fmol per mg protein (Fig. 2*b*). The competitive inhibition of ^{3}H-neurotensin binding by neurotensin appeared to be monophasic with a Hill coefficient not significantly different from 1 (Fig. 2*c*). The relative potencies of various neurotensin fragments in displacing ^{3}H-neurotensin were consistent with the potency obtained for these fragments in biological assays[11] (Tal indicating that the binding properties of ^{3}H-neurotensi consistent with the binding to a physiological neurotensin tor. When sections of the cat striatum were incubate ^{3}H-diprenorphine which labels all opiate receptor subty the concentration used[12], local patches of dense labelling e ded in a less dense background were observed (Fig. 1*a* labelling pattern was completely abolished in the prese 1 μM naloxone. When stained for acetylcholinestera regions rich in ^{3}H-diprenorphine receptors (Fig. 1*c*) co with the acetylcholinesterase-poor striosomes (Fig. 1*a* results obtained by histochemistry and receptor autora phy were further evaluated using microdensitometry (Ta The neurotensin- and [Met]-enkephalin-like immun tivities were six times more concentrated in the striosom in the surrounding tissue, whereas the neurotensin re were higher by 25% in the tissue around the strioson the opiate receptors 20% more numerous within the stric The acetylcholinesterase staining was 30% higher in th striosomal tissue.

The present findings indicate that neurotensin recept unevenly distributed throughout the caudate nucleus adult cat with areas of dense labelling corresp to acetylcholinesterase-rich regions. This contrast

Table 1 Inhibition of specific ^{3}H-neurotensin binding to cat caudate nucleus membranes by neurotensin fragments

Peptide	IC_{50}(nM)	Relative potency %
NT_{1-13}	2.80	100
NT_{8-13}	2.60	107
NT_{9-13}	265	1.1
NT_{10-13}	>10,000	<0.04
NT_{1-6}	>10,000	<0.04
NT_{1-8}	>10,000	<0.04
NT_{1-11}	>10,000	<0.04

The binding of tritiated neurotensin (NT) was assayed at a concentration of 2 nM. IC_{50} was defined as the concentration of unlabelled peptide required to inhibit the specific binding of ^{3}H-neurotensin by 50% and it was derived from the Hill transformation of the displacement curves. Similar IC_{50} values were obtained in three separate experiments. The relative potencies were calculated by comparing the IC_{50} values with that of NT_{1-13}. NT_{9-13} and NT_{10-13} were synthesized by Dr B. Williams (MRC Neurochemical Pharmacology Unit), NT_{8-13} was purchased from Peninsula Laboratories and all other peptides were obtained from Cambridge Research Biochemicals.

Table 2 Relative distribution of acetylcholinesterase activity, [Met]-enkephalin-like immunoreactivity, neurotensin-like immunoreactivity, opiate receptors and neurotensin receptors in striosomes and background matrix of the cat caudate nucleus

	Striosomes	Background matrix
Acetylcholinesterase	100 ± 3.10%	129.55 ± 4.38%*
[Met]-enkephalin-like immunoreactivity	100 ± 6.18%	14.00 ± 3.77%*
^{3}H-diprenorphine binding	100 ± 4.77%	81.41 ± 5.01%†
Neurotensin-like immunoreactivity	100 ± 11.52%	16.12 ± 7.05%*
^{3}H-neurotensin binding	100 ± 0.85%	125.75 ± 1.81%*

Striosomes and background matrix of the dorsal anterior part of the cat caudate nucleus (where the striosomal organization is the most marked) stained for acetylcholinesterase, [Met]-enkephalin- or neurotensin-like immunoreactivity[8] and incubated with ^{3}H-diprenorphine or ^{3}H-neurotensin were quantitatively assessed by using microdensitometry, as described previously[21]. The intensity in staining in the striosomal region was arbitrarily defined as 100% and the staining in the matrix expressed relative to it. Background staining for the immunohistochemistry and receptor binding was obtained from adjacent absorption control sections and subtracted for both striosomes and background matrix. For acetylcholinesterase background was taken to be the staining present in the internal capsule. The results represent the mean ±s.e.m. ($n = 5$).

* $P < 0.001$; † $P < 0.05$.

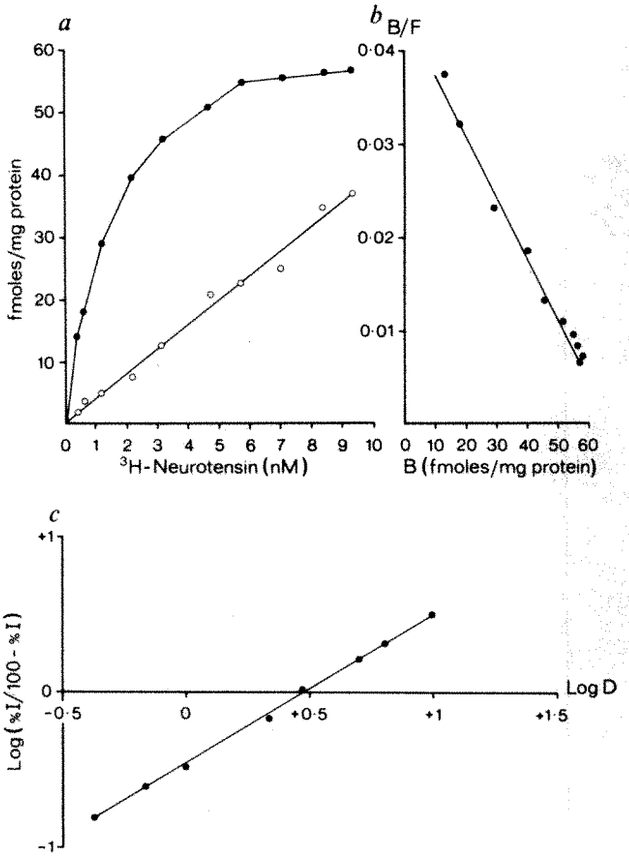


Fig. 2 ^{3}H-neurotensin binding to a membrane preparation of the cat caudate nucleus. *a*, ^{3}H-neurotensin binding as a function of the radiolabelled peptide concentration. Values were obtained in a single experiment. Similar results were obtained in four separate experiments. *b*, Scatchard plot of specific ^{3}H-neurotensin binding data. *c*, Hill transformation of ^{3}H-neurotensin binding by increasing concentrations of unlabelled neurotensin. A typical experiment is shown; similar results were obtained in four separate experiments.

Methods: The caudate nucleus from adult cats was homogenized in 10 volumes (w/v) of ice-cold 50 mM Tris-HCl, *p*H 7.40, with a Polytron at setting 7 for 15 s. After 20-min centrifugation at 50,000*g* the pellet was resuspended in 10 volumes of 50 mM Tris-HCl, incubated at 37 °C for 30 min and centrifuged again. The washed pellet was resuspended in 50 mM Tris-HCl, *p*H 7.40, containing 0.1% bovine serum albumin, 40 mg l^{-1} bacitracin and 1 mM EDTA to yield a concentration of 10 mg tissue per ml buffer. One ml of homogenate was incubated in the presence of ^{3}H-neurotensin (New England Nuclear) at 25 °C for 10 min. Non-specific binding (○) was defined as binding in the presence of 1 μM neurotensin and specific binding (●) was obtained by subtracting the nonspecific from the total binding. At the end of the incubation period the tubes were transferred onto ice and filtered immediately under reduced pressure through GF/B glass fibre filters (Whatman) pretreated with 0.2% polyethyleneimine (Sigma) in water. Each of the filters was washed four times with 5 ml ice-cold buffer and radioactivity determined by liquid scintillation spectrometry. A detailed characterization of the binding assay will be published elsewhere[22]. Proteins were determined according to Lowry *et al.*[23] using bovine serum albumin as the standard.

neurotensin-like immunoreactivity which is distributed like the previously described striosomes[8] defined by a low acetylcholinesterase activity and a high content of enkephalin-like immunoreactivity[1]. In the rat[4], opiate receptors coincide with [Met]-enkephalin-like immunoreactivity in the caudate nucleus. Lesion studies have indicated that rat opiate receptors are located on both intrinsic striatal neurones and dopamine-containing terminals, the receptor patches being mainly located in striatal neurones[13,14]. Similarly, we have found that 60% of rat striatal neurotensin receptors are localized on neurones intrinsic to the striatum[15].

The reason for the inverse relationship between neurotensin receptors and neurotensin-like immunoreactivity is unknown at present. Possibly, it could arise from different rates of turnover of neurotensin-like immunoreactivity in striosomes as compared to acetylcholinesterase-rich regions. Such regional differences have been observed for the turnover of dopamine in the rat striatum[16]. Discrepancies between the localization of either classical neurotransmitters or neuropeptides and their respective receptors have been noticed before. For instance, there exists a mismatch between noradrenaline levels and beta-adrenergic receptor numbers in the rat striatum[17,18] and between the content of substance P-like immunoreactivity and the number of substance P receptors in the rat substantia nigra[19,20]. Our present finding that neurotensin-like immunoreactivity and neurotensin receptors have different topographical localizations within an anatomical structure characterized by distinct histochemical compartments may have general implications for the function of basal ganglia.

We thank Dr B. Williams for two of the neurotensin fragments and Chloë Brown and Dr I. L. Martin for many helpful discussions. M.G. is supported by the British Council and P.W.M. is a NIH fellow.

Received 29 June; accepted 11 November 1983.

1. Graybiel, A. M. in *Cytochemical Methods in Neuroanatomy* (eds Chan-Palay, V. & Palay, S. L.) 45–67 (Raven Press, New York, 1982).
2. Graybiel, A. M. & Ragsdale, C. W. *Proc. natn. Acad. Sci. U.S.A.* **75,** 5723–5726 (1978).
3. Graybiel, A. M., Ragsdale, C. W., Yoneoka, E. S. & Elde, R. P. *Neuroscience* **6,** 377–397 (1981).
4. Herkenham, M. & Pert, C. B. *Nature* **291,** 415–418 (1981).
5. Graybiel, A. M., Ragsdale, C. W. & Moon Edley, S. *Exp Brain Res.* **34,** 189–195 (1979).
6. Ragsdale, C. W. & Graybiel, A. M. *Brain Res.* **208,** 259–266 (1981).
7. Goedert, M. & Emson, P. C. *Brain Res.* **272,** 291–297 (1983).
8. Goedert, M., Mantyh, P. W., Hunt, S. P. & Emson, P. C. *Brain Res.* **274,** 176–179 (1983).
9. Young, W. S. & Kuhar, M. J. *Brain Res.* **179,** 255–270 (1979).
10. Lewis, P. R. *Bibl. anat.* **2,** 11–20 (1961).
11. Kitabgi, P. *Ann. N.Y. Acad. Sci.* **400,** 37–55 (1982).
12. Wamsley, J. K., Zarbin, M. A., Young, W. S. & Kuhar, M. J. *Neuroscience* **7,** 595–613 (1982).
13. Pollard, H., Llorens, C., Schwartz, J. C., Gros, C. & Dray, F. *Brain Res.* **151,** 392–398 (1978).
14. Murrin, L. C., Coyle, J. T. & Kuhar, M. J. *Life Sci.* **27,** 1175–1183 (1980).
15. Goedert, M., Pittaway, K. & Emson, P. C. *Brain Res.* (in the press).
16. Olson, L., Seiger, Å. & Fuxe, K. *Brain Res.* **44,** 283–288 (1972).
17. Alexander, R. W., Davis, J. N. & Lefkowitz, R. J. *Nature* **258,** 437–440 (1975).
18. Palacios, J. M. & Kuhar, M. J. *Science* **208,** 1378–1380 (1980).
19. Quirion, R. *et al. Nature* **303,** 714–716 (1983).
20. Mantyh, P. W., Pinnock, R. D., Downes, C. P., Goedert, M. & Hunt, S. P. *Soc. Neurosci. Abstr.* **13,** 1206 (1983).
21. Hunt, S. P. & Mantyh, P. W. *Brain Res.* (in the press).
22. Goedert, M., Pittaway, K., Williams, B. & Emson, P. C. *Brain Res.* (in the press).
23. Lowry, D. H. Rosebrough, N. H., Farr, A. L. & Randall, R. J. *J. biol.* **193,** 265–275 (1951).

Suppression of sprouting at the neuromuscular junction by immune sera

M. E. Gurney

Department of Pharmacological and Physiological Sciences, University of Chicago, 947 East 58th Street, Chicago, Illinois 60637, USA

Injury of afferent motor axons or pathological loss of motoneurones from the spinal cord causes the remaining axons within a muscle to sprout and to reinnervate the denervated muscle fibres. Sprouting occurs at two sites along intramuscular axons, at nodes of Ranvier (nodal sprouting) and at the neuromuscular junction (terminal sprouting)[1,2]. Terminal sprouting is also produced by treatment with botulinum toxin and by other agents that render muscle inactive[3–7]. The muscle probably provides a signal for terminal sprouting as restoration of muscle activity by direct electrical stimulation prevents sprouting[8–10]. Such a signal might be a local change on the muscle fibre surface or a 'soluble' sprouting factor[2,4], although the failure to induce terminal sprouting in one muscle by denervating adjacent muscles argues against the latter hypothesis[11–13]. I now report that rabbit antisera against a 56,000 (56K)-molecular weight protein secreted by denervated rat muscle suppress botulinum toxin-induced terminal sprouting in the mouse gluteus muscle. An immune response against this protein has also been detected in serum of patients with amyotrophic lateral sclerosis (ALS), a disease in which loss of motoneurones from the spinal cord is not accompanied by the degree of sprouting and reinnervation seen in other motoneurone diseases[14].

In 1977, Lømo, Brockes and Raff produced a rabbit antiserum against dialysed medium conditioned by an organ-cultured rat hemidiaphragm that was freshly denervated by being placed in culture. An IgG fraction from the antiserum thus obtained was used to construct an immunoaffinity column over which was passed medium conditioned (DdiaCM) by a hemidiaphragm that had been denervated 4 days *in vivo* before being placed in culture. The material that passed through the column was used to produce a second antiserum. In 1981, preliminary results obtained by Kemplay and Stolkin suggested that the second antiserum suppressed partial denervation-induced terminal sprouting in the rat sternocostalis muscle by about one-half (personal communication). I obtained a similar effect using the rat fourth deep lumbrical muscle (denervation protocol as in ref. 5). None of the first antiserum remained for testing.

To produce a second blocking antiserum, I fractionated the DdiaCM protein by gel filtration and took two size fractions for immunization (see Fig. 2 legend). Material eluting in the void volume of the column was used to immunize two rabbits (D5 and D6), and material eluting at ~60–40K was used to immunize a second pair (D7 and D9). These molecular weight ranges were chosen on the basis of preliminary immunoblot analysis of the antigens in the DdiaCM reacting with the blocking antiserum produced by Lømo, Brockes and Raff. A fifth rabbit (D8) was immunized with the unfractionated DdiaCM. The five antisera were tested for suppression of botulinum toxin-induced terminal sprouting in the mouse gluteus muscle (see Fig. 1 legend). Only antisera from D7 and D9 significantly suppressed sprouting (by 60% and 40%, respectively; see Table 1). D7 antiserum also suppressed terminal sprouting in C5-deficient, DBA/2NCox mice (Laboratory Supply Co.), but failed to suppress sprouting if delivered intraperitoneally (i.p.) rather than subcutaneously (s.c.) over the surface of the gluteus. Immunoblot analysis of the antigens in the DdiaCM reacting with the various antisera revealed that an antigen of molecular weight ~56K reacted with the D7 and D9 antisera, but not with any of the non-blocking antisera (Fig. 2). The antigen was a minor component of the DdiaCM which was not visualized in Coomassie blue-stained gels. To purify this antigen for immunization, 1 mg of

Table 1 Effects of antisera on botulinum toxin-induced terminal sprouting in the mouse gluteus

Serum	Toxin	SP%	NSP%	P (SP%)	P (NSP%)
D6pre	0712	55 ± 5	101 ± 9	NS	NS
D10pre	0911	42 ± 9	84 ± 18	NS	NS
D8	0712	43 ± 5	79 ± 9	NS	0.05
D5	0630	60 ± 6	105 ± 10	NS	NS
D6	0630	56 ± 7	98 ± 13	NS	NS
D7	0401	17 ± 3	36 ± 6	0.0001	0.0001
D9	0712	33 ± 4	61 ± 8	0.005	0.0005
D10	0910	32 ± 4	68 ± 8	0.005	0.005
D11	0823	28 ± 3	69 ± 7	0.0005	0.005

The data base consisted of the nine groups of eight mice each listed (D6pre and D10pre were pre-immune sera), six groups of mice that received sera from neurologically normal human subjects, six groups that received sera from patients with diabetic peripheral neuropathy and 19 groups that received sera from sporadic ALS patients. The data for the human sera tested are reported in detail elsewhere[20]. One-way analysis of variance (Statistics Package for the Social Sciences[28]) was used to establish that within each control treatment the distribution of SP% (% of axon terminals with sprouts) for the mice within individual groups did not differ (toxin alone, pre-immune rabbit, normal human, diabetic), and that the distribution of mean SP% for the groups within the different control treatments did not differ across the treatments (d.f. 3, 14; $F = 1.189$; $P = 0.35$). Thus, the controls were homogeneous and the data could be pooled. The distribution of SP% for the pooled data (138 muscles) was normal (χ^2 goodness of fit test) with mean 47.9%, σ 16.1%. To assess which sera significantly suppressed sprouting, the z-score for each experimental group was tested against the parameters of the normal distribution for SP% of the pooled controls. During the study, six dilutions of the 10 $\mu g\,ml^{-1}$ toxin stock were used (listed by date in the second column). The SP% averaged among control groups that received each dilution did not differ significantly (F-test), but ranged from 41 to 57%. To remove this source of variation, the mean SP% for each group was normalized against the average SP% for the control groups receiving the same dilution of toxin (NSP%). The parameters of the distribution of NSP% for the pooled controls were mean 100%, σ 33%. The probabilities of the calculated z-scores for SP% and NSP% are given. Values shown are ±s.e. NS, not significant.

0028–0836/84/060546—03$01.00

© 1984 M

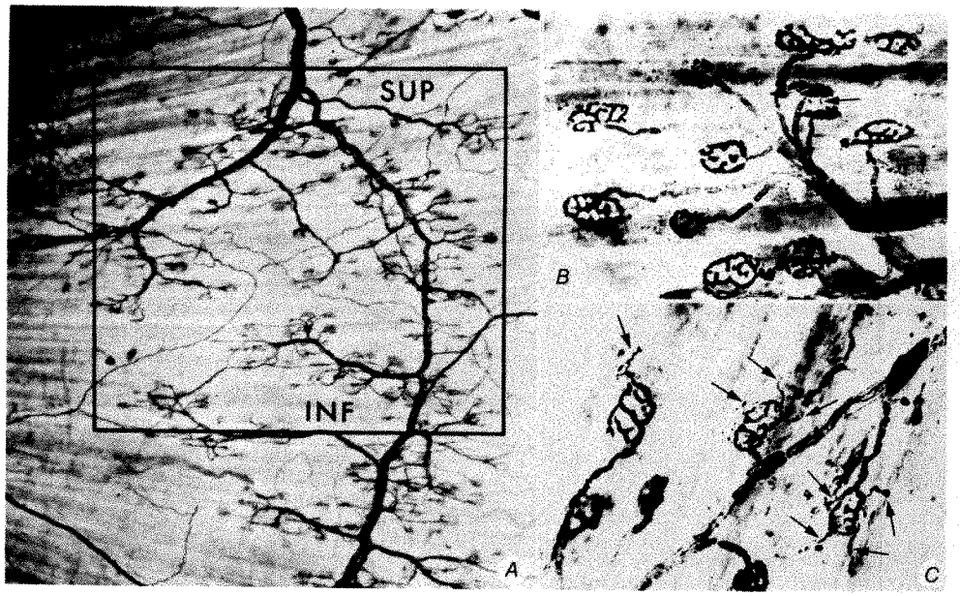


Fig. 1 *A*, Whole mount of the mouse gluteus muscle stained by a combined silver–cholinesterase technique[23] to indicate details of its innervation. The gluteus was chosen for the sprouting assay as both toxin and experimental antisera could be injected directly over the surface of the relatively thin sheet of muscle fibres. Also, the muscle could be viewed in whole mount, which left the terminals undisturbed by the dissection (magnification ~×35). *B*, Zinc iodide–osmium tetroxide-(Z10)-stained nerve terminals in a botulinum-treated muscle exposed to an antiserum (D7) that suppressed terminal sprouting. *C*, Same as *B* but exposed to an antiserum (D8) having no effect. Fine processes are seen sprouting from some of the axon terminals (arrows in *B* and *C*; magnification ~×190). Except for the presence of fewer sprouting terminals in *B* than in *C*, no other histological differences were apparent in the Z10-stained material. The superior (SUP) and inferior (INF) gluteal nerves can be seen to meet across the midsection of the muscle in *A*. In each muscle examined, the SP% (see Table 1) was determined in the zone of innervation of the superior gluteal nerve within the region delimited by the box. An average of 100 terminals was scored for sprouting in each muscle.

Methods: To induce terminal sprouting, female ICR mice (Spraque–Dawley, 20–24 g) were injected s.c. over the surface of the right gluteus muscle with ~250 pg of botulinum toxin type A (Sigma) in 5 μl of 70 mM NaH_2PO_4, 0.2% gelatin buffer (*p*H 6.5). The toxin was diluted from a 10 μg ml^{-1} stock and titred by injecting five groups of eight mice each with 62.5, 125, 250, 500 and 800 pg of toxin, respectively. The index of terminal sprouting (SP%) increased linearly over the 62.5–250 pg doses (from 25 ± 4% to 49 ± 4%). The 500 and 800 pg doses produced 37% and 75% lethality, respectively, while the 250 pg dose chosen for the study produced only 4% lethality overall (512 mice injected). Visible signs of paralysis were flacidity of the ipsilateral abdominal wall and diminution of the toe spreading reflex in the injected leg. On each of the 6 days after toxin injection, groups of eight mice were injected s.c. over the gluteus with 0.1 ml of a heat-inactivated (60 °C, 30 min) test serum. Groups of mice injected with toxin alone, or that received the series of injections with serum, were killed 7 days after toxin injection. The gluteus was excised and stained with Z10[24] for 3 h in the dark at 23 °C. Muscles were cleared in xylene and whole-mounted in Permount. Before microscopic examination (×250 magnification with a Leitz Pl Apo 25/0.65 objective), slides were randomly coded in batches of 40–80 (from 5–10 experimental groups). Data for six muscles that stained too darkly and six that stained too lightly were eliminated from the study before decoding (out of 488 muscles examined); no other selection of the data was made.

protein from the 60–40K AcA-44 fraction was separated on a 7.5% SDS-polyacrylamide gel (see Fig. 2 legend). After brief staining with Coomassie blue to visualize the major proteins in the fraction, the region of ~60–54K was excised from the gel, homogenized in saline, then used to immunize another pair of rabbits (D10 and D11). Antigenic material was recovered from the gel as both rabbits developed antisera that suppressed terminal sprouting (Table 1). Both D10 and D11 antisera reacted with the 56K antigen on immunoblotting, although neither was monospecific (Fig. 2). D11 reacted with some poorly resolved material of 66K and 60K which also reacted with D9, but not with D7 or D10. D10 reacted with some material of ~63K which also reacted with D7, but not with D9 or D11. Thus, the 56K antigen still seeemed the most likely candidate for the antigen of interest. The other material, but not the 56K antigen, was also detected in immunoblots of rat serum reacted with D10 or D11.

Clinical studies have been unable to determine whether the decreased reinnervation seen in ALS is an active process of the disease, or simply due to motoneurone death outpacing the rate of reinnervation[14–19]. Evidence that ALS sera suppressed terminal sprouting would favour the first hypothesis. To test this, serum samples were obtained from 19 sporadic ALS patients with both upper and lower motoneurone signs and tested for suppression of sprouting in the mouse gluteus assay. Sprouting was significantly suppressed by 8/19 ALS sera (NSP%, $P < 0.05$; see Table 1 legend), but not by six sera from healthy individuals[20]. The three most effective sera (N.L., R.V. and E.Z.; NSP%: 9 ± 2% (±s.e.), 47 ± 10%, 71 ± 10%, respectively) were reacted with immunoblots of the rat DdiaCM. Each reacted with the rat 56K antigen and little else in the DdiaCM, while none of the six normal human sera reacted with the antigen (data for N.L. and R.V. are shown in Fig. 2). These data provide evidence for active suppression of sprouting in some cases of ALS. The detection of antibody against the rat 56K antigen in several blocking ALS sera further implies that the disease may have an autoimmune component directed against a human equivalent of the rat antigen, and provides independently derived evidence corroborating the results obtained with the experimental antisera.

Due to the polyclonal nature of the sera used in the study, identification of the 56K antigen as the effective antigen for eliciting a blocking antiserum has not been unequivocally established—that will require the use of a monoclonal reagent, which is now being produced. Nor can the biological role of the 56K antigen be defined with the data in hand. However, the molecular weight of the antigen on gel filtration (60–40K) and on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) is similar to that reported for activities in conditioned medium that stimulate neurite outgrowth from spinal cord explants[21] and dissociated spinal cord neurones[22]. Preliminary experiments have shown that the 56K antigen co-purifies 100–200-fold with an activity(s) that promotes sprouting of neurites and enhances survival of dissociated spinal cord cells in culture, but elucidation of the antigen's biological activity awaits its final purification.

I acknowledge the support of the MRC Neuroimmunology Project (University College, London) where preliminary studies were completed; and thank M. Raff, J. Brockes and T. Lømo for their gift of the initial antiserum used in the study; S. Kemplay and C. Stolkin for sharing their preliminary results; M. Brown, W. Hopkins, S. Bevan, M. Duxson, J. Winter, D. Lawson and G. Rougon for advice; A. Belton for her assistance; J. Antel and N. Cashman for providing the ALS sera; and P. Grobstein, D. Van Essen and K. Drickamer for reading the manuscript.

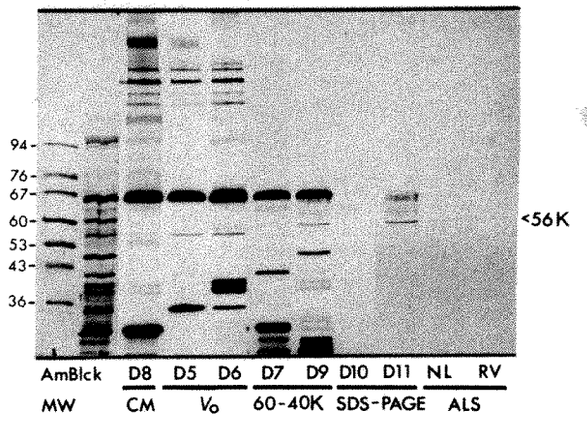


Fig. 2 Immunoblot analysis of the sera described in the text. Molecular weight markers were loaded in the lane marked MW and the other lanes were loaded with 35 μg of protein obtained from medium conditioned by a denervated rat hemidiaphragm (DdiaCM). The first two lanes of the filter were stained for protein with amido-black, the rest were allowed to react individually with one of the sera described in the text. Bound γ-globulin was detected with ^{125}I-labelled *Staphylococcus* protein A and the autoradiogram of the filter is shown. The antisera produced in rabbits D8, D5 and D6 failed to suppress terminal sprouting, while those from D7, D9, D10 and D11 all exhibited suppression. Sera from two ALS patients (N.L. and R.V.) also suppressed terminal sprouting. All sera that suppressed terminal sprouting reacted with an antigen of ~56K (56.6 ± 0.5K, ±s.d. for three determinations) which is believed to be the effective antigen in the immunizations.

Methods: The left hemidiaphragms of male Sprague–Dawley rats (150–200 g) were denervated by cutting the phrenic nerve under ether anaesthesia, and cultures were prepared as described by Merlie *et al.*[25], except that no serum was added to the medium. The medium consisted of Dulbecco's modified Eagle's medium (Flow Labs) supplemented with 33 μg ml^{-1} bovine insulin (Sigma) and 35 μg ml^{-1} *p*-aminobenzoic acid with buffering maintained by bicarbonate in an atmosphere of 5% CO_2 in O_2 at 37 °C. The hemidiaphragms were pinned out on Sylgard (Dow Corning) slants poured in 100-mm Petri dishes and were rocked twice per min. After 2–4 h in culture, the medium was replaced, then collected after a further 24 h (DdiaCM). Protein content ranged from 1 to 2 mg ml^{-1} (Bio-Rad protein assay; horse γ-globulin protein standard). The DdiaCM was stored frozen at −20 °C for up to 6 months. Antisera were produced in female SPF New Zealand White rabbits (12–14 kg). D8 antiserum was raised against the dialysed (50 mM NaH_2PO_4, 1 mM Na_2EDTA, *p*H 7.5, 4 °C), unfractionated DdiaCM. To fractionate the DdiaCM by gel filtration for immunization of D5, D6, D7 and D9, 100 ml of DdiaCM (45 mg protein) were dialysed against 0.1 M NaH_2PO_4, 0.2 M NaCl, 5 mM Na_2EDTA (*p*H 7.5) at 4 °C, concentrated by ultrafiltration to 6 ml (Amicon stirred cell, YM-10 membrane), then applied to a 2.5 × 100 cm column packed with AcA-44 (LKB) and eluted in the same buffer at a flow rate of 17.5 ml hr^{-1}; 8-ml fractions were collected. The column was calibrated with thyroglobulin to indicate the void volume (V_0), bovine serum albumin (67K), ovalbumin (44K), and carbonic anhydrase (29K). Fractions containing material that eluted in the V_0 (15.6 mg protein) were pooled as were fractions eluting between 60 and 40K (6.3 mg protein). The two pooled fractions were dialysed against 20 mM ammonium bicarbonate, then lyophilized. Rabbits D5 and D6 were immunized with the V_0 AcA-44 fraction, while D7 and D9 received the 60–40K AcA-44 fraction. For immunization, 1 mg of protein (dry weight) was dissolved in phosphate-buffered saline (PBS; 5 mM NaH_2PO_4, 0.9% NaCl, *p*H 7.5) and emulsified in an equal volume of adjuvant. Freund's complete adjuvant was used for the first immunization (10–15 intradermal sites) and in a second immunization at 2 weeks. After 6 weeks, booster injections were given in incomplete adjuvant; blood was obtained two weeks after each boost. After collection, blood was clotted, the serum separated, heat-inactivated at 60 °C for 30 min, then stored frozen at −20 °C in 1-ml aliquots for use in the sprouting assay. To purify antigen by preparative SDS-PAGE for the D10 and D11 immunizations, 1 mg of the lyophilized 60–40K AcA-44 fraction was boiled for 2 min in 10% (w/v) glycerol, 0.08 M Tris-HCl *p*H 6.8, 2.0% (w/v) SDS, 5.0% (v/v) β-mercaptoethanol and electrophoresed through a 1.5 mm-thick, 7.5% SDS-polyacrylamide gel[26]. The gel was briefly stained in Coomassie blue (0.125% in 10% acetic acid, 50% methanol) then destained in 10% acetic acid, 15% methanol. The 60–40K AcA-44 fraction contained the prominent doublet at ~60K seen in the DdiaCM stained with amido-black, as well as the next prominent band of ~54K. The region between these two markers was cut from the gel, briefly rinsed in several changes of PBS, minced, then Dounce-homogenized in 1–2 ml of PBS. The gel slurry was emulsified in Freund's complete adjuvant for the first immunization, then in incomplete adjuvant for immunizations at 10 and 13 weeks. D10 was exsanguinated at 17 weeks. D11 was bled at 15 weeks, at which time it exhibited muscle wasting and had lost about one-third of its body weight; this rabbit died 1 week later and the carcass was unintentionally destroyed. All of the other rabbits remained healthy and a muscle biopsy of D7 at 10 weeks showed no signs of denervation (K. Stefansson, data not shown). For immunoblotting, 35 μg of DdiaCM protein in culture medium was boiled in the above SDS sample buffer and electrophoresed through a 1.5 mm-thick, 7.5% polyacrylamide (bis, 2.5% w/w of total monomer) slab gel[26] with cooling. The protein was electrophoretically transferred[27] from the gel to a nitrocellulose filter (BA85; Schleicher and Schuell) at 3.5 V cm^{-1}, 0.2 A for 1 h. After transfer, staining of the trichloroacetic acid-fixed gel with Coomasie blue demonstrated complete transfer of all protein having a molecular weight <94K. Individual lanes were cut from the filter and stained for protein with amido-black (0.1% in 10% acetic acid, 50% methanol) or soaked in 5% haemoglobin in PBS–NaN_3 for 1 h to block reactive sites. The 'blocked' strips were reacted overnight at 23 °C with sera diluted 1:50 in 1% haemoglobin. IgG bound to the filter was detected by incubation for 3 h with *Staphylococcus* protein A (Sigma) iodinated by the chloramine-T method to 5–10 μCi per μg protein, followed by exposure to Kodak XRP-5 film overnight at 4 °C. Molecular weight markers (Sigma) were: phosphorylase B (94K), human transferrin (76K), bovine serum albumin (67K), catalase (60K), glutamic dehydrogenase (53K), ovalbumin (43K) and lactate dehydrogenase (36K).

The work was supported in part by NIH HD05940 and by the Searle Scholars Program.

Received 15 June; accepted 2 November 1983.

1. Edds, M. V. *Q. Rev. Biol.* **28,** 260–276 (1953).
2. Brown, M. C., Holland, R. L. & Hopkins, W. G. *A. Rev. Neurosci.* **4,** 17–42 (1981).
3. Brown, M. C. & Ironton, R. *Nature* **265,** 459–461 (1977).
4. Betz, W. J., Caldwell, J. H. & Ribchester, R. R. *J. Physiol., Lond.* **303,** 281–297 (1980).
5. Duchen, L. W. & Tonge, D. A. *J. Anat.* **124,** 205–215 (1977).
6. Holland, R. L. & Brown, M. C. *Science* **207,** 649–651 (1980).
7. Duchen, L. W. & Stritch, S. J. *Q. J. exp. Physiol.* **53,** 84–89 (1968).
8. Ironton, R., Brown, M. C. & Holland, R. L. *Brain Res.* **156,** 351–354 (1978).
9. Brown, M. C., Holland, R. L. & Ironton, R. *J. Physiol., Lond.* **306,** 493–570 (1980).
10. Brown, M. C. & Holland, R. L. *Nature* **282,** 724–726 (1979).
11. Weiss, P. & Edds, M. V. *Am. J. Physiol.* **145,** 587–607 (1946).
12. Van Harreveld, A. *Am. J. Physiol.* **150,** 670–676 (1947).
13. Brown, M. C., Holland, R. L., Hopkins, W. G. & Keynes, R. J. *Brain Res.* **210,** 145–151 (1980).
14. Stålberg, E. in *Human Motor Neuron Diseases* (ed. Rowland, L. P.) 47–59 (Raven, New York, 1981).
15. Stålberg, E., Schwartz, M. S. & Trontelj, J. V. *J. neurol. Sci.* **24,** 403–415 (1975).
16. Swash, M. & Schwartz, M. S. *J. neurol. Sci.* **56,** 185–197 (1982).
17. Schwartz, M. S. & Swash, M. *Acta neurol. scand.* **65,** 424–431 (1982).
18. Butler, R. C., Gawel, M., Rose, F. C. & Sloper, J. C. in *Motor Neuron Disease* (ed. Rose, F. C.) 79–93 (Churchill-Livingston, London, 1976).
19. Schwartz, M. S., Stålberg, E. & Swash, M. *J. Neurol. Neurosurg. Psychiat.* **43,** 150–155 (1980).
20. Gurney, M. E., Belton, A. C., Cashman, N. & Antel, J. P. *Neurology* **33** (Suppl. 2), 155 (1983).
21. Dribin, L. B. & Barrett, J. N. *Devl Brain Res.* **4,** 435–441 (1982).
22. Henderson, C. E., Huchet, M. & Changeux, J-P. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2625–2629 (1981).
23. Hopkins, W. G. & Slack, J. R. *J. Neurocytol.* **10,** 537–556 (1981).
24. Brown, M. C. & Ironton, R. *J. Physiol., Lond.* **278,** 325–348 (1978).
25. Merlie, J. P., Heinemann, S. & Lindstrom, J. M. *J. biol. Chem.* **254,** 6320–6327 (1979).
26. Laemmli, U. K. *Nature* **227,** 680–695 (1970).
27. Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76,** 4350–4354 (1979).
28. Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K. & Bent, D. H. in *Statistical Package for the Social Sciences* (McGraw-Hill, New York, 1970).

Myelin-associated glycoprotein in human retina

K. Stefansson*, M. L. Molnar*, L. S. Marton*, G. K. Molnar†, M. Mihovilovic*, R. C. Tripathi‡ & D. P. Richman*

* Department of Neurology and The Brain Research Institute, and
‡ Eye Research Laboratory, University of Chicago, Chicago, Illinois 60637, USA
† Department of Neurology, University of Turku, Turku 20110, Finland

The human retina is unmyelinated, but structural similarities have been noted between Müller cells, the main glial cell type of retina, and oligodendrocytes, the myelin-forming cells of the central nervous system[1]. We now show that antibodies against myelin-associated glycoprotein, a minor component of central and peripheral myelin so far found only in myelin and myelin-forming cells[2–4], also stain Müller cells. Immunoblot analysis of retinal proteins indicates that the antigen detected is myelin associated glycoprotein. These results suggest a closer relationship between Müller cells and oligodendrocytes than previously suspected and raise questions about the functional role of myelin-associated glycoprotein.

0028–0836/84/060548—03$01.00

© 1984 Macmillan Journals Ltd

Rat monoclonal antibodies against myelin-associated glycoprotein were obtained as a by-product of attempts to raise monoclonal antibodies specific for membrane-bound nicotinic acetylcholine receptor (AChR) from the electric organ of *Torpedo californica*. Rats were immunized with AChR from the electric organ, tissue known to be rich in myelinated axons. The preparation of acetylcholine receptor used for the immunization was later shown to be contaminated with myelin. The monoclonal antibodies were raised by fusing spleen cells from the immunized animals to the non-secreting mouse myeloma cell line SP2/O-Ag14 using the hybridoma technique of Köhler and Milstein[5], slightly modified[6]. The hybridoma supernatants were screened against AChR-containing membrane preparations from the electric organ using an enzyme-linked immunoassay[7]. Two IgG monoclonal antibodies produced in this way ($BRIC_{37}$ and $BRIC_{38}$) that did not bind to the acetylcholine receptor reacted on a Western blot[8] with isolated human MAG (given by Dr R. H. Quarles). When Western blots of homogenates of adult human spinal cord or human peripheral nerves were studied, the antibodies reacted only with bands having relative mobilities identical to that of MAG (Fig. 1). The monoclonal antibodies showed no reaction with Western blots of homogenates from human liver, kidney or striated muscle.

The immunohistochemical distribution of the antigen with which $BRIC_{37}$ and $BRIC_{38}$ react is identical to that described for MAG[3,4]. The antigen is present in myelin sheaths of the central and peripheral nervous systems as well as in the cytoplasm of oligodendrocytes just prior to and during myelination. As controls for $BRIC_{37}$ and $BRIC_{38}$, both on the Western blots and in immunohistochemical work, we used several monoclonal antibodies against the nicotinic AChR[6]. We also used serum from a patient (M) with IgM monoclonal gammopathy and neuropathy whose monoclonal IgM is directed against human MAG[9] (Fig. 1). As controls for this serum we used sera from several patients with IgM monoclonal gammopathy unaccompanied by neuropathy. It has been demonstrated by peptide mapping that $BRIC_{37}$ and $BRIC_{38}$ may react with the same epitope on MAG but one which differs from that which reacts with IgM from the patient's (M) serum (L.S.M. and K.S., in preparation).

Sections of formalin-fixed, paraffin-embedded human retinas obtained from autopsy and surgical material were stained immunohistochemically with $BRIC_{37}$ and $BRIC_{38}$ using the peroxidase–antiperoxidase (PAP) method of Sternberger[10]. They were also stained with the patient's serum described above, using the biotin–avidin peroxidase technique[11]. There was intense staining of processes throughout the retina, reaching down to the inner limiting membrane and up to the outer limiting membrane (Fig. 2). At the outer limiting membrane there was staining of the fine processes of the Müller cells that reach between the inner segments of the photoreceptors. A few nuclei in the inner nuclear layer were outlined by a darkly staining rim (Fig. 3). These nuclei were located where nuclei of Müller cells are known to be present. This staining pattern is similar to that described for monoclonal antibodies raised against Müller cells[12]. The controls gave no staining (Fig. 2).

Fresh normal human retinas were homogenized, solubilized, electrophoresed on a polyacrylamide gel, and blotted onto nitrocellulose paper as described previously[9]. The nitrocellulose paper was subsequently exposed to $BRIC_{37}$, $BRIC_{38}$ or the patient's (M) serum. The anti-MAG antibodies identified a band having a relative mobility identical to that of MAG (molecular weight (M_r) ~ 100,000), another band of ~130,000 M_r, a third at ~200,000 M_r and a few minor bands of larger molecular weights (Fig. 1). The controls identified nothing on the blots. It has been shown previously that MAG from developing rats and myelin-deficient *quaking* mice has a molecular weight larger (but only by 10,000 to 25,000) than MAG isolated from normal adult animals (refs 13, 14). Therefore, we examined the molecular weights of proteins with which the anti-MAG antibodies react in the developing human central nervous system (CNS) and in the brain stem: for example, at 31 weeks of gestation the antibodies react with only two pairs of bands, one of M_r >250,000, the other of M_r ~200,000. At 37 weeks of gestation the anti-MAG antibodies react with three bands: one of M_r ~225,000, another of ~200,000 and the third one ~100,000. At 7 months postnatal the anti-MAG antibodies react only with an antigen having a molecular weight of 100,000 (L.S.M. and K.S., in preparation). It seems, therefore, that the 'immature' MAG in the human CNS is larger than that in rodents. All the bands identified by the anti-MAG antibodies in the human retina, including the 130,000-M_r protein, are

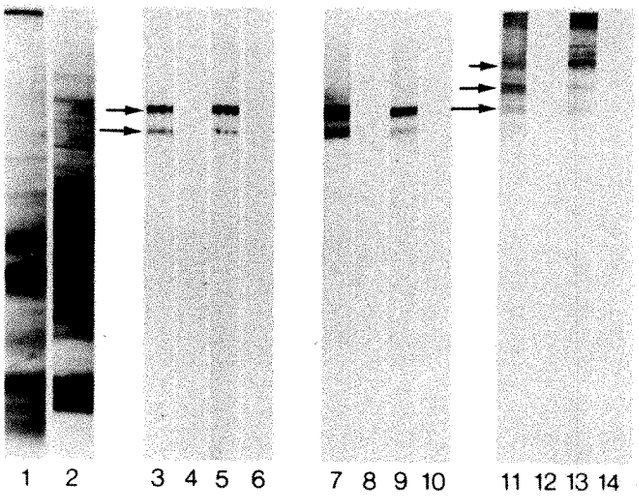


Fig. 1 Western blots of isolated MAG (lanes 3–6), homogenate of adult human spinal cord (lanes 7–10) and homogenate of human retina lanes (11–14). The isolated MAG, the spinal cord homogenate and the retinal homogenate were run side by side on a 3-well SDS-polyacrylamide gel using the Fairbanks system[19] (lane 1 is a Coomassie blue-stained gel of the spinal cord homogenate and lane 2 is the same for retinal homogenate). The antigens were subsequently transferred electrically to nitrocellulose sheets. The nitrocellulose sheet was then cut into strips and the rest of the staining procedure was carried out with each of the strips in a capped test tube (for experimental details see ref. 9). Next, gel lanes 3, 7 and 11 were incubated with $BRIC_{37}$; 4, 8 and 12 with control monoclonal antibody; 5, 9 and 13 were incubated with the patient's (M) serum; and 6, 10 and 14 with a control serum from a patient with IgM monoclonal gammopathy without accompanying neuropathy. Then strips 3, 4, 7, 8, 11 and 12 were incubated with peroxidase-labelled rabbit anti-rat immunoglobulins and strips 5, 6, 9, 10, 13 and 14 were incubated with peroxidase-labelled rabbit anti-human IgM. The bound immunoglobulins were revealed by dipping the strips into diaminobenzidine–H_2O_2 solution. $BRIC_{37}$ (lane 3) and IgM from the patient's serum (lane 5) bound to isolated MAG (short arrow) and dMAG (long arrow) (dMAG is a degradation product of MAG[20]) and only to MAG and dMAG in the spinal cord homogenate (lanes 7 and 9). In the retinal homogenate both $BRIC_{37}$ (lane 11) and IgM from the patient's (M) serum (lane 13) identified three major bands: one with a relative mobility identical to MAG (longest arrow), another with a M_r ~ 130,000 (middle arrow) and the third with a M_r of ~200,000 (shortest arrow). The control monoclonal antibody (lanes 4, 8, 12) and the control serum (lanes 6, 10, 14) were totally negative. There are some differences between $BRIC_{37}$ and IgM from the patient's (M) serum with respect to relative intensity of the bands. $BRIC_{37}$, for example, stains dMAG in the spinal cord homogenate (lane 7) more intensely than does IgM from the patient's serum (lane 9). $BRIC_{37}$ also brings out dMAG in the retinal homogenate (11) whereas IgM from the patient's (M) serum does not (13). However, IgM from the patient's (M) serum stains the 200,000-M_r protein in the retinal homogenate (13) more darkly than does $BRIC_{37}$ (11). These differences are probably a reflection of the fact that $BRIC_{37}$ and the IgM from the patient's (M) serum do not react with the same epitopes on MAG. $BRIC_{38}$, which appears to bind to the same epitope as $BRIC_{37}$, gives the same relative intensities of bands on blots of retina and spinal cord as does $BRIC_{37}$.

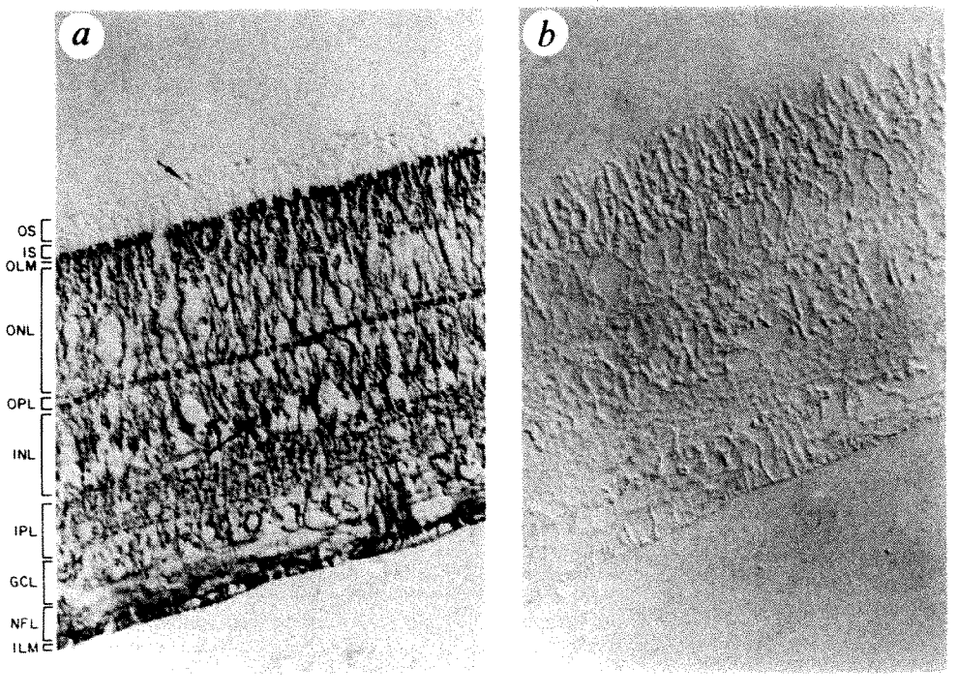


Fig. 2 *a*, Section of formalin-fixed, paraffin-embedded human retina stained with $BRIC_{37}$ (PAP technique). The photograph is taken through differential interference optics (Nomarski), ×400 before photographic conversion. There is intense staining of processes throughout the retina. The long arrow points to the location of nuclei of Müller cells, the short arrow to unstained photoreceptors. OS, outer segment of photoreceptors; IS, inner segment of photoreceptors; OLM, outer limiting membrane; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NFL, nerve fibre layer; ILM, inner limiting membrane. *b*, A section from the same block as that shown in *a*, stained with one of the controls and photographed in the same manner as *a*.

identified in the human CNS at one time point or another during development. Thus, it appears that the antigens in the retina with which the anti-MAG antibodies react may be a mixture of the 'immature' and 'mature' forms of MAG.

The normal human retina is unmyelinated throughout life[1] yet the retina is the only site at which MAG has been found outside the myelin sheath and myelin-forming cells—this observation underlines the need to look beyond myelination in the search for a functional role for MAG. It is also of interest that in the retina MAG appears to be present in Müller cells. Barnstable[12] has described a monoclonal antibody which in the rat retina reacts only with Müller cells and also binds to astrocytes in all regions of the brain. There are several other features that astrocytes and Müller cells have in common, such as close apposition to neuronal processes[15], potassium ion transport[16] and γ-aminobutyric acid transport[17]. However, it has been demonstrated that in the nerve fibre layer of chicken retina, Müller cells form a myelin-like sheath around thick axons[18]. Therefore it could be argued that they bear some resemblance to oligodendrocytes and Schwann cells. The presence of MAG on human Müller cells adds a new feature to that resemblance and provides another reason for considering the possibility that the Müller cells may be more than simply specialized retinal astrocytes.

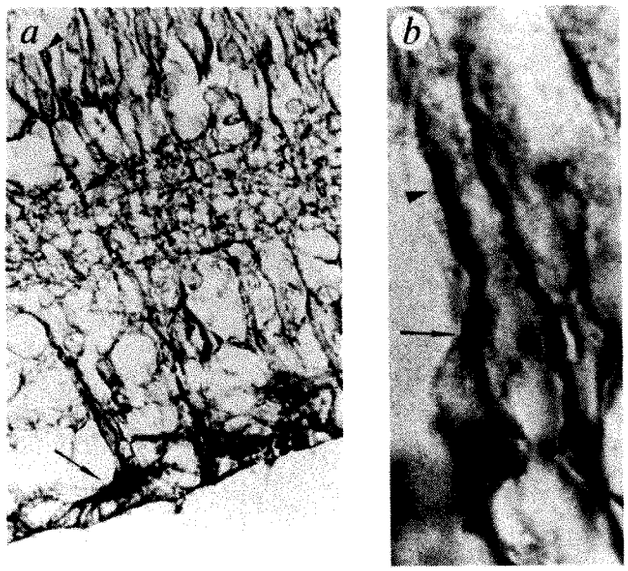


Fig. 3 *a*, Section of paraffin-embedded human retina stained with $BRIC_{37}$ (PAP technique). The long arrow points to the footplate of a Müller cell; the arrowhead to a perinuclear area of a Müller cell which is shown at higher magnification in *b*. A process from this cell can be followed all the way to the inner limiting membrane (thick arrow) (Nomarski ×300). *b*, The perikaryal area of a Müller cell (arrow) and a characteristically broad ascending process (arrowhead). Only the plasma membrane is stained. There is no staining of the nucleus or the cytoplasm (Nomarski ×1,200, oil immersion).

We thank Professors B. G. W. Arnason and R. W. Guillery for constructive criticism. This work was supported in part by a grant from the National Multiple Sclerosis Society, MS:RG 1130-D-17, and NIH grant 5 R01 NS-15462.

Received 9 September; accepted 23 November 1983.

1. Sigelman, J. & Ozanics, V. in *Ocular Anatomy, Embryology and Teratology* (ed. Jakobiec, F. A.) 441–485 (Harper and Row, Philadelphia, 1982).
2. Quarles, R. H., Everly, J. L. & Brady, R. O. *Biochem. biophys. Res. Commun.* **47**, 491–497 (1972).
3. Sternberger, N. H., Quarles, R. H., Itayama, Y. & Webster, H. deF. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1510–1514 (1979).
4. Trapp, B. D. & Quarles, R. H. *J. Cell Biol.* **92**, 877–882 (1982).
5. Kohler, G. & Milstein, C. *Nature* **256**, 495–497 (1976).
6. Gomez, C. M. *et al. Biochem. biophys. Res. Commun.* **88**, 575–582 (1979).
7. Kenneth, R. H. in *Monoclonal Antibodies, Hybridomas* (eds Kenneth, R. H., McKearn, T. J. & Rechtol, K. B.) 376–377 (Plenum, New York, 1981).
8. Towbin, H., Saehlin, T. & Cordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4356 (1979).
9. Stefansson, K. *et al. Acta neuropath.* **59**, 255–261 (1983).
10. Sternberger, L. A. in *Immunocytochemistry* 2nd edn, 104–130 (Wiley, New York, 1979).
11. Hsu, S. M. & Raine, L. *J. Histochem. Cytochem.* **29**, 1349–1353 (1981).
12. Barnstable, C. J. *Nature* **286**, 231–235 (1980).
13. Matthieu, J.-M., Brady, R. O. & Quarles, R. H. *Devl Biol.* **37**, 146–152 (1974).
14. Matthieu, J.-M., Brady, R. O. & Quarles, R. H. *J. Neurochem.* **22**, 291–296 (1974).
15. Peters, A., Palay, S. L. & Webster, H. deF. in *The Fine Structure of the Nervous System* 231–278 (Saunders, Philadelphia, 1976).
16. Kuffler, S. W., Nicholls, J. G. & Orkland, R. K. *J. Neurophysiol.* **36**, 855–868 (1966).
17. Iverson, L. L. & Kelly, J. S. *Biochem. Pharmac.* **24**, 933–938 (1975).
18. Inoue, Y., Sugihara, Y., Nishimura, Y. & Shimai, K. *Okajimas Folia anat. jap.* **57**(2–3), 79–88 (1980).
19. Fairbanks, G., Steck, T. L. & Wallach, D. H. *Biochemistry* **10**, 2606–2617 (1971).
20. Sato, S., Quarles, R. H. & Brady, R. O. *J. Neurochem.* **39**, 97–105 (1982).

Modulation of the response of a photosensitive muscle by β-adrenergic regulation of cyclic AMP levels

L. J. Rubin

Department of Ophthalmology, Box 8096, Washington University Medical School, 660 S. Euclid St, St Louis, Missouri 63110, USA

J. F. Nolte

Department of Anatomy, University of Colorado Health Sciences Center, Denver, Colorado 80262, USA

The light-induced constriction of the irises of some vertebrates is mediated by photosensitive pupillary sphincter cells, which have rhodopsin molecules in their sarcolemmas[1–6]. Light-induced isomerization of these rhodopsin molecules leads to the relase of Ca^{2+} from an internal pool, which in turn activate the contractile proteins[2,4,7]. A central nervous reflex is therefore not essential for the light responsiveness of these irises, but they do appear to be innervated[8–14]. The photosensitive iris of the toad receives sympathetic (adrenergic) innervation. Stimulation of sympathetic nerves to the eye[11] or application of adrenergic agonists to the iris[8,14] cause pupillary dilation due to relaxation of the sphincter muscle. We show here that β-adrenergic stimulation of toad sphincter cells modulates their photoresponses by elevating the intracellular levels of cyclic AMP. However, cyclic AMP does not appear to be involved in the transduction event but rather alters the availability of Ca^{2+} for contraction.

Irises of the toad *Bufo marinus* were isolated by opening the eye cup posteriorly and removing the lens and sclera, except for a small circumferential stric of sclera attached to the iris. This scleral strip was pinned with the anterior iridial surface upwards to a clear silicone rubber substrate in a perfusion chamber and placed in an IR pupillometer[13]. The output of the pupillometer was calibrated as pupillary area (mm^2) and provided continuous monitoring of iridial strip responses to both light and drug treatment. The scleral strip and attached iris were pinned so as to avoid any external tension on the sphincter muscle. The toad iris does possess a dilator muscle (contrary to the results of Armstrong and Bell[8]) but it is unresponsive to either light or adrenergic stimulation[15].

Addition of adrenergic agonists to irises causes a pronounced pupillary dilation and variable changes in the size of light responses. This response is elicited most easily by the β-adrenergic agonist isoprenalin and least easily by the α-agonist phenylephrine, suggesting that the effect of adrenergic stimulation is mediated by β-adrenergic receptors. This is supported by the observation: (1) the effect of isoprenaline, adrenalin or phenylephrine is blocked by the β-antagonist propranolol (Fig. 1), but not by the α-antagonists phentolamine or phenoxybenzamine; (2) the response is elicited by a 1,000-fold lower concentration of isoprenaline than phenylephrine; and (3) propranolol antagonizes the pupillary response elicited by high-potassium depolarization and subsequent release of endogenous neurotransmitter[15]. Low concentrations of isoprenaline (10^{-9}–10^{-8} M) always cause pupillary dilation of dark-adapted, previously untreated irises ($n = 23$). However, the sensitivity of the light response to isoprenaline treatment varies slightly between animals. Low concentrations of isoprenaline affect light responses by either increasing ($n = 16$) or decreasing ($n = 7$) the peak-to-peak amplitude of responses. In cases where isoprenaline causes an increase in the amplitude of light responses, higher concentrations of isoprenaline (5–10 times) cause a decrease in response amplitude. However, in most cases the decrease of response amplitude caused by isoprenaline is transient (Fig. 2). One possible explanation is that the β-adrenergic receptors desensitize rapidly. However, since this effect is not transient (up to 1 h) if a cyclic AMP-phosphodiesterase (PDE) inhibitor is also present, a more likely explanation is an increase in PDE activity and subsequent reduction of cyclic AMP levels.

The toad iris appears to resemble other systems in that β-adrenergic receptors affect cells by activating adenylate cyclase

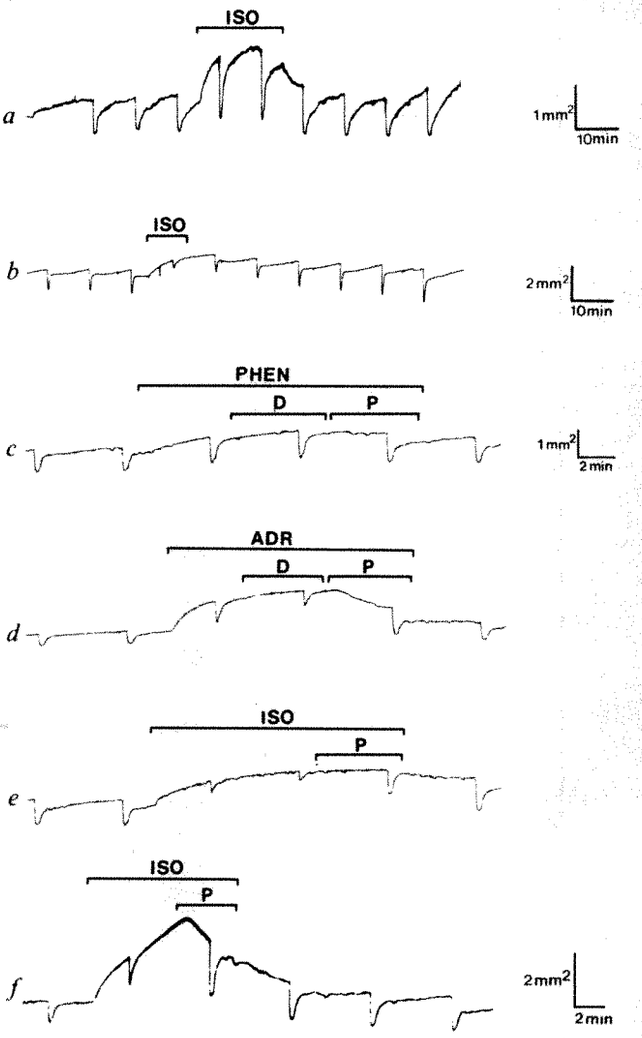


Fig. 1 Tracings of the output of the pupillometer (pupillary area). Stimulation with light causes a downward deflection of the tracing and indicates a decrease in pupillary area equivalent to pupillary constriction. Effect of the adrenergic agonist isoprenaline on pupillary size and light response in four different irises. *a*, *b*, Addition of isoprenaline (ISO, 10^{-8} M) caused pupillary dilation in both cases. However, in one case isoprenaline increased the peak-to-peak amplitude of light responses (*a*) and in the other case it decreased the amplitude of light responses (*b*). *c*–*e*, Tracings from the same preparation. The iris dilates in response to addition of phenylephrine (PHEN, 10^{-6} M), adrenaline (ADR, 10^{-6} M) or isoprenaline (ISO, 10^{-7} M) to the perfusate. Effect of phenylephrine and adrenaline is not blocked by the α-blocker phenoxybenzamine (D, 10^{-6} M) but is reversed by the β-antagonist, propranolol (P, 10^{-6} M), although the reversing effect on phenylephrine response in this iris is slower. Propranolol (P, 10^{-6} M) blocks the effect of isoprenaline (10^{-7} M) on light responses but does not rapidly return the dark-adapted iris to pretreated levels of dilation. Concentrations of propranolol 100 times the isoprenaline concentration do block and reverse the effect of isoprenaline (*f*). All drug solutions are prepared fresh from frozen stock solutions (10^{-3} M dissolved in 2–5 mM HEPES Ringer's, *p*H 4.8). Stock solutions of agonists are supplemented with ascorbic acid (50 mg l^{-1}) to retard oxidative degradation[29]. Perfusion rate was one to four times the chamber volume per min. Light stimulus intensity was 16.45 mW cm^{-2}.

0028-0836/84/060551—03$01.00

© 1984 Macmillan Journals Ltd

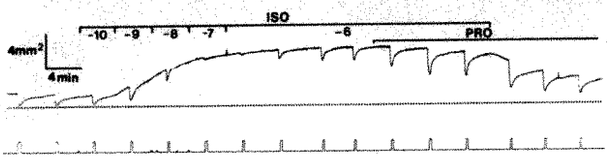


Fig. 2 Effect of progressively higher doses (log concentrations) of isoprenaline (ISO) on pupillary responses to light. Lower trace is the output of a light monitor. Dotted line is an arbitrary reference line. Low concentrations of isoprenaline (10^{-9} M) cause pupillary dilation and slightly increase the amplitude of the light response. To decrease the amplitude of light responses the concentration of isoprenaline was increased successively. High concentrations of isoprenaline (10^{-6} M) still only transiently reduce the size of light responses. Addition of propranolol (PRO, 10^{-6} M) to an isoprenaline-treated iris partially antagonizes the effect of isoprenaline.

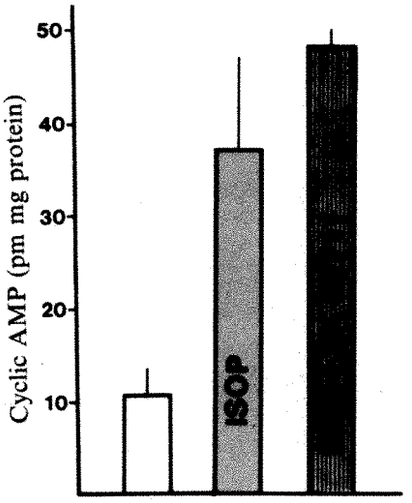


Fig. 3 Cyclic AMP levels of dark-adapted toad sphincters. The irises of 24 toads (*Bufo marinus*) were dissected in dim room light, pinned to a clear silicone rubber base in a Petri dish and then dark-adapted for 15–20 min while maintained in Ringer's solution. Because of the relatively small amount of tissue available for these measurements the largest toads (usually female) were used. In addition, each measurement was made from a pooled sample of four irises from four different animals, thus minimizing individual animal variations. Values represent the mean and standard deviation of the mean of untreated ($n=6$), isoprenaline-treated (ISOP, $n=3$, 10^{-7} M) and ZK62711 with isoprenaline-treated ($n=3$, 10^{-6} M) sphincters. After dark-adaptation the sphincter region was dissected from the remainder of the iris using IR viewers (FJW, Find-R-Scope) mounted on a dissection microscope. For drug treatment, tissue was then immersed in Ringer's solution with drug for 30–60 s and then frozen in liquid Freon at liquid nitrogen temperature. Untreated tissue was placed in fresh Ringer's for 30–60 s and then frozen. Frozen samples were homogenized in cold 5% trichloroacetic acid, centrifuged and the cyclic nucleotide content of the supernatant was determined according to the radioimmunoassay technique of Harper and Brooker[17]. The protein precipitate was processed according to the method of Lowry *et al.*[30].

and thereby increasing cellular concentrations of cyclic AMP[16]. Measurements of cyclic nucleotide levels by radioimmunoassay[17] indicate that isoprenaline causes a fourfold increase in cyclic AMP levels (Fig. 3). Therefore, the pupillary dilation and decrease in the size of light responses observed with isoprenaline treatment may be due to an increase in cyclic AMP levels. This is supported by the enhancement of the effect of isoprenaline on light responses by other agents which increase cyclic AMP levels. Treating irises with PDE inhibitors such as isobutyl methylxanthine (IBMX, $n=3$) or the cyclic AMP-specific PDE inhibitors ZK62711 ($n=10$, Shering)[18,19] or RO201724 ($n=3$, Hoffman LaRoche)[18–20] but not the cyclic GMP-specific PDE inhibitor MB22948 ($n=5$, May and Baker)[18–21] causes a decrease in the size of light responses

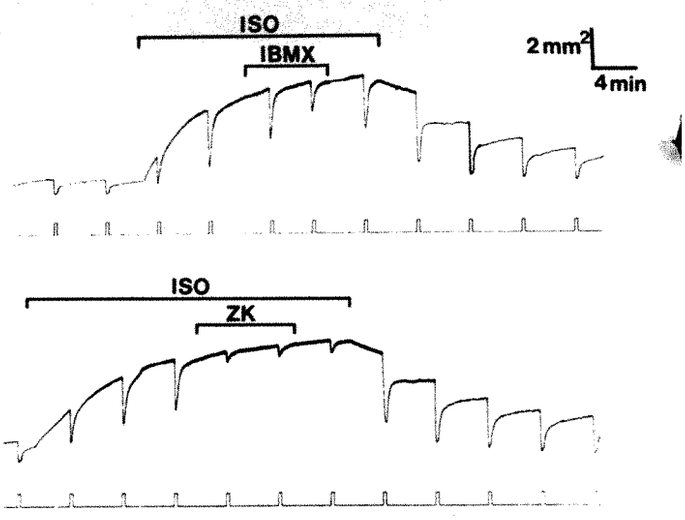


Fig. 4 Potentiating effect of the phosphodiesterase inhibitors ZK62711 and isobutyl methylxanthine (IBMX) on the response of the iris to isoprenaline. Isoprenaline (ISO, 5×10^{-8} M) added to the perfusate bathing the iris caused pupillary dilation and increased the peak-to-peak amplitude of light responses. Treating the iris with IBMX (10^{-5} M) in the presence of isoprenaline reduces the size of light responses. ZK62711 (ZK, 10^{-6} M) in the presence of isoprenaline produced an even greater decrease in light responses which persisted for as long as the inhibitor was present with isoprenaline. ZK62711 alone had no effect on either the dark-adapted iris or on light responses.

beyond that caused by isoprenaline alone (Fig. 4). Irises treated with isoprenaline and ZK62711 contain slightly higher levels of cyclic AMP than irises treated with isoprenaline alone (Fig. 3). In addition, the effect of isoprenaline on light responses is potentiated by cholera toxin ($n=2$). Cholera toxin is known to enter cells and activate adenylate cyclase irreversibly thereby increasing the intracellular concentration of cyclic AMP[22,23]. Potentiation of the effect of isoprenaline on iridial light responses by specific cyclic AMP–PDE inhibitors and by cholera toxin suggests that cyclic AMP is involved in an inhibition of light-induced contractions.

Although cyclic AMP appears to modify the iridial response to light, preliminary results of another study indicate that cyclic nucleotide levels are not regulated by light in this particular photoreceptor. Furthermore, in the absence of stimulation of β-adrenergic receptors, PDE inhibitors have little or no effect on either the size of the dark-adapted iris or on light responses, suggesting that the basal activity levels of the cyclase as well as the PDE are low. Thus it seems unlikely that activation of a PDE[24,25] and subsequent lowering of cyclic nucleotide levels are involved in phototransduction in the sphincter muscle. The most likely explanation is that cyclic AMP exerts its effect on light responses after the initial transduction events.

There are at least two places where cyclic AMP can act to modulate a contractile response in smooth muscle. Cyclic AMP can inhibit the contractile response by initiating the phosphorylation of myosin light-chain kinase, thus preventing the activation of myofilaments[26]. That is, Ca^{2+} released as a result of exposure to light would have no effect on inactivated myoproteins. Cyclic AMP also causes sequestration of Ca^{2+} by the sarcoplasmic reticulum, thereby making fewer Ca^{2+} available for contraction[27]. Either or both of these mechanisms could be responsible for the effects observed in the present case. However, several pieces of evidence suggest that increased sequestration of Ca^{2+} rather than prevention of activation of myofilaments is the primary cause of the decrease in size of light responses. First, irises that are maximally dilated by agents such as isoprenaline still respond to light. This suggests that the myoproteins are functional. Second, normal irises respond to sustained illumination with a prolonged contraction, but isoprenaline-treated irises

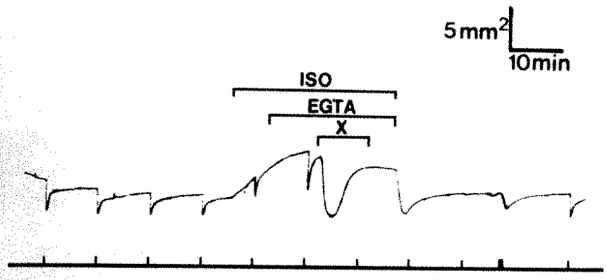


Fig. 5 Effect of calcium ionophore X-537A on the pupillary dilation caused by isoprenaline. In this experiment the iris was treated first with isoprenaline (ISO, 10^{-7} M) in normal Ringer's solution and then by isoprenaline in a low calcium, EGTA (EGTA, 10^{-4} M) solution before addition of the ionophore (X, 5×10^{-6} M) to the same solution. Further experiments indicated that the iris responds to isoprenaline treatment even in the absence of calcium in the perfusate. Therefore, in several experiments the calcium free-EGTA perfusate was started before the application of isoprenaline and ionophore. The results are the same as presented here. The ionophore X-537A causes maximum pupillary constriction and eliminates the response to light.

respond with only a transient contraction, as though Ca^{2+} released by light is rapidly resequestered. Third, the light-induced contraction is larger after either the removal of isoprenaline or the addition of propranolol to an isoprenaline-treated iris. These findings suggest that calcium pools had been loaded during isoprenaline treatment and the stimulus for Ca^{2+} sequestering was then removed.

If the effect of elevated cyclic AMP levels on the sphincter is to increase the sequestering of Ca^{2+} and not affect the contractile proteins directly, then release of Ca^{2+} from these pools should cause a maximum contraction, even in the presence of elevated cyclic AMP levels. This appears to be the case, because perfusion of an isoprenaline-treated iris with the calcium ionophore X-537A[28] in a calcium-free EGTA Ringer's solution causes a rapid maximal pupillary constriction, followed by dilation and complete loss of the light response ($n = 5$, Fig. 5). These results suggest that the myofilaments are capable of contracting during treatment with isoprenaline, but that Ca^{2+} is unavailable due to increased sequestration. The increased size of the light response immediately after some of the treatments with isoprenaline and the temporary absence of light responses after treatment with the ionophore may indicate that at least some of the Ca^{2+} stores augmented by cyclic AMP are sensitive to light.

These results indicate that the autonomous light response of the pupillary sphincter is modified by stimulation of β-adrenergic receptors. Thus, sympathetic innervation of the sphincter appears to regulate at least the amplitude of light responses by regulating the availability of Ca^{2+} for light-induced release. The most likely mechanism for calcium regulation in this system is a cyclic AMP-mediated sequestration of Ca^{2+} into stores; some of these stores are light sensitive. These results also imply that cyclic nucleotides are not directly involved in the transduction process of this muscle.

Received 23 May; accepted 21 November 1983.

1. Bell, A. L. thesis, State Univ. New York (1967).
2. Bito, L. J. & Turansky, D. G. *Comp. Biochem. Physiol.* **50,** 407–413 (1975).
3. Steinach, E. *Arch ges. Physiol.* **47,** 289–340 (1890).
4. Barr, L. & Alpern, M. J. *Gen. Physiol.* **46,** 1249–1265 (1963).
5. Blaustein, D. thesis, State Univ. New York (1977).
6. Seliger, H. H. *J. gen. Physiol.* **46,** 333–342 (1962).
7. Zucker, R. & Nolte, J. *Nature* **274,** 78–81 (1978).
8. Armstrong, P. B. & Bell, A. L. *Am. J. Physiol.* **214,** 566–573 (1968).
9. Bell, A. L. & DiStefano, H. S. *Anat. Rec.* **154,** 498 (1966).
10. Kuchnow, K. P. *Vis. Res.* **10,** 955–964 (1970).
11. Morris, J. L. *Am. J. Physiol.* **231,** 1272–1278 (1976).
12. Nilsson, S. *J. comp. Physiol.* **138,** 149–155 (1980).
13. Rubin, L. & Nolte, J. *Curr. Eye Res.* **1,** 543–551 (1982).
14. Young, J. Z. *Proc. R. Soc.* **B112,** 242–249 (1933).
15. Rubin, L. thesis, Univ. Colo. Hlth Sci. Cent. (1982).
16. Klainer, L. M., Chi, Y.-M., Freidberg, S. L., Rall, T. W. & Sutherland, E. *J. biol. Chem.* **237,** 1239–1243 (1962).
17. Harper, J. F. & Brooker, G. *J. Cyclic Nucleotide Res.* **1,** 207–218 (1975).
18. Fredholm, B. B., Brodin, K. & Strandberg, K. *Acta pharmac. tox.* **45,** 336–344 (1979).
19. Schwabe, U., Miyaka, M., Ohga, Y. & Daly, J. W. *Molec. Pharmac.* **12,** 900–910 (1976).
20. Bergstrand, H., Kristoffersson, J., Lundquist, B. & Schurmann, A. *Molec. Pharmac.* **13,** 38–43 (1977).
21. Broughton, B. J. *et al. Nature* **25,** 650–652 (1974).
22. Gill, D. M. *Adv. Cyclic Nucleotide Res.* **8,** 85–118 (1977).
23. Lai, C.-Y. *Crit. Rev. Biochem.* **9,** 171–206 (1980).
24. Bitensky, M. W. *et al. Adv. Cyclic Nucleotide Res.* **5,** 213–255 (1975).
25. Chader, G., Herz, L. R. & Fletcher, R. T. *Biochim. biophys. Acta* **247,** 491–493 (1974).
26. Conti, M. A. & Adelstein, R. S. *Fedn Proc.* **39,** 1569–1573 (1980).
27. Nilsson, K. B., Andersson, R. G. G., Mohme-Lundholm, E. & Lundholm, L. *Acta pharmac. tox.* **41,** 53–64 (1977).
28. Pressman, B. C. *A. Rev. Biochem.* **45,** 501–530 (1976).
29. Hamberger, B. *Acta physiol. scand. Suppl.* **295,** 7–56 (1967).
30. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193,** 265–275 (1951).

Complementation in monocyte hybrids revealing genetic heterogeneity in chronic granulomatous disease

Mic N. Hamers, Martin de Boer, Louis J. Meerhof, Ron S. Weening* & Dirk Roos

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands
* Department of Pediatrics, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Chronic granulomatous disease (CGD) is a rare syndrome, found predominantly in male children and characterized by life-threatening, recurrent infections. The superoxide (O_2^-)/hydrogen peroxide (H_2O_2) generating system in the granulocytes and monocytes of CGD patients is completely defective[1]. Furthermore, a novel type of cytochrome *b*, detected by the optical spectrum of phagocytes from healthy subjects[2,3], is lacking in those of most male CGD patients[4]. In female CGD patients, the cytochrome *b* is present[4], but cannot, as in normal cells[5], be reduced on metabolic stimulation of the phagocytes in anaerobic conditions. Here, to demonstrate the importance of cytochrome *b* in this system and to investigate the genetic background of the various forms of CGD, we have hybridized monocytes from a cytochrome *b* negative, X-linked male CGD patient with monocytes from a cytochrome *b* positive, male CGD patient with unknown genetic background. Monocytes were used because they are the only blood phagocytes that show an active protein synthesis, whereas fibroblasts or lymphocytes do not express the O_2^-/H_2O_2 generating system. The heterologous hybrids were positive in the nitroblue tetrazolium (NBT) slide test, indicating the complementation of the O_2^-/H_2O_2 generating system, whereas the homologous hybrids remained negative, as did the non-fused cells of these patients. We thus conclude that cytochrome *b* is part of the O_2^-/H_2O_2 generating system and that somatic cell hybridization experiments with monocytes provide a means of studying the genetic background of CGD patients. We believe this to be the first report of genetic complementation by somatic cell hybridization experiments using monocytes instead of fibroblasts.

Because two patterns of inheritance of CGD seem to exist—one X-linked[6] and one autosomal recessive[7]—Segal and co-workers[3] have suggested that the absence of cytochrome *b* is correlated with the X-linked form of CGD and that the malfunction of cytochrome *b* is correlated with the autosomal recessive form. However, segregation between the expression of cytochrome *b* and the X-linked form of CGD has recently been described[8]. Moreover, we have discovered three cytochrome *b* negative patients (one male and two females) within one family with the autosomal recessive form of CGD, as well as one cytochrome *b* negative female CGD patient with an unknown genetic background. Table 1 shows the genetic heterogeneity of 12 male and 5 female CGD patients. The CGD families are unrelated to each other in the last four generations. It is clear

0028-0836/84/060553—03$01.00

© 1984 Macmillan Journals Ltd

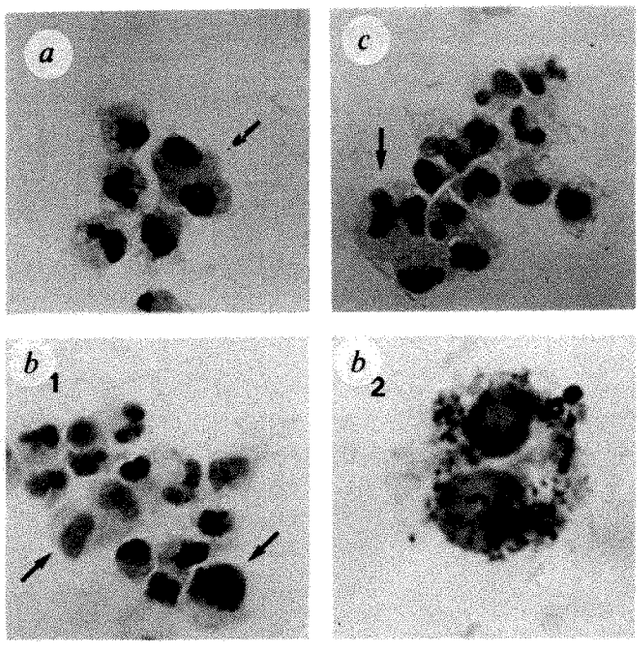


Fig. 1 NBT slide test on somatic cell hybrids of monocytes from two different CGD patients. Patient 1: X-linked, cytochrome *b* negative male; patient 2: cytochrome *b* positive male, mode of disease inheritance unknown. *a*, Fusion product of monocytes from patient 1 (negative in NBT test); b_1, fusion products of monocytes from patient 1 and patient 2 (one negative and one positive in NBT test); b_2, NBT-positive heterologous hybrid (higher magnification); *c*, fusion product of monocytes from patient 2 (negative in NBT test).

Methods: Monocytes (90–95% monocytes, 5–10% lymphocytes, 0–2% basophils) were isolated from mononuclear leukocytes by elutriation[16], 3×10^7 cells were fused with polyethylene glycol (58%, molecular weight 4,000) for 1 min in Dulbecco medium[17] and kept in maintenance culture in round-bottom wells of a microtitre plate in Iscove's modified medium with 10% fetal calf serum. Cells were collected at the indicated times and resuspended in medium of *p*H 7.4 containing 138 mM NaCl, 2.9 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.6 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.5 mM glucose and 0.5% (w/v) human albumin. Nitroblue tetrazolium (Sigma) was added at 0.5 mg ml^{-1}, and the cells were stimulated for 30 min with 100 ng ml^{-1} phorbol myristate acetate (Consolidated Midland Corporation). Thereafter, the cells were fixed in suspension with paraformaldehyde (1% v/v), spun onto a glass slide with a Shandon-Elliot Cytospin centrifuge (10 min at 600 r.p.m.), and stained with nuclear fast red (Merck Darmstadt) for 20 min for counting of the NBT-positive hybrids. Part of the unstimulated cells were stained with May-Grünwald (5 min) Giemsa (1 h) to count the total number of monocyte hybrids.

from Table 1 that considerable heterogeneity exists in CGD: 5 of the 12 male patients do not show the X-linked inheritance (compared with 4 out of 20 patients in ref. 4) and 3 (two sibs) of the 5 female patients were cytochrome *b* negative (compared with 2 cousins with partial cytochrome *b* deficiency out of 7 female patients in ref. 3). Furthermore, the discovery of the family with the autosomal recessive trait and absence of cytochrome *b* in their neutrophils, as well as the X-linked CGD patient with cytochrome *b* in this study and the one reported recently[8], preclude correlation between the absence of cytochrome *b* and the X-linked form of CGD. However, the correlation between the absence of cytochrome *b* and CGD in general still holds. The one male patient without cytochrome *b* in 'unknown' category (Table 1) is comparable with the three male patients in ref. 3 without any affected relatives. Because the disease is often fatal in early childhood, a high incidence of spontaneous mutations in the X-linked form may be expected. This might explain why some male patients without cytochrome *b* have mothers and sisters without any sign of heterozygosity. The one female patient without cytochrome *b* in the 'unknown' category is more difficult to explain. She might belong to the autosomal recessive form of CGD without cytochrome *b*, or to the X-linked form of CGD. In the latter instance, she would be heterozygous, with an extreme lyonization pattern (as yet undetected), while her mother would be heterozygous with an extreme lyonization pattern in the opposite way.

Whatever the mode of inheritance in these patients, Table 1 clearly shows that the expression of the O_2^-/H_2O_2 generating system is controlled by at least three genetic loci, one or more of which is located on the short arm of the X chromosome[9,10].

As long as the molecular defects remain unknown, the only way to study the genetic background of the various CGD patients is by complementation analysis of somatic cell hybrids. Generally, fibroblasts are used for this purpose; however, these cells do not express the O_2^-/H_2O_2 generating system, as measured with the NBT reduction test (ref. 11, confirmed by us). Lymphocytes have also been reported to express the O_2^-/H_2O_2 generating system[12], but these cells, too, failed to reduce NBT in our hands (unpublished). The only way to circumvent this problem is to perform complementation studies with bone marrow cells or monocytes, because these cells display an active protein synthesis. For obvious reasons, the use of bone marrow cells is excluded, leaving the possibility of monocytes. However, this implies that very few hybridization experiments can be performed, because for one experiment 100 ml of fresh blood from two patients are required. Cryopreserved monocytes cannot be used, because in control experiments we found that the latter cells yielded somatic hybrids that quickly deteriorate in culture.

The large blood sample is needed for the following reasons: (1) the fusion efficiency of monocytes is 0.4–1%, and at least 2×10^7 monocytes per fusion are required for a good recovery of viable hybrids; (2) the monocytes must be highly purified (>90%) to avoid fusion between monocytes and contaminating cells, which would lead to false-negative results; (3) to meet the above-described criteria and to perform two homologous and one heterologous fusion experiment, 3×10^7 purified monocytes are needed, equivalent with 100 ml of whole blood.

To establish the different complementation groups, multiple blood samples from some patients may be needed. This would pose problems because most patients are small children. Therefore, we decided to try the most obvious complementation experiment, fusion of monocytes from an X-linked, cytochrome *b* negative male (patient 1) with those of a cytochrome *b* positive male (patients 2 and 3), to show not only the feasibility of monocyte hybridization but also the importance of cytochrome *b* for the activity of the O_2^-/H_2O_2 generating system. The results of these experiments are shown in Fig. 1 and Table 2.

Figure 1 clearly shows that NBT-positive hybrids were only found in the heterologous situation (expt *b*). The number of positive hybrids observed directly (0 h, Table 2, expt *d*) or shortly after (2.5 h, Table 2, expt *b*) fusion was already 30% of all hybrids, indicating that protein synthesis was not required for complementation in these particular experiments. Indeed, in an experiment parallel to expt *d* (Table 2), inhibition of protein synthesis with cycloheximide (1 μg ml^{-1}) had no effect on the percentage of positive hybrids up to 40 h (not shown). This finding, however, does not preclude the use of monocytes, because protein synthesis might be needed in other complementation groups. The decline in the number of positive hybrids after 40 h in expt *b* is probably due to deterioration of the hybrids. The percentage of positive hybrids at 15 h is very close to the expected maximal value of 50%, indicating that complementation only occurs when heterologous hybrids are formed. The homologous hybrids remained NBT negative, as did the non-fused cells of both patients. In separate experiments, we found that all monocyte hybrids from a single normal donor remained NBT positive, as did non-fused normal cells. Thus, we conclude that both types of CGD patient are genetically different, and that cytochrome *b* is most probably one of the corrective factors. Furthermore, monocyte hybridization experiments can provide information on the genetic background of

Table 1 Genetic heterogeneity in 17 patients with chronic granulomatous disease

	Mode of inheritance					
	X-Chromosomal cytochrome *b*		Autosomal recessive cytochrome *b*		Unknown cytochrome *b*	
	Positive	Negative	Positive	Negative	Positive	Negative
No. of male patients	(1)*	6	—	1	3	1
No. of female patients	—	—	—	2	2	1

CGD patients were diagnosed clinically by recurrent infections of skin, lungs and lymph nodes with catalase-positive bacteria and yeasts, and biochemically by failure of their blood granulocytes to mount a respiratory burst (O_2 consumption[13], NBT reduction (see legend to Fig. 1), H_2O_2 formation[14]) on incubation with opsonized zymosan or phorbol myristate acetate, and reduced capacity of these cells to kill opsonized *Staphylococcus aureus in vitro*. X-chromosome linkage is concluded from the detection of heterozygosity in the mothers and sisters of the patients (subnormal values for O_2 consumption and H_2O_2 production, subnormal amount of cytochrome *b*, and mosaicism in the NBT slide test). Autosomal recessive inheritance is concluded from the following findings. In this family one male and two female CGD patients were found; a sister showed heterozygosity in the metabolic parameters and in the amount of cytochrome *b* (41% of normal), but no mosaicism in the NBT slide test; the father and the mother (first cousins) had low-normal metabolic activity in their neutrophils, no mosaicism in the NBT slide test and 59% and 64%, respectively, of the normal amount of cytochrome *b*. 'Unknown' comprises the category of GCD patients whose parents, brothers and sisters were found to be normal with respect to metabolic and functional tests. The presence of cytochrome *b* was evaluated from optical difference spectra of dithionite-reduced neutrophils minus native neutrophils; the amount of cytochrome *b* was calculated from these spectra by using an absorbance coefficient of 106 mM^{-1} cm^{-1} at 425 nm and of 21.7 mM^{-1} cm^{-1} at 558 nm (ref. 15).

* The amount of cytochrome *b* in the neutrophils from this patient was not measured accurately due to a slight contamination of the cells with haemoglobin; this resulted in a double peak at 425 nm and 433 nm in the redox spectrum; thus, a partial deficiency of cytochrome *b* may exist.

Table 2 Expression of the NBT reductase activity in somatic cell hybrids of monocytes from three CGD patients

Time after fusion (h)	% of NBT-positive hybrids in fusion experiment				
	a(1×1)	*b*(1×2)	*c*(2×2)	*d*(1×3)	*e*(3×3)
0	—	—	—	32.1 (*n* = 6)	0 (*n* = 3)
2.5	2.5 (*n* = 2)	30.1 (*n* = 4)	1.7 (*n* = 3)	—	—
15	0 (*n* = 2)	43.6 (*n* = 10)*	0 (*n* = 2)	45.1 (*n* = 3)†	0 (*n* = 4)
40	0 (*n* = 2)	22.6 (*n* = 7)*	0 (*n* = 2)	35.1 (*n* = 4)†	0 (*n* = 2)
136	0 (*n* = 1)	25.9 (*n* = 4)*	0 (*n* = 1)	—	—

The percentage of NBT-positive hybrids was calculated as the percentage of the number of hybrids counted per 5,000 cells per slide (4–40 hybrids); 1 to 10 slides were evaluated. Results are in geometric means derived from the log-normally distributed values. Expts *a*, *b* and *c* are as in Fig. 1; expts *d* and *e* were performed 1 month later with a second blood donation of patient 1.

* Student's *t*-tests performed on the log-normally distributed values, corrected for separate variance estimates, indicated two-sided *P* values of 0.08 for 2.5 h versus 15 h, 0.003 for 15 h versus 40 h, and 0.5 for 40 h versus 136 h.

† Student's *t*-tests performed as described above. $P = 0.06$ for 0 h versus 15 h, and 0.2 for 15 h versus 40 h.

CGD patients. We feel that only a multi-centre follow-up of this study will lead to the elucidation of a full set of complementation groups within reasonable time, because it is impossible to obtain these large quantities of blood frequently from the relatively small number of patients in one institute. The establishment of complementation groups may well lead to a better understanding of the genetic defects underlying CGD.

We thank Dr A. Westerveld for valuable advice, Dr J. W. M. van der Meer for submission of two patients under his care to the hybridization experiments and Mr W. Schaasberg for statistical analysis. This study was supported by grant 13–39–40 from the Foundation for Medical Research FUNGO, which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

Note added in proof: Fusion of monocytes from patients 2 and 3, and of monocytes from patient 1, with another X-linked patient did not result in complementation even after prolonged culturing.

Received 14 July; accepted 24 October 1983.

1. Klebanoff, S. J. & Clark, R. A. *The Neutrophil: Function and Clinical Disorders*, 641–696 (North-Holland, Amsterdam, 1978).
2. Segal, A. W. & Jones, O. T. G. *Nature* **276**, 515–517 (1978).
3. Segal, A. W., Garcia, R., Goldstone, A. H., Cross, A. R. & Jones, O. T. G. *Biochem. J.* **196**, 363–367 (1981).
4. Segal, A. W. *et al. New Engl. J. Med.* **308**, 245–251 (1983).
5. Segal, A. W. & Jones, O. T. G. *FEBS Lett.* **110**, 111–114 (1980).
6. Windhorst, D. B., Page, A. R., Holmes, B., Quie, P. G. & Good, R. A. *J. clin. Invest.* **47**, 1026–1034 (1968).
7. Azimi, P., Bodenbender, J. G., Hintz, R. L. & Kotras, S. B. *Lancet* **i**, 208–209 (1968).
8. Borregaard, N. *et al. Eur. J. clin. Invest.* **13**, 243–247 (1983).
9. Wolff, G., Müller, C. R. & Jobke, A. *Hum. Genet.* **54**, 269–271 (1980).
10. Denson, P. *et al. Blood* **58**, 34–37 (1980).
11. Seger, R. & Steinmann, B. *Lancet* **i**, 1216 (1981).
12. Tsan, M. F., Winkelstein, J. A., Hsu, S. H. & Newman, B. *Blood* **50**, 178A (1977).
13. Weening, R. S., Roos, D. & Loos, J. A., *J. Lab. clin. Med.* **83**, 570–576 (1974).
14. Homan-Müller, J. W. T., Weening, R. S. & Roos, D. *J. Lab. clin. Med.* **85**, 198–207 (1975).
15. Hamers, M. N., Wever, R., Van Schaik, M. L. J., Weening, R. S. & Roos, D. in *Developments in Biochemistry* Vol. 11b (eds Bannister, W. H. & Bannister, J. V.) 242–251 (Elsevier, New York, 1980).
16. Figdor, C. G., Bont, W. S., De Vries, J. E. & Van Es, W. L. *J. immun. Meth.* **40**, 275–288 (1981).
17. Galtré, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. *Nature* **266**, 550–552 (1977).

Amino acid sequence of rat submaxillary tonin reveals similarities to serine proteases

Claude Lazure, Richard Leduc, Nabil G. Seidah, Gaétan Thibault, Jacques Genest & Michel Chrétien

Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Canada H2W 1R7

Tonin[1], an esteroprotease isolated from rat submaxillary gland, is a serine protease with trypsin- and chymotrypsin-like activity. The substrate specificity of tonin shows that it differs from kallikreins and is definitely not a renin-like enzyme or an angiotensin-converting enzyme[2]. Tonin can produce directly the vasoactive peptide angiotensin II, from angiotensin I, angiotensinogen and the synthetic tetradecapeptide substrate of renin by cleavage of a Phe-His bond. It has also been found to cleave some Phe and Arg bonds in various substrates such as β-lipotropin (β-LPH), adrenocorticotropin (ACTH), pro-

0028-0836/84/060555—06$01.00

© 1984 Macmillan Journals Ltd

opiomelanocortin (POMC)[3] and substance P[4]. Here we describe the complete amino acid sequence of rat submaxillary gland, tonin. Comparison of the sequence of 219 amino acids with other serine proteases, particularly kallikreins, γ-subunit of nerve growth factor (NGF) and the recently described γ-renin, reveals extensive similarities. More interestingly, it also reveals the substitution of an Asp residue always found in the serine protease active site triad (Asp, His, Ser) by a Leu residue. This unusual substitution does not seem to affect the proteolytic activity of the enzyme.

Previous reports on the primary structure of rat tonin have shown that isoleucine and proline occupy the amino and carboxy termini respectively and that sequence analysis of 95 residues showed a very high homology to the serine protease family, especially to the γ-subunit of mouse NGF[5,6]. The complete amino acid sequence of submaxillary gland tonin has now been determined (Fig. 1). Tonin is a single polypeptide chain of 219 amino acids including 10 cysteines and having a molecular weight of 23,796. This molecular weight is much lower than those previously reported which were all in the region of 28,000–30,000 and obtained experimentally by gel filtration, high pressure gel permeation, sedimentation equilibrium and gel electrophoresis experiments[7,8]; this, in turn, led to an overestimation of the actual number of amino acids previously reported as 272[5]. The deduced sequence shown in Fig. 1 was obtained through analysis of the reduced and alkylated chain, of fragments derived from autolysis cleavages and from cyanogen bromide fragmentation. Details of the sequence, most of it done at the 10–20 nmol range, will be reported elsewhere (C.L. *et al.* in preparation).

Comparisons of tonin amino acid sequence with the published sequences of serine proteases (Table 1) show a very high homology (over 50%) between tonin and the various sequence of kallikrein including the pig pancreatic kallikrein[9], the murine glandular kallikrein deduced from a cloned cDNA fragment[10], the rat submaxillary kallikrein[11], which itself proved entirely homologous (117 out of 118 residues) to the rat pancreatic kallikrein deduced from the nucleotide sequence of the mRNA[12]. Moreover, the tonin sequence is also very similar to the γ-subunit of murine NGF[13] as proposed earlier[6] and also to another enzyme found recently in the mouse submaxillary gland, γ-renin[14]. Interestingly, the γ-renin can produce angiotensin I from the renin substrate tetradecapeptide, but not from the natural renin substrate, whereas tonin can act on both substrates to produce angiotensin II. It is thus quite clear, as judged by Table 1, that tonin, NGF γ-subunit, γ-renin and probably epidermal growth factor (EGF) binding protein, form a closely related group within the kallikrein family. That relationship has already been documented by Bothwell *et al.*[15] for glandular kallikrein and growth factor proteases of mouse submaxillary gland.

Looking at the actual sequence, it can be seen that the 10 half-cystine residues in tonin, when aligned to maximize homology can be found in similar positions in the NGF γ-subunit and in trypsin. From this alignment, the linkage of the disulphide bridges in tonin can thus be inferred so that Cys-7 is linked to Cys-131 (corresponding to trypsin 13 to 143), Cys-24 to Cys-40 (trypsin 31 to 47) which forms the 'histidine loop' responsible for proper orientation of the His residue in the active site, Cys-110 to Cys-177 (trypsin 115 to 216), Cys-142 to Cys-156 (trypsin 154 to 168) which forms the 'methionine loop', Cys-167 to Cys-192 (trypsin 179 to 203) which link together the primary and secondary binding sites. It can also be deduced that tonin, like the kallikreins, should not be expected to bind calcium ions since those residues in trypsin, namely Gln-58, Asn-60, Val-63 and Gln-68, are replaced in tonin by Arg-51, Asn-53, Lys-56 and Ala-61. It can also be deduced that tonin should not contain any carbohydrates, at least not the Asn-linked type, since the typical Asn-Xaa-Ser or Asn-Xaa-Thr recognition sequence cannot be found. This differs from pig pancreatic kallikrein where two glycosylation sites were found[9], from the NGF γ-subunit where one site was found[13] and possibly also from the rat

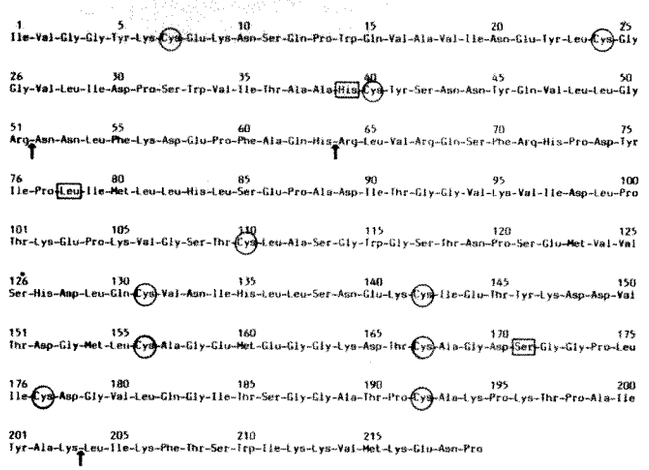


Fig. 1 Amino acid sequence of rat submaxillary gland, tonin. Cys residues are encircled and the residues localized at the active site are enclosed in boxes. Arrows indicate potential sites of autolysis. Positions 1–66 were obtained by sequencing the reduced and alkylated protein. Positions 64–86 were obtained by sequencing the autolysis fragment beginning at position 64. Positions 81–120 were given by the sequence of the cyanogen bromide-derived fragment starting at 81 and also of a fragment comprising residues 81–123. Positions 124–154 were obtained by sequencing the first 29 residues; the last two residues were localized as Gly-Met because position 154 is a CNBr cleavage site. Positions 155–200 and 161–200 were deduced by sequencing the respective cyanogen bromide fragments; positions 201–203 being deduced by amino acid composition. The last 15 residues were obtained by sequence of an autolysis fragment starting at position 204; the C-terminal Pro is deduced by amino acid composition and confirmed by previous studies[5].

pancreatic kallikrein where a glycosylation recognition sequence can be seen (see note added in proof).

All but two of the 29 invariant residues of the serine protease family are conserved in the tonin sequence including the His and Ser residues found in the active site. Indeed, the sequence Val-Leu-Thr-Ala-Ala-His-Cys, found in every serine protease, is present; it does contain a conservative substitution, namely the isoleucine at position 35 in place of the common leucine residue. The complete sequence Cys-Xaa-Gly-Asp-Ser-Gly-Gly-Pro-Leu surrounding the active site Ser is also found in tonin at positions 167–175. One of the exceptions is at position 135 where an His residue replaces Pro. This particular position is characteristically variable in kallikrein and kallikrein-related enzymes as shown by the presence of an Asp in rat pancreatic kallikrein, a Thr in the pig pancreatic and mouse submaxillary kallikrein and a Lys in the γ-subunit of mouse NGF and in the γ-renin. The other exception, at position 78, is more interesting since it involves the replacement of the Asp residue localized at the active site (corresponding to Asp-102 of chymotrypsin) by a Leu residue; this change will be discussed later. The Asp-189 of trypsin, thought to determine the substrate specificity, is also present at position 165 in tonin thus explaining its trypsin-like character. The secondary binding site corresponding to the sequence Ser-Trp-Gly (trypsin position 214–216) is localized at position 186–188 where the Trp is replaced by a Gly. The Gly at position 216 is conserved in tonin (position 188) whereas the Gly at position 226 is replaced by an Ala in tonin (position 199). Both residues are located at the entrance of the binding cavity.

When aligning the sequence to maximize homology, it is necessary to introduce a two-residue deletion between position 20 and 21 with respect to all kallikreins and kallikrein-related enzymes. Another deletion of the same length is introduced between position 120 and 121 compared with pig kallikrein. One of the most striking features of the tonin sequence is the presence of a sizeable deletion (Fig. 2) between residue 73 and 74. To accommodate other kallikrein sequences, is is necessary

to introduce a 16-residue deletion which accounts for the shortened chain length of tonin corresponding to 219 amino acids instead of the 237 of rat pancreatic kallikrein and the 233 of the NGF γ-subunit. The only other serine protease having a comparable deletion is human plasmin (Fig. 2). Moreover, this region of the molecule is particularly sensitive to autodigestion of the so-called 'kallikrein autolysis loop' leading to cleavages illustrated in Fig. 2. In the case of tonin, autodigestion was not observed in that region even though three sites of cleavage, some of them probably due to autolysis were identified at positions 51–52, 63–64, and finally 203–204 (Fig. 1). The most often encountered being the one at 63–64, which could indicate a conformation different from the one found in kallikreins. Presence of autolysis sites seems to occur in all members of the kallikrein family and in some serine proteases. The presence of fragments arising from autolysis which complicates the sequence determination greatly, can be clearly seen upon reduction and alkylation of the disulphide bridges of tonin, of the γ-subunit[16] and two serine proteases from the mouse submaxillary gland[17].

An important striking feature of the tonin sequence is the absence of the Asp residue corresponding to Asp-102 (chymotrypsin numbering) present at the active site and its replacement by a Leu residue. This latter substitution is also accompanied by substitutions of the amino acids surrounding it (Fig. 2). This result was entirely unexpected since, in all serine proteases known so far, the Asp residue is thought to participate actively in the enzyme mechanism by forming what is known as the 'charge-relay system'. The existence of such a relay has been challenged, but examination of crystallographic data shows that the Asp residue is sufficiently close to the His to favour intramolecular interactions. On the other hand, some recent theoretical considerations by Genest and Ptak[18] have shown that reinforced interactions between His and Ser, the latter being involved in the formation of the acyl-enzyme intermediate, must be accomplished with destabilization of the Asp-His diad. Also, Polgar and Bender[19] have suggested that the Asp-102 does not take part in the charge-relay system but may act solely to stabilize the imidazolinium intermediate. Moreover, the finding that papain, a thiol-protease containing His and Cys in the active site, reacts in a similar fashion to serine proteases could indicate that the Asp-102 might not be essential for proteolytic activity even though it might contribute to maintenance of a favourable conformation of the active site. On the other hand, it is clear that, even with such a substitution, tonin can act as a serine protease[2]. With angiotensin I as substrate, the *p*H optimum of tonin was 6.8[1] whereas, with small synthetic substrates *p*H 8–9 was obtained[2] which is similar to values obtained with other serine proteases. It is inactivated by, for example,

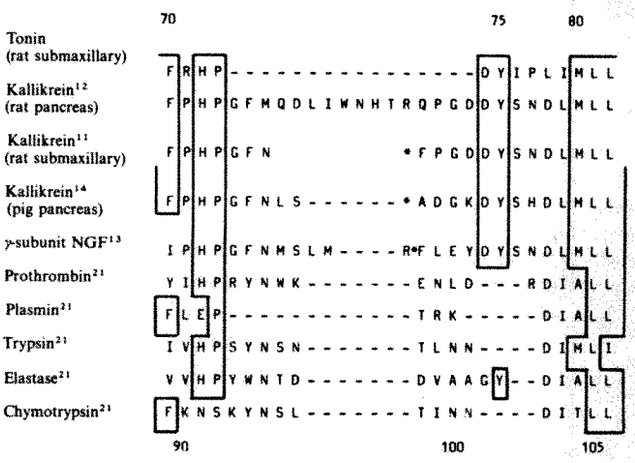


Fig. 2 Alignment of tonin 70–82 with corresponding segments of other serine proteases according to Dayhoff[15] (in the single letter code[24]). For ease of comparison, the numbering of chymotrypsin has been added below the alignment. The sign * in front of a sequence indicates the beginning of an observed autolytic fragment. Absence of dashes following a sequence indicates lack of sequence information.

diisopropylfluorophosphate (DFP) even though it requires much more DFP and longer incubation time for complete inactivation which could indicate modification around the active site as compared to other serine proteases. It is also able to cleave a certain number of Phe-Arg, Phe-Lys, Arg-Tyr, Arg-Trp, Phe-His, Phe-Phe and Phe-Gly bonds in various substrates. The only other substitutions known so far in active site residues concern the serine protease homologue, haptoglobin devoid of any proteolytic activity, where the triad His, Asp, Ser is replaced by Lys, Asp, Ala[20]. Another explanation could be that the 16-residue deletion together with other amino acid substitutions could induce a conformational change sufficiently important as to bring close to the histidine and serine, another acidic residue not adjacent to Asp-102 in the sequence, but close in the three-dimensional structure. Even though intriguing, this explanation is difficult to reconcile with a deletion of similar size localized in the region of the plasmin enzyme that contains the normally encountered aspartic residue.

Final conclusions concerning both the importance of the deletion and of the substitution of Asp by Leu, will have to rely on current research on the three-dimensional structure of the enzyme.

Table 1 Homologies between serine proteases and rat submaxillary tonin

Enzymes	Species	No. of residues Compared	No. of residues Identical	Homology %	Chain length
Kallikrein[9] (submaxillary gland)	Rat	118	89	75.4	—
Kallikrein[10] (pancreatic)	Rat	237	170	71.1	237
Kallikrein[11] (submaxillary gland)	Murine	149	97	65.1	—
γ-Subunit NGF[12]	Murine	233	150	64.4	233
γ-Renin[13]	Murine	73	46	63.0	—
Kallikrein[14] (pancreatic)	Porcine	232	119	51.2	232
Trypsin[21]	Bovine	229	92	40.2	223
Plasmin[21]	Human	237	83	35.0	229
Chymotrypsin[21]	Bovine	240	76	31.7	231
Factor X[21]	Human	241	67	27.8	—
Elastase[21]	Porcine	245	68	27.8	240
Prothrombin[21]	Human	262	71	27.1	—
Chymotrypsin[22]	Insect	234	61	26.1	216
Trypsin[21]	Bacterial	237	61	25.7	221
Haptoglobin[20] (β-chain)	Human	252	55	21.8	245
Factor B[23]	Human	288	46	16.0	—

The number of residues compared includes gap positions assigned to maximize homologies between the tonin sequence and the various proteases. Superscript numbers indicate the reference number.

This work was supported by a Group Grant and a Program Grant (PG-2) from the Medical Research Council of Canada and NIH (MS 16-315-03).. C.L. is a 'chercheur-boursier' of the 'Fonds de la Recherche en Santé du Québec'. We thank Ms D. Marcil for secretarial assistance.

Note added in proof: Since submission of this manuscript work has clarified uncertainties at position 119–123. The results (included in Fig. 1) revealed the presence of an Asn-Pro-Ser-Glu-Met which could lead to N-glycosylation even though Bause and Legler[25] have shown that the Asn residue in such a sequence cannot be glycosylated because of the vicinal Pro.

Received 6 June; accepted 16 November 1983.

1. Demassieux, S., Boucher, R., Grisé, C. & Genest, J. *Can. J. Biochem.* **54,** 788–795 (1976).
2. Thibault, G. & Genest, J. *Biochim. biophys. Acta* **660,** 23–29 (1981).
3. Seidah, N. G. *et al. Biochem. biophys. Res. Commun.* **86,** 1002–1013 (1979).
4. Chrétien, M. *et al. FEBS Lett.* **113,** 173–176 (1980).
5. Seidah, N. G. *et al. Can. J. Biochem.* **56,** 920–925 (1978).
6. Lazure, C. *et al. Nature* **292,** 383–384 (1981).
7. Ikeda, M., Gutkowska, J., Thibault, G., Boucher, R. & Genest, J. *Hypertension* **3,** 81–86 (1981).
8. Lazure, C., Dennis, M., Rochemont, J., Seidah, N. G. & Chrétien, M. *Analyt. Biochem.* **125,** 406–414 (1982).
9. Tschesche, H. *et al.* in *Kinins-II* (eds Fujii, S., Moriya, H. & Suzuki, T.) 245–260 (Plenum, New York, 1979).
10. Richards, R. I., Catanzaro, D. F., Mason, A. J., Morris, B. J., Baxter, J. D. & Shine, J. *J. biol. Chem.* **257,** 2758–2761 (1982).
11. Lazure, C., Seidah, N. G., Thibault, G., Genest, J. & Chrétien, J. in *Proceedings of the seventh American Peptide Symposium* (eds Rich, D. H. & Gross, E.) 517–519 (Pierce Chemical, New York, 1981).
12. Swift, G. H., Dagorn, J. C., Ashley, P. L., Cummings, S. W. & MacDonald, R. J. *Proc. natn. Acad. Sci. U.S.A.* **79,** 7263–7268 (1982).
13. Thomas, K. A., Baglan, N. C. & Bradshaw, R. A. *J. biol Chem.* **256,** 9156–9166 (1981).
14. Poe, M. *et al. J. biol. Chem.* **258,** 2209–2216 (1982).
15. Bothwell, M. A., Wilson, W. H. & Shooter, E. M. *J. biol. Chem.* **254,** 7287–7294 (1979).
16. Thomas, K. A., Silverman, R. E., Jeng, I., Baglan, N. G. & Bradshaw, R. A. *J. biol. Chem.* **256,** 9147–9155 (1981).
17. Schenkein, I., Franklin, E. C. & Frangione, A. *Archs Biochem. Biophys.* **209,** 57–62 (1981).
18. Genest, M. & Ptak, M. *Int. J. Peptide Protein Res.* **19,** 420–431 (1982).
19. Polgar, L. & Bender, M. L. *Proc. natn. Acad. Sci. U.S.A.* **64,** 1335–1342 (1969).
20. Kurosky, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **77,** 3388 (1980).
21. Dayhoff, M. D. (Ed.) in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 3 (1978).
22. Jany, K. D., Bekelar, K., Pfleiderer, G. & Ishay, J. *Biochem. biophys. Res. Commun.* **110,** 1–7 (1983).
23. Christie, D. L. & Gagnon, J. *Biochem. J.* **209,** 61–70 (1983).
24. *Eur. J. Biochem.* **5,** 151–153 (1968).
25. Bause, E. & Legler, G. *Biochem. J.* **195,** 639–644 (1981).

Computer-based characterization of epidermal growth factor precursor

Russell F. Doolittle, Da Fei Feng & Mark S. Johnson

Department of Chemistry, University of California, San Diego, La Jolla, California 92093, USA

The cDNA sequence of the precursor of mouse epidermal growth factor (EGFP) has recently been reported by two groups[1,2], both of whom noted the presence of repeated similar segments, each about 40 residues long. One of these repeat units overlaps with the sequence of epidermal growth factor itself. The sequence of epidermal growth factor has been reported to be similar to that of pancreatic secretory trypsin inhibitor[3] (PSTI) and a somewhat better match has been found with part of the sequence of bovine factor X[4], one of the blood coagulating factors. We report here that there is an even stronger similarity between the sequences of some of the repeat units of epidermal growth factor precursor and certain segments in factor X. This sequence similarity is also apparent in comparisons with other blood coagulation factors. On the basis of these sequence comparisons we suggest a scheme for the evolution of the epidermal growth factor precursor. We have also identified certain structural features in the precursor sequence that bear on the way in which the mature epidermal growth factor is generated.

The EGFP precursor sequence was searched against 1,081 sequences (~120,000 residues) in the 1978 Dayhoff *Atlas*[4] and about 800 sequences (~160,000 residues) in Newat[5]. The search identified bovine factors X and IX (in the Dayhoff *Atlas*), and human factors X and IX and bovine protein C (in Newat) as having matchable segments. In addition the search patterns revealed a repeat segment of 41 ± 2 residues in the queried sequence, in good agreement with the size of the homologous segments reported by Gray *et al.*[1] and Scott *et al.*[2].

Table 1 Resemblances between homologous units in mouse EGFP and two adjacent segments of bovine factor X

EGFP Segment (residue)	Factor X_A (residues 44–84) % Identity	NAS	(s.d.)	Factor X_B (residues 85–126) % Identity	NAS	(s.d.)
a (22–64)	17.1	159	(0.2)	21.4	238	(2.7)
b (327–361)	34.3	314	(3.6)	37.1	357	(2.6)
c (362–402)	34.2	402	(4.8)	31.7	463	(10.5)
d (403–442)	22.5	288	(2.0)	32.5	475	(9.7)
e (443–484)	17.1	220	(0.4)	42.9	488	(10.2)
f (747–788)	29.3	354	(6.2)	35.7	500	(9.8)
g (838–876)	23.1	359	(4.1)	18.0	282	(1.5)
h (877–918)	31.7	354	(4.3)	26.2	286	(2.0)
i (919–960)	34.2	402	(6.2)	14.3	238	(1.3)
j (977–1,020)	31.7	378	(4.7)	19.1	238	(0.9)

Segments were compared by a computer program using a similarity measure based on the original algorithm of Needleman and Wunsch[6]. The normalized alignment score (NAS) reflects a penalty 2.5 × the value of every match (10 points) (Cys matches, 20 points); it is adjusted for 100 residues. Scores over 150 usually indicate common ancestry[5]. The statistical likelihood of chance resemblance was empirically determined by comparing multiple jumbled sequences of the same lengths and compositions. If the alignment score of the genuine sequences is greater than 3.0 standard deviations (s.d.) above the mean of the jumble comparisons, common ancestry is generally inferred[5]. In contrasting *A* and *B*, the higher NAS is underlined in each case. Note that EGFP segments *a–f* are more closely related to factor X segment *B*, whereas *g–j* more closely resemble factor X segment *A*. EGFP segment *j* overlaps the known sequence for EGF itself.

The nine segments from EGFP noted by Gray *et al.*[1] were designated *b–j*; a tenth homologue was denoted '*a*' (Fig. 1). The two corresponding segments in the zymogen proteases were denoted *A* and *B* (Fig. 2). The sequences were compared by both similarity[6] and difference[7,8] types of computer alignment schemes. Each segment was compared with every other, and various phylogenetic trees were constructed[9]. Comparisons of the 10 homologous EGFP units with the factor X segments are given in Table 1. Interestingly, the units nearer the amino terminus more closely resemble segment *B* of factor X than they do segment *A*, whereas the inverse is true of the units nearer the carboxy terminus.

The similarities of EGFP units *c–f* to factor X segment *B* are such that the authentic alignment scores lie +10 standard deviations above the average of comparison sets made with jumbled sequences of the same lengths and compositions (Table 1). These similarities are of the same degree as lysozyme–lactalbumin or insulin–relaxin[5] and are significantly better matches than the previously reported homology of EGF and PSTI (3) or, for that matter, EGF and factor X (4).

Several other homologies in EGFP were overlooked in the recent cDNA reports. There is a subtle but very significant homology between two large sections of the precursor sequence that are not in the cysteine-rich units (Fig. 1). The segment denoted *x* (residues 121–310) has a 30% identity with the segment denoted *y* (residues 561–746) (Fig. 1). It seems very likely that these two segments arose by internal duplication, a score of greater than +10 standard deviations being achieved when jumbled sequences of the same lengths and compositions were compared. In addition, there are four other shorter sequences that have similar sequences to each other; these are denoted *q–t* in Fig. 1.

There are a number of alternative explanations for the present structure of the EGF precursor. For example, it may have begun with a translocation of an 80-residue segment from the amino terminal region of factor X or a related zymogen. In this case the existence of the already duplicated segment (X_{AB}), would increase the chances of further mismatching and unequal crossing over. It is also possible that EGFP is the indirect result of

0028–0836/84/060558—03$01.00

© 1984 Macmillan Journals Ltd

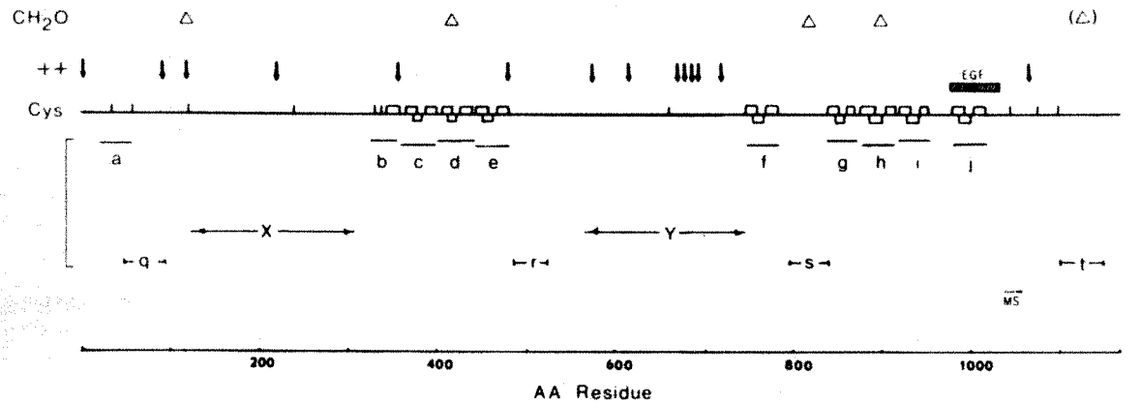


Fig. 1 Schematic depiction of mouse epidermal growth factor precursor (EGFP). The putative protein has 1,168 amino acids[1] (another report[2] suggests a sequence of 1,217 residues, but a comparison of the two cDNA sequences indicates that a difference of one base at position 3,751 puts the sequences out of register near the carboxy terminus). Three different kinds of homologous segments have been identified (*a–j*; *x–y*; *q–t*). These include nine previously noted homologous units designated *b–j*, and a tenth unit, less obvious, denoted *a*; EGF (residues 977–1,029) overlaps unit *j*. Two longer segments in EGFP are also homologous and are designated *x* and *y*. Finally, four other short segments are similar to each other and are denoted *q-t*. The disulphide bond arrangements in units *c–i* are patterned on the known arrangement in EGF (unit *j*). Other cysteines (Cys), including one in a predicted membrane-spanning segment (residues 1,039–1,059), have been left unpaired even though most are likely in the form of disulphide bonds. Triangles indicate consensus carbohydrate-attachment points; if the model described in the text is correct, only those situated outside the plasma membrane will be derivatized. Arrows denote 'double-basic' (Arg–Arg, Arg–Lys, and so on) potential proteolysis points.



Fig. 2 Alignment of segments *A* and *B* from blood coagulation zymogens and 10 homologous segments from epidermal growth factor precursor (EGFP). Cysteine residues are shown in lower case. Underlined segments are predicted as turns by two different computer programs (broken lines predicted by only one of the two predictive programs). See Fig. 1 for locations of EGFP segments.

two separate exchanges with the factor X gene. This interpretation is in better agreement with the observation that all the homologus EGFP peptide segments nearer the amino terminus resemble unit *B* in factor X more than they do unit *A*, whereas the inverse is true of the segments nearer the carboxy terminus. If a DNA segment encoding an 80-residue previously duplicated twin had been the initiating material, then one might have expected, in whole or part, an alternating arrangement of homologous segments according to how much they resembled factor X_A and X_B, respectively.

The EGFP sequence was subjected to analysis by computer programs thought to be predictive of secondary structure[10,11]. We were particularly interested in predicting 'turns', which are among the more accurately assignable features in proteins, for if the disulphides in each of the homologues exist in the same arrangements as occur in EGP[12], then they would be constrained to turn back on themselves three times. In fact, the remarkable agreement of predicted turns in the 10 homologues (Fig. 2) suggests that all of the units are tightly folded and that the disulphide pairings within them are similar. The six-cysteine 40-residue segments being examined here should not be confused with the six-cysteine 80-residue configurations commonly referred to as 'kringles', of which prothrombin contains two, but factor IX, factor X or protein C apparently contain none[13].

The 1,168-residue sequence of EGFP was also analysed by a computer program that provides a moving average of its hydropathy[14]. The profile closely resembled those of known membrane-attached glycoproteins[15] in that a likely membrane-spanning segment was predicted for residues 1,039–1,058. In addition, a characteristic signal peptide was projected for residues 9–29, as suggested by Scott *et al.*[2]. EGFP may exist on the surface of the cells of its origin, anchored by the spanning segment, and with residues 1,060–1,168 remaining inside the cell. Release of active EGF would occur as the result of limited proteolysis of the tethered precursor.

The known active EGF molecule corresponds to the 53-residue segment numbered 977–1,029. The segment is obviously released from the precursor (or some fragment thereof) by cleavage of an arginyl–asparagine bond at its amino terminus and an arginyl–histidine bond at its carboxy terminus. Neither of these dipeptidyl junctions occurs at equivalent positions around the other nine homologous segments, nor, do arginyl junctions of any type. In fact, if the disulphide pairing is analogous to that known to exist in EGF (segment *j*), then there is no possibility that tryptic-like cleavages would release EGF-like fragments from the nine homologues.

The topology of a computer-derived tree indicates that EGFP evolved before or about the time of the divergence of factors IX, X and protein C but definitely after their divergence from prothrombin. These particular blood coagulation zymogens belong to a small subset of vertebrate plasma proteins that contain clusters of the unusual amino acid γ-carboxyglutamic acid near their amino termini. The duplicated segments under discussion here are actually adjacent to the γ-carboxyglutamic acid region. Protein C and factors IX and X also contain the unusual amino acid β-hydroxyaspartic acid[16,17], and in this case the unusual amino acid is actually situated in segment *A*. None of the EGFP homologous units has aspartic acid at the equivalent positions, however.

The divergence times can also be estimated from a consideration of the sequence differences between mouse and human EGF[4]. Knowing that rodents and primates last shared a common ancestor 80 Myr ago, and that the sequences of their EGFs differ in 17 of 53 residues, we can estimate an approximate rate of change. If we assume that the EGF-like units in the rest of the precursor protein have been changing at about the same rate, then the two most similar of these units, *c* and *d*, which differ by about 60%, diverged about 200 Myr ago. Two quite dissimilar units, *b* and *g*, are 80% different and by this measure would have diverged about 500 Myr ago. However, the (presumably) non-functional homologues may have been changing significantly faster than EGF itself. Nonetheless several of the homologous units resemble the zymogen segments more closely than does EGF. Given common ancestry, they have either been more rigorously conserved or have diverged on an independent and more recent occasion.

By either of these criteria EGF is an early vertebrate creation. The data agree with a gene encoding a segment of some primitive factor X-like molecule having been partially duplicated and translocated elsewhere in the genome. The transferred unit, whether corresponding to a 40- or an 80-residue segment, may have included parts of surrounding introns that subsequently became expressible as a result of the translocation. It seems reasonable that this new unit encoded a membrane-anchored protein from its inception and that limited proteolysis released a primitive EGF-like molecule. By chance the gene produced must have bound, however weakly, to an existing receptor for some other (growth) factor. Interestingly, factor IX binds to several cell types including fibroblasts[18]. From that point on the receptors and growth factors diverged in parallel, as is observed for many other biological systems. The existence of two similar sequences in the originally transposed unit would have facilitated further mismatching and tandem elongation.

It has recently been reported that a tumour growth factor from several sources is homologous with EGF[19]. The degree of similarity found is less than that observed between several of the EGP homologues and the corresponding segments in the zymogen clotting factors. Although the full sequences of these tumour growth factors are not known, the data available suggest that they are about 30% similar to EGF itself; the resemblance is less when the tumour growth factors are compared with any of the EGFP homologues (*a–i*), suggesting that the tumour growth factors have not arisen directly from any of the other homologus units in EGFP. Rather, it is consistent with the tumour growth factor and EGFP arising as the result of duplication of a common ancestral gene. As such it can be expected that they will also have precursors that are anchored in the membranes of their cells of manufacture and will be activated by a scheme of extracellular proteolysis.

This work was supported by NIH grants HL 18,576 and RR 00757.

Received 26 August; accepted 2 December 1983.

1. Gray, A., Dull, T. J. & Ullrich, A. *Nature*, **303**, 722–725 (1983).
2. Scott, J. *et al. Science* **221**, 236–240 (1983).
3. Hunt, L. T., Barker, W. & Dayhoff, M. O. *Biochem. biophys. Res. Commun.* **60**, 1020–1028 (1974).
4. Dayhoff, M. O. *Atlas of Protein Sequences and Structures* Vol. 5 & Suppl. 1–3 (1978).
5. Doolittle, R. F. *Science* **214**, 149–159 (1981).
6. Needleman, S. B. & Wunsch, C. D. *J. molec. Biol.* **48**, 443–53 (1970).
7. Waterman, M. S., Smith, T. F. & Beyer, W. A. *Adv. Math.* **20**, 367–387 (1976).
8. Gotoh, O. *J. molec. Biol.* **162**, 705–8 (1982).
9. Fitch, W. M. & Margoliash, E. *Science* **155**, 279–284 (1967).
10. Chou, P. Y. & Fasman, G. D. *A. Rev. Biochem.* **47**, 251–276 (1978).
11. Garnier, J., Osguthorpe, D. J. & Robson, B. *J. molec. Biol.* **120**, 97–120 (1978).
12. Savage, C. R., Hash, J. H. & Cohen, S. *J. biol. Chem.* **248**, 7669–7672 (1973).
13. Katayama, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 4990–4994 (1979).
14. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105–132 (1982).
15. Rose, J. K. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 3884–3888 (1980).
16. Stern, D. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4119–4123 (1983).
17. Drakenberg, T. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 1802–1806 (1983).
18. McMullen, B. A. *et al. Biochemistry* **22**, 2875–2884 (1983).
19. Marquardt, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4684–4688 (1983).

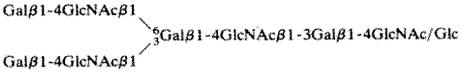
Erythrocyte receptors for *Mycoplasma pneumoniae* are sialylated oligosaccharides of Ii antigen type

Lesley M. Loomes*, Kei-ichi Uemura*§, Robert A. Childs*, James C. Paulson†, Gary N. Rogers†, Peter R. Scudder*, Jean-Claude Michalski‡, Elizabeth F. Hounsell*, David Taylor-Robinson* & Ten Feizi*

* Division of Communicable Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK
† Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, California 90024, USA
‡ Laboratoire de Chimie Biologique, Université des Sciences et Techniques de Lille I, Villeneuve d'Ascq Cedex, France

Among the pathological effects in man following infection with *Mycoplasma pneumoniae* is a transient autoimmune disorder characterized by the presence of high-titre erythrocyte autoantibodies (cold agglutinins)[1,2]. These autoantibodies are usually directed against the carbohydrate antigen termed I (ref. 3) which consists of a branched oligosaccharide[4,5]. The mechanism by which the anti-I antibodies are elicited is unknown. However, sialic acid-containing receptors have been implicated in the adherence of *M. pneumoniae* to erythrocytes and other cell types[6], and both I and the related antigen i occur on erythrocytes in sialylated form[7]: i is the predominant antigen on fetal erythrocytes and I is predominant in adults[8]. Anti-I antibodies might arise in *M. pneumoniae* infection in response to a modification of the 'self' antigen-I as a result of its interaction with this agent[9,10]. Here we report our study of the specificity of the interaction of *M. pneumoniae* with human erythrocytes. We found that this interaction is mediated by long chain oligosaccharides of sialic acid joined by α2–3 linkage to the terminal galactose residues of poly-*N*-acetyllactosamine sequences of Ii antigen type.

Antigen I is built of repeating *N*-acetyllactosamine (Galβ1-4G1cNAc) units as in structure *a* below[4,5] and antigen i of linear poly-*N*-acetyllactosamine sequences[11] as in structure *b* (Gal, galactose; Glc, glucose; GalNac, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid).



Structure *a*

Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc/Glc Structure *b*

The importance of sialic acid as a component of the erythrocyte receptor for *M. pneumoniae* was confirmed by the reduction of the binding of neuraminidase-treated erythrocytes to 10% of that of untreated erythrocytes (Table 1). The preferen-

§ Present address: Department of Biochemistry, Institute of Adaptation Medicine, Shinshu University, Matsumoto 390, Japan.

0028–0836/84/060560—04$01.00

© 1984 Macmillan Journals Ltd

Table 1 Comparison of the binding of native, 'asialo' and resialylated erythrocytes to sheet cultures of *M. pneumoniae*

Erythrocytes	*N*-acetylneuraminic acid (nmol ml^{-1}) Content	Incorporated	c.p.m. added ($\times 10^{-3}$)	c.p.m. bound ($\times 10^{-3}$)	% Binding
Native	720		5,100	97.5	2
'Asialo'	40		4,500	8.9	0.2
Resialylated					
Galβ1-4/3GlcNAc |α2,3 NeuAc		75	1,100	151	13.6
Galβ1-4GlcNAc |α2,6 NeuAc		75	4,500	71	1.6
Galβ1-3GalNAc |α2,3 NeuAc		260	3,900	19	0.5

Human blood group O erythrocytes of I type were desialylated[38,39] using neuraminidase from *Vibrio cholerae* (Behringwerke) which released approximately 95% of the total sialic acid. Resialylation was achieved using donor CMP-NeuAc and one of the following purified enzymes: Galβ1-3/4GlcNAc α2-3-sialyltransferase[40,41], Galβ1-4GlcNAc α2-6-sialyltransferase[40,41] and Galβ1-3GalNAc α2-3-sialyltransferase[42]. The *N*-acetylneuraminic acid (NeuAc) content (per ml of packed cells) was estimated as described previously[38]. For radiolabelling, erythrocytes (1×10^9) were washed twice in Tris-buffered saline (TBS) containing 0.14 M NaCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.01 M Tris-HCl *p*H 7.2 and incubated at 37 °C for 45 min with 100 μl of TBS containing 500 μCi sodium ^{51}Cr-labelled chromate (350–600 mCi mg^{-1} Cr) (Amersham). The cells were washed three times and resuspended in 100 μl of TBS. ^{51}Cr incorporation was in the range $1–10\times10^7$ c.p.m. per 1×10^9 erythrocytes as determined using a LKB Wallac 80,000 γ-counter. Sheet cultures were obtained using a strain of *M. pneumoniae*, MY 9862, which had been passed 10 times in mycoplasma liquid medium[43], once in hamster lung and then twice more in mycoplasma medium. Sheet cultures were prepared in 16×24 multiwell plates (Costar) by inoculating with 0.25 ml actively growing *M. pneumoniae* liquid culture (2.5×10^6 colour-changing units) per well containing 1 ml mycoplasma liquid medium. Plates were incubated at 37 °C until confluent sheets were obtained (3–4 days). The protein content of washed mycoplasma sheets, after solubilization in 1 M NaOH, was 20–30 μg per well; this was determined[44] using bovine serum albumin as a standard. For binding assays, the mycoplasma monolayers or uninoculated wells containing medium alone were washed twice with 1 ml TBS, then ^{51}Cr-labelled erythrocytes (1×10^7 cells in 300 μl) were added (in triplicate). The c.p.m. added varied with the erythrocyte preparations due to their differing incorporation of ^{51}Cr. The plates were incubated at 37 °C for 1 h and unattached erythrocytes were removed by washing each well with 3×1 ml of TBS. The attached erythrocytes in each well were solubilized in 1 ml of 1 M NaOH and the radioactivity counted for 30 s. The radioactivity bound to medium control wells (0.2–0.5% of c.p.m. added) was subtracted from that bound to the mycoplasma monolayers.

tial binding of *M. pneumoniae* to sialic acid joined by α2–3 linkage to galactose was clearly shown with the resialylated erythrocytes as follows. First, compared with 'asialo' erythrocytes, those having the NeuAcα2-3Galβ1-4/3GlcNAc sequence had a 68-fold greater binding while those having the NeuAcα2-6Galβ1-4GlcNAc and NeuAcα2-3Galβ1-3GalNAc sequences had only 8- and 2.5-fold greater binding, respectively (Table 1). Thus, erythrocytes having the NeuAcα2-3Galβ1-4/3GlcNAc sequence had 8.5-fold greater binding than those with the NeuAcα2-6Galβ1-4GlcNAc sequence. Second, compared with the native erythrocytes, the NeuAcα2-3Galβ1-4/3GlcNAc-derivatized erythrocytes showed almost sevenfold greater binding despite their lower sialic acid content. A major sialylated product obtained with the Galβ1-4/3GlcNAc α2-3-sialyltransferase (unpublished observations) as with the Galβ1-4GlcNAc α2-6-sialyltransferase[12], corresponds to band 3 glycoprotein. Thus, the supranormal binding of erythrocytes derivatized using the Galβ1-4/3GlcNAc α2-3-sialyltransferase can be explained in terms of the derivatization of band 3 oligosaccharides that are not naturally sialylated to this extent. Third, erythrocytes derivatized using the Galβ1-3GalNAc α2-3-sialyltransferase showed negligible binding above the 'asialo' erythrocytes despite their greater than six-fold content of sialic acid. This enzyme acts predominantly on the 0-glycosidically linked oligosaccharides of glycophorin A[12].

The specificity of the *M. pneumoniae*–erythrocyte interaction was next investigated using oligosaccharides and glycoproteins as inhibitors of binding (Table 2). The preference for sialic acid α2-3-linked rather than α2-6-linked to galactose as a receptor, sequence was confirmed with the two α2-3-linked isomers (Table 2, structures *d* and *f*) which were seven and nine times, respectively, more active as inhibitors than the corresponding α2-6 isomers (Table 2, structures *e* and *g*). Moreover, sialic acid α2-6-linked to *N*-acetylgalactosamine was ruled out as a receptor sequence for *M. pneumoniae* as none of the three submaxillary mucins tested gave inhibition. These glycoproteins are known to contain sialic acid predominantly α2-6-linked to *N*-acetylgalactosamine[13–16].

In its strong preference for sialic acid α2-3-linked to galactose residues, *M. pneumoniae* differs from *Mycoplasma gallisepticum* which does not distinguish between sialic acid α2-3-linked to galactose or α2-6-linked to *N*-acetylgalactosamine[17]. In contrast to our findings with *M. pneumoniae* (Table 2), the submaxillary mucins of ovine, bovine and porcine origins were found[17] to be effective inhibitors of the binding of *M. gallisepticum* to erythrocytes.

The trisaccharide sequence α2-3-*N*-acetylneuraminosyllactosamine (Table 2 as in structure *d*) was almost three times as active as α2-3-*N*-acetylneuraminosyllactose (structure *f*) indicating that *N*-acetylglucosamine is part of the receptor sequence. The inhibition experiments summarized in Table 2 show the strikingly strong inhibitory activity of oligosaccharides and glycoproteins containing the repeating *N*-acetyllactosamine sequence. Thus, oligosaccharide structure *c* containing two *N*-acetyllactosamine units was five times more active than structure *d* with a single unit. Similarly, among the glycoproteins tested, the most potent inhibitors were the polyglycosyl peptides of human erythrocytes (largely derived from bands 3 and 4.5) and the bovine erythrocyte glycoprotein GP-2, both of which are rich in branched poly-*N*-acetyllactosamine sequences of I antigen type (refs 18–20 and Y. Suzuki, H. C. Gooi & T.F., unpublished observations). Human glycophorin A and α_1-acid glycoprotein were substantially less active and fetuin was inactive, in accordance with their lack of poly-*N*-acetyllactosamine sequences[21–26]. Inhibition experiments with glycolipids (to be described elsewhere) have also indicated that sialylated poly-*N*-acetyllactosamine sequences are the preferred sequences for *M. pneumoniae* binding and that the sialic acid residues can be of *N*-acetyl or *N*-glycolyl type.

Although our results and those of others[27,28] have shown that solubilized glycophorin A as well as glycolipids and oligosaccharides having short chain sequences can inhibit the binding

of erythrocytes to *M. pneumoniae*, the following experiments showed that they do not serve as receptors for this agent on intact erythrocyte membranes. First, glycophorin-deficient cells, termed En(a$^-$), showed normal mycoplasma binding (Table 3); these cells have a 30–50% reduction in their sialic acid levels[29,30]. Second, following Pronase digestion of erythrocytes, which results in the loss of almost all the glycosylated portions of the major sialoglycoproteins (D. J. Anstee & M. J. A. Tanner, personal communication) but not of band 3 protein[31], the binding of mycoplasma was not reduced and was even slightly increased (Table 3). The carbohydrate chains of band 3 protein, but not those of the sialoglycoproteins, are known to contain poly-*N*-acetyllactosamine sequences having I antigen activity[19,20]. Third, erythrocytes treated with endo-β-galactosidase showed reduced binding to the *M. pneumoniae* sheets (Table 3), indicating that long chain, poly-*N*-acetyllactosamine sequences of Ii type[32] are the main receptors for this agent. The reduction in binding reflected the content of endo-β-galactosidase-susceptible poly-*N*-acetyllactosamine sequences in the three cell types as follows: the binding of normal and En(a$^-$) erythrocytes of I type was reduced to 44% and 43%, respectively, of untreated cells (Table 3), in accordance with the predominance in these cells of branched oligosaccharides of poly-*N*-acetyllactosamine type which are only partially susceptible[20,33] to endo-β-galactosidase. The availability of the variant erythrocytes of i-antigen type provided the strongest evidence for the importance of sialylated poly-*N*-acetyllactosamine sequences as receptors for *M. pneumoniae*. Cells of i type have a high content of linear poly-N-acetyllactosamine sequences which are more susceptible to digestion with endo-β-galactosidase than the corresponding branched sequences on erythrocytes of I type[20,33]. The binding of these cells was reduced by 85% following treatment with endo-β-galactosidase. This is remarkable as only 5% of the total erythrocyte sialic acid is released by this glycosidase[20]. Thus, minor sialylated oligosaccharides of the poly-*N*-acetyllactosamine series, such as those carried by glycoprotein bands 3 and 4.5, and glycolipids[20] are the main receptors for *M. pneumoniae* rather than the carbohydrate chains of the major sialoglycoproteins which are not susceptible to digestion by endo-β-galactosidase.

Table 2 Inhibition of binding of ^{51}Cr-labelled erythrocytes to sheet cultures of *M. pneumoniae* by oligosaccharides and glycoproteins

Designation	Inhibitors: Oligosaccharides	Concentration*
c	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc-Asn \|α2,3 NeuAc	16
d	Galβ1-rGlcNAc-Asn \|α2,3 NeuAc	88
e	Galβ1-4GlcNAc-Asn \|α2,6 NeuAc	640
f	Galβ1-4Glc \|α2,3 NeuAc	245
g	Galβ1-4Glc \|α2,6 NeuAc	2,136
h	NeuAc	NA
j	Galβ1-4Glc†	NA
	Glycoproteins	
	Polyglycosyl peptides	0.9
	Bovine erythrocyte sialoglycoprotein (GP-2)	2.5
	Glycophorin A	12.6
	α_1-acid glycoprotein	47.7
	Bovine fetuin	NA
	Submaxillary mucins; bovine, ovine, porcine	NA
	Bovine serum albumin†	NA

Structures *c*, *d* and *e* (asparaginyl oligosaccharides) were isolated from the urine of patients with aspartylglucosaminuria (J-C.M., J. Montreuil, G. Strecker, H. van Halbeek and J. Vliegenthart, in preparation). Structure *g*, NeuAcα2-6 isomer of sialosyllactose, isolated from human milk, was given by the late Dr Adeline Gauhé. Structure *f*, NeuAcα2-3 isomer of sialosyllactose, and structure *j*, *N*-acetylneuraminic acid and lactose, were from Sigma. The preparation of α2-6 isomer of sialosyllactose was found to contain 11% free *N*-acetylneuraminic acid and the preparation of α2-3 isomer contained 10% α2-6 isomer as assessed by HPLC[45]. The inhibition values shown for these two isomers of sialosyllactose have been corrected to allow for the impurities. Polyglycosyl peptides, obtained by pronase digestion of human erythrocyte membranes[18], were given by Dr J. Finne; the sugar composition and their molar ratios were fucose 3.6: mannose 3.0: galactose 13.9: *N*-acetylglucosamine 15.5: *N*-acetylneuraminic acid 2.8, (approximately 3 mol mannose per mol glycopeptide). The bovine erythrocyte sialoglycoprotein, GP-2, which is a strong inhibitor of haemagglutination by Sendai virus[46], was given by Dr Y. Suzuki. This glycoprotein contains 70% carbohydrate by weight; the monosaccharides *N*-acetylgalactosamine, *N*-acetylglucosamine, galactose and sialic acid (*N*-glycolylneuraminic acid 96%; *N*-acetylneuraminic acid 4%) are present in molar ratios of 1.0:4.0:5.2:2.9. The backbone sequence is deduced to be a branched poly-*N*-acetyllactosamine structure from the strong I antigen activity expressed on asialo GP-2 (Y. Suzuki, H. C. Gooi & T.F., unpublished observations). Glycophorin A from human erythrocyte membranes[47] was a gift of Dr C. G. Gahmberg. Human α_1-acid glycoprotein was a gift from Dr K. Schmid. Bovine fetuin type IV was from Sigma. Ovine submaxillary mucin was from Dr D. van den Eijnden; bovine and porcine submaxillary mucins from Drs A. Corfield and R. Schauer; and bovine serum albumin (fraction V) from Miles Laboratories. Inhibition of binding assays were performed as follows: washed mycoplasma monolayers were preincubated with 300 μl of serial dilutions of inhibitors(in triplicate) in TBS at 37 °C for 1 h before the addition of 300 μl of ^{51}Cr-labelled erythrocytes (1×10^6 cells containing $1–10\times10^4$ c.p.m.). All subsequent steps were as for the binding assays (Table 1). Inhibition was calculated as the per cent reduction in binding relative to control wells which contained no inhibitor.

NA, No inhibition at the highest levels tested as follows: NeuAc 3,236 nmol; bovine fetuin 292 nmol sialic acid; submaxillary mucins; bovine 1,070, ovine 1,050 and porcine 470 nmol sialic acid. The relatively high inhibitory activities of the glycoproteins reflect the cooperative effects of multivalence of their carbohydrate chains.

* Concentration expressed as nmol sialic acid ml^{-1} giving 50% inhibition.

† Lactose, structure *j* (2,900 nmol oligosaccharide) and the non-glycosylated protein; bovine serum albumin (1 mg) served as non-sialylated controls.

Table 3 Effect of Pronase and endo-β-galactosidase treatment on binding of ^{51}Cr-labelled erythrocytes of OI, Oi and En(a$^-$) types to sheet cultures of *M. pneumoniae*

Erythrocytes	c.p.m. added ($\times10^{-2}$)	c.p.m. bound ($\times10^{-2}$)	% Binding
OI-untreated	800	57	7.1
Pronase	800	72	9.0
Endo-β-galactosidase	1,000	31	3.1
Oi adult-untreated	1,000	68	6.8
Endo-β-galactosidase	600	6	1.0
En(a$^-$)-untreated	1,000	71	7.1
Endo-β-galactosidase	1,000	30	3.0

Blood group OI erythrocytes were from a normal donor. Blood group O erythrocytes of the rare i phenotype[48] were from an adult deficient in I antigen [Oi (adult)]. Erythrocytes from an adult genetically deficient in the major erythrocyte sialoglycoprotein, glycophorin A (designated En(a$^-$)[49]) were given by Dr D. Anstee. For treatment with endo-β-galactosidase, 150 μl packed erythrocytes were washed three times in PBS and incubated at 37 °C for 3 h with 50 μl of PBS containing 6 mU of endo-β-galactosidase from *Bacillus fragilis*[50]. This enzyme cleaves poly-*N*-acetyllactosamine-type oligosaccharides containing the sequence GlcNAcβ1-3Galβ1-. The cells were then washed three times in PBS. For treatment with Pronase (Sigma, type VI), 150-μl packed cells were incubated at 37 °C for 30 min with 150 μl PBS containing 2 mg ml^{-1} Pronase; they were then washed three times in PBS. Radiolabelling of the untreated and enzyme-treated erythrocytes with ^{51}Cr and binding to mycoplasma monolayers was as described in the legend to Table 1; 3×10^6 erythrocytes were added to each monolayer.

The present studies further support the concept that the post-infective autoantibodies (anti-I) elicited following infection with *M. pneumoniae* occur as a result of the interaction of I antigen with the mycoplasma and the presentation of this 'self' antigen in an immunogenic form to the immune system of the host. This was first suggested in 1969 when we (T.F., D.T-R. and colleagues) immunized rabbits with human group OI erythrocytes which had been treated with live *M. pneumoniae* and about 30% of the rabbits developed high titres of anti-I antibodies[34]. More recently, we have obtained evidence that *M. pneumoniae* cultures absorb I-active glycolipids from serum-containing culture media. These observations now account for previous reports of I-antigen activity associated with laboratory strains of *M. pneumoniae*[35,36].

In view of the observation that *M. pneumoniae* can react with erythrocytes of both I and i type, it is intriguing that anti-I

antibodies rather than anti-i antibodies are elicited following infection with this agent. A possible explanation is that *M. pneumoniae* infection occurs usually in children and adults rather than neonates[37] and the i type oligosaccharides of fetal erythrocytes are largely replaced by I type structures in the first year of life[8,20]. Thus *M. pneumoniae* would encounter and adhere to I- rather than i-type erythrocytes and possibly other cells of I type in the inflamed bronchial epithelium.

The principles established with the *M. pneumoniae*–erythrocyte receptor system could apply to a variety of receptor systems where inhibition and cell binding data have been difficult to reconcile. Also, the complexing of adhesive molecules of infective agents to specific saccharides of host cell membranes may be an important mechanism for the eliciting of auto-antibodies with anti-receptor specificities.

L.M.L. holds a MRC PhD studentship; K.U. was a Guest Research Fellow of the Royal Society and P.R.S. is supported by the Cancer Research Campaign. We thank Drs A. Corfield, D. van den Eijnden, J. Finne, C. G. Gahmberg, J. Montreuil, Y. Nagai, R. Schauer, K. Schmid and G. Strecker for providing valuable glycoproteins, glycolipids and oligosaccharides and Mrs Maureen Moriarty for help in preparing the manuscript.

Received 20 September; accepted 16 November 1983.

1. Chanock, R. M., Mufson, M. A., Somerson, N. L. & Couch, R. B. *Am. Rev. Resp. Dis.* Suppl. 2, **88,** 218–231 (1962).
2. Feizi, T. *Ann. N.Y. Acad. Sci.* **143,** 801–812 (1967).
3. Feizi, T. & Taylor-Robinson. D. *Immunology* **13,** 405–409 (1967).
4. Watanabe, K., Hakomori, S., Childs, R. A. & Feizi, T. *J. biol. Chem.* **254,** 3221–3228 (1979).
5. Feizi, T., Childs, R. A., Watanabe, K. & Hakomori,, S. *J. exp. Med.* **149,** 975–980 (1979).
6. Razin, S., Kahane, I., Banai, M. & Bredt, W. *Ciba Fdn Symp.* **80,** 98–118 (1981).
7. Feizi, T., Childs, R. A., Hakomori, S. & Powell, M. E. *Biochem. J.* **173,** 245–254 (1978).
8. Marsh, W. L. *Br. J. Haemat.* **7,** 200–209 (1961).
9. Feizi, T. *Med. Biol.* **58,** 123–127 (1980).
10. Feizi, T. *Ciba Fdn Symp.* **80,** 117–118 (1981).
11. Niemann, H., Watanabe, K., Hakomori, S., Childs, R. A. & Feizi, T. *Biochem. biophys. Res. Commun.* **81,** 1286–1293 (1978).
12. Paulson, J. C., Sadler, J. E. & Hill, R. L. *J. biol. Chem.* **254,** 2120–2124 (1979).
13. Tettamanti, G. & Pigman, W. *Archs Biochem. Biophys.* **124,** 41–50 (1968).
14. Bertolini, M. & Pigman, W. *Carbohyd. Res.* **14,** 53–63 (1970).
15. Carlson, D. M. *J. biol. Chem.* **243,** 616–626 (1968).
16. Glathmann, W. D. & Aminoff, D. *Biochem. biophys. Res. Commun.* **100,** 1453–1458 (1981).
17. Glasgow, L. R. & Hill, R. L. *Infect. Immunity* **30,** 353–361 (1980).
18. Krusius, T., Finne, J. & Rauvala, H. *Eur. J. Biochem.* **92,** 289–300 (1978).
19. Childs, R. A., Feizi, T., Fukuda, M. & Hakomori, S. *Biochem. J.* **173,** 333–336 (1978).
20. Fukuda, M. N., Fukuda, M. & Hakomori, S. *J. biol. Chem.* **254,** 5458–5465 (1979).
21. Tomita, M., Furthmayr, H. & Marchesi, V. T. *Biochemistry* **17,** 4756–4769 (1978).
22. Yoshima, H., Furthmayr, H. & Kobata, A. *J. biol. Chem.* **255,** 9713–9718 (1980).
23. Thomas, D. B. & Winsler, R. J. *J. biol. Chem.* **244,** 5943–5946 (1969).
24. Schmid, K. in *Plasma Proteins* Vol. 1. (ed. Putman, F.) 183–228 (Academic, New York, 1975).
25. Schmid, K. *et al. Biochim. biophys. Acta* **581,** 356–359 (1979).
26. Nilsson, B., Norden, N. E. & Svensson, S. *J. biol. Chem.* **254,** 4545–4553 (1979).
27. Chandler, D. K. F., Grabowski, M. W. & Barile, M. F. *Infect. Immun.* **38,** 598–603 (1982).
28. Banai, M., Razin, S., Bredt, W. & Kahane, I. *Infect. Immun* **30,** 628–634 (1980).
29. Tanner, M. J. & Anstee, D. J. *Biochem. J.* **153,** 271–277 (1976).
30. Gahmberg, C. G., Myllyla, G., Leikola, J., Pirkola, A. & Nordling, S. *J. biol. Chem.* **251,** 6108–6116 (1976).
31. Cabantchick, Z. I. & Rothstein, A. *J. Membrane Biol.* **15,** 227–248 (1974).
32. Reizi, T. *Immunol. Commun.* **10,** 127–156 (1981).
33. Scudder, P., Uemura, K., Fukuda, M. N. & Feizi, T. in *Proc. 7th int. Symp. on Glycoconjugates* (eds Chester, M. A., Heinegard, D., Lundblad, A. & Svensson, S.) 484–485 (Rahms, Lund, 1983).
34. Feizi, T., Taylor-Robinson, D., Shields, M. D. & Carter, R. A. *Nature* **222,** 1253–1256 (1969).
35. Costea, N., Yakulis, V. J. & Heller, P. *Proc. Soc. exp. Biol. Med.* **139,** 476–479 (1972).
36. Lind, K. *Acta path. microbiol. scand. Sect. B,* 4966–4975 (1973).
37. Clyde, W. A. Jr in *The Mycoplasmas* Vol. II (eds Tully, J. G. & Whitcomb, R. F.) (Academic, New York, 1979).
38. Carroll, S. M., Higa, H. H. & Paulson, J. C. *J. biol. Chem.* **256,** 8357–8363 (1981).
39. Rogers, G. N. & Paulson, J. C. *Virology* **127,** 361–373 (1983).
40. Weinstein, J., De Souza-e-Silva, U. & Paulson, J. C. *J. biol. Chem.* **257,** 13835–13844 (1982).
41. Weinstein, J., De Souza-e-Silva, V. & Paulson, J. C. *J. biol. Chem.* **257,** 13845–13853 (1982).
42. Sadler, J. E., Rearick, J. I., Paulson, J. C. & Hill, R. L. *J. biol. Chem.* **254,** 4434–4443 (1979).
43. Manchee, R. J. & Taylor-Robinson, D. *J. gen. Microbiol.* **50,** 465–478 (1968).
44. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193,** 265–275 (1951).
45. Burgh, M. L., Koppen, P. & van den Eijnden, D. H. *Carbohyd. Res.* **94,** 225–229 (1981).
46. Suzuki, Y., Suzuki, T. & Matsumoto, M. *J. Biochem.* **93,** 1621–1633 (1983).
47. Gahmberg, C. G., Jokinen, M. & Andersson, L. C. *J. biol. Chem.* **254,** 7442–7448 (1979).
48. Race, R. R. & Sanger, R. in *Blood Groups in Man* 6th edn, 447–462 (Blackwell, Oxford, 1975).
49. Anstee, D. J., Barker, D. M., Judson, P. A. & Tanner, M. J. A. *Br. J. Haemat.* **35,** 309–320 (1977).
50. Scudder, P., Uemura, K., Dolby, J., Fukuda, M. N. & Feizi, T. *Biochem. J.* **213,** 485–494 (1983).

Stability of expression-linked surface antigen gene in *Trypanosoma equiperdum*

Gregory A. Buck*, Shirley Longacre*, Anna Raibaud*, Urszula Hibner*, Christine Giroud†, Theo Baltz†, Dominique Baltz† & Harvey Eisen*

* Unité d'Immunoparasitologie, Institut Pasteur, 75724 Paris, Cedex 15, France
† Laboratoire d'Immunologie et de Biologie Parasitaire, Université de Bordeaux II, 33000 Bordeaux, France

African trypanosomes evade clearance in immune-competent hosts by periodically replacing their major surface glycoprotein with an antigenically different glycoprotein[1–4]. Expression of many of these variant surface glycoproteins (VSGs) is associated with the duplication and transposition of silent basic copy genes (BCs) into unlinked genomic expression sites[5–10]. The new expression-linked VSG gene copies (ELCs) are oriented with their 3′ ends proximal to chromosome telomeres[11–14]. Other VSG genes are activated without the production of an ELC[10,15–18]. The 3′ ends of these VSG genes are near chromosome telomeres both when they are active and when they are inactive. Recently, we have shown that activation of the VSG-1 gene in the BoTaR (Bordeaux trypanozoon antigen repertoire) serodeme of *Trypanosoma equiperdum* involves the duplication and transposition of a telomeric BC gene into one of at least three unlinked telomeric sites[9]. Here we show that the VSG-1 ELC is inactivated but not eliminated in some antigenic variants derived from a VSG-1 expressor. In addition, a subsequent variant that again expresses VSG-1 has not reactivated the residual VSG-1 ELC (R-ELC), but instead contains a new, active VSG-1 ELC in an unlinked telomeric site. These results show that the simple presence of an ELC in a potential expression site is not sufficient for its expression.

We examined the fate of the VSG-1 ELC in *T. equiperdum* variants isolated from a rabbit infected with VSG-1 expressor BoTat-1 (Bordeaux trypanozoon antigen type-1). Figure 1*a* explains the genealogy of these trypanosome clones. Trypanosomes expressing VSG-1 always appear in the first peak of parasitaemia in rabbits infected with any variant from the BoTaR serodeme of *T. equiperdum*[4]. VSG-2 and -4 expressors also appear very early, whereas VSG-20, -28 and -78 expressors appear later in such infections. Figure 1*b* compares the hybridization profiles of the DNAs from these trypanosomes after digestion with *Bam*HI endonuclease, which does not cleave within the VSG-1-coding sequence, and hybridization to a ^{32}P-labelled VSG-1-specific cDNA clone.

As described previously[9], the profile of BoTat-1 showed four fragments, one of which contained the VSG-1 ELC (14 kilobases, kb), whereas the profiles of BoTats-20, -28 and -78 each showed only three fragments hybridizing to the VSG-1 cDNA. Surprisingly, the hybridization patterns of BoTats-2 and -4 also contained a fourth VSG-1-related band. DNA from BoTat-4[(20)], a second VSG-4-expressing variant derived from BoTat-20, showed only three *Bam*HI fragments hybridizing to the VSG-1 probe (Fig. 1*b*). Thus, the supplementary VSG-1-related sequences in the DNAs of BoTats-2 and -4 were not necessary for expression of the early VSGs.

The above results suggested that the supplementary VSG-1 sequences in BoTats-2 and -4 represented inactive residual VSG-1 ELCs (R-ELCs). To examine this possibility, restriction maps of the regions containing the apparent R-ELCs in these variants were constructed as described in Fig. 2 legend. The 5′ genomic environments of the R-ELCs seemed to be identical to that of the original VSG-1 ELC in BoTat-1, at least as far as the *Sal*I site more than 9 kb upstream from the gene. The restriction maps of the coding regions of the R-ELCs and the BoTat-1 ELC were also identical. Moreover, like both the

0028–0836/84/060563—04$01.00

© 1984 Macmillan Journals Ltd

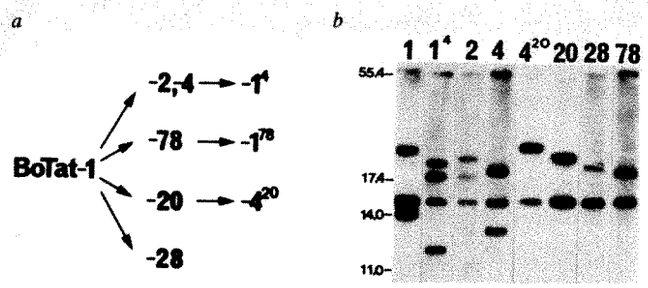


Fig. 1 *a*, Genealogy of trypanosome variants. All the *T. equiperdum* variants described here were derived from BoTat-1 as described by Capbern *et al.*[4]. Arrows represent infection of a rabbit with the designated variant. The variants were then isolated from the rabbits and cloned in mice. The variants are labelled here and in subsequent figures according to their numbers: 2 refers to BoTat-2 which expresses VSG-2, etc. BoTats-2 and -4 were isolated from the same rabbit. BoTat-1$^{(4)}$ was isolated from a rabbit infected with BoTat-4. The rabbit infected with BoTat-20 from which BoTat-4$^{(20)}$ was isolated, was immunized against VSG-1 to prevent passage through a VSG-1-expressing intermediate. *b*, *Bam*HI restriction profiles of VSG-1-related sequences in the DNAs of various variants. DNA was isolated from each variant, digested with *Bam*HI which does not cut the VSG-1-coding sequence, electrophoresed in a 0.5% agarose gel, blotted to nitrocellulose filters and hybridized to ^{32}P-labelled VSG-1-specific cDNA plasmid pTe 1.6 as described previously[9]. The numbers above the lanes denote the variants from which the DNA was taken. The large (>60 kilobase pair) fragment is present in all of these variants, but is partially degraded in some of the DNA preparations. Molecular weight standards were from a *Bgl*II digest of coliphage T4 DNA and sizes are given in kb.

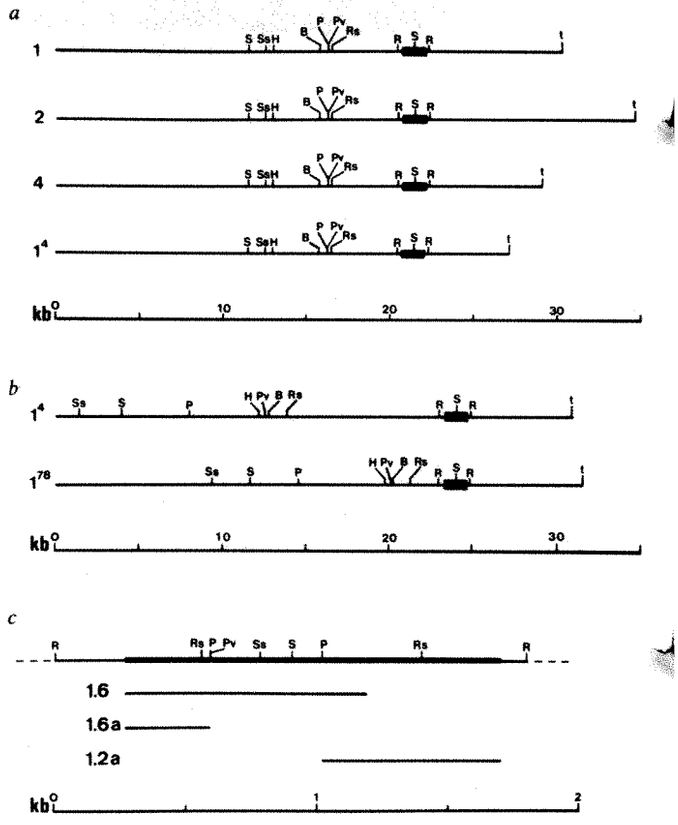


Fig. 2 Restriction maps of DNA containing VSG-1 genes. *a*, Maps of VSG-1 ELC in BoTat-1 and VSG-1 R-ELC in BoTats-2, -4 and -1$^{(4)}$. *b*, Maps of active VSG-1 ELCs in BoTats-1$^{(4)}$ and -1$^{(78)}$. *c*, Map of expanded coding sequence of the VSG-1 gene. The derivations of the various probes have been described previously[9] and are shown beneath the expanded map: 1.6, pTe 1.6; 1.6a, 5′-specific pTe 1.6a; 1.2a, 3′-specific pTe 1.2a. The maps were generated by treating the DNAs with single or multiple restriction endonucleases and hybridizing separately with ^{32}P-labelled 3′- or 5′-specific VSG-1 probes. Sites mapping within the sequences homologous to the VSG-1 cDNA clones (solid boxes) are not shown in *a* or *b* but are given in the expanded map in *c*. Transcription is from left to right. Sites that are 'universally' sensitive to restriction endonucleases and are presumed chromosome telomeres are marked (t). Symbols: S, *Sal*I; Ss, *Sst*I or isoschizomer *Sac*I; H, *Hin*dIII; B, *Bam*HI; P, *Pst*I; Pv, *Pvu*II; Rs, *Rsa*I; R, *Eco*RI.

VSG-1 ELC and BC in BoTat-1[14], the 3′ ends of the VSG-1 R-ELCs were proximal to apparent chromosome telomeres. This was inferred from the presence of 'universal' restriction sites at the presumed chromosome termini in the DNA sequences flanking the 3′ ends of these R-ELCs (see maps in Fig. 3) and from the selective sensitivity of these 3′-specific fragments to *Bal*31 exonuclease (data not shown). The only differences observed in the maps of the original VSG-1 ELC and the R-ELCs were in the lengths of the DNA sequences between the VSG-1-coding sequence and the apparent chromosome telomeres.

To assess the level of activity of the R-ELCs in BoTats-2 and -4, the trypanosomes were screened by immunofluorescence with anti-VSG-1 antibody and their purified RNAs were examined by Northern filter hybridization with VSG-1-specific cDNA probes; no VSG-1 glycoprotein or VSG-1 mRNA was detected (data not shown). Finally, because it was shown recently that chromatin containing the ELC of an expressed VSG gene is selectively sensitive to DNase I[7], we examined the DNase I sensitivity of chromatin containing the VSG-1 R-ELCs in BoTats-2 and -4. Chromatin was isolated from BoTats-1, -2 and -4, treated with DNase I, and analysed as described in Fig. 3 legend. As expected from previous work[9], the 8.6-kb *Sac*I fragment containing the 5′ end of the VSG-1 ELC in BoTat-1 was selectively degraded by the DNase I treatment (Fig. 3*a*). In contrast, the 8.6-kb fragments containing the 5′ end of the VSG-1 R-ELC in BoTat-2 (not shown) and BoTat-4 (Fig. 3*b*) were not selectively sensitive to DNase I. From these results we conclude that the VSG-1 R-ELCs are inactive and that their inactivation was accompanied by alterations of the chromatin containing the gene.

As the VSG-1 ELC was inactivated but not lost on antigenic variation to BoTats-2 and -4, we asked whether it would be reactivated after a change back to VSG-1 expression. Thus, a rabbit was infected with BoTat-4 and the VSG-1-expressing variant BoTat-1$^{(4)}$ was isolated. The hybridization profile of the VSG-1-specific sequences in BoTat-1$^{(4)}$ DNA after cleavage with *Bam*HI is shown in Fig. 1*b*. Clearly, BoTat-1$^{(4)}$ contained two VSG-1-specific sequences not present in BoTats-20, -28

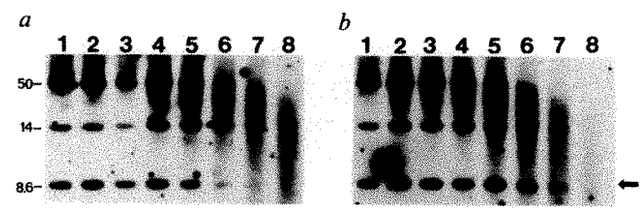


Fig. 3 DNase I-sensitivity of VSG-1 R-ELCs. *a*, DNA from BoTat-1; *b*, DNA from BoTat-4. The procedure for DNase I treatment of trypanosome chromatin has been described in detail elsewhere[7]. Briefly, nuclei were isolated from 2×10^9 trypanosomes and treated at 20 °C with 80 ng ml^{-1} RNase-free DNase I (Worthington Biochemicals). Aliquots were taken at 0 min (lane 1), 0.5 min (lane 2), 1 min (lane 3), 2 min (lane 4), 5 min (lane 5), 10 min (lane 6), 20 min (lane 7) and 40 min (lane 8). The DNA was extracted, treated with *Sac*I, electrophoresed in a 0.6% agarose gel, blotted and hybridized with ^{32}P-labelled 5′-specific cDNA probe pTe 1.6a. The arrow shows the position of the 8.6-kb fragment containing the 5′ end of the VSG-1 ELC in BoTat-1 or the VSG-1 R-ELC in BoTat-4. Sizes of the fragments are in kb.

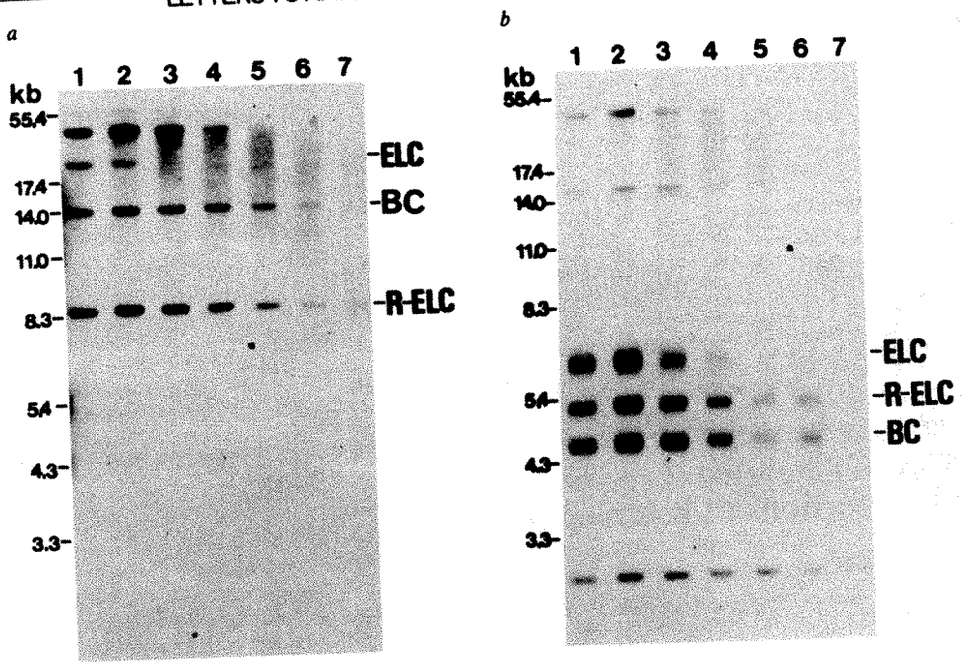


Fig. 4 DNase I-sensitivity of new VSG-1 ELC and R-ELC in BoTat-1(4). *a*, Sensitivity of 5′-specific sequences; *b*, sensitivity of 3′-specific sequences. Nuclei were isolated from BoTat-1(4) and treated with DNase I as described in Fig. 3 legend. Aliquots were taken at 0 min (lane 1), 0.5 min (lane 2), 1 min (lane 3), 2 min (lane 4), 5 min (lane 5), 10 min (lane 6) and 20 min (lane 7). The DNA was extracted, cut with *Sac*I, electrophoresed and blotted as described in Fig. 3 legend. The blot in *a* was hybridized with the 5′-specific VSG-1 probe pTe 1.6a; that in *b* was hybridized with the 3′-specific VSG-1 probe pTe 1.2a. The positions of the fragments containing the 3′ or 5′ ends of the new VSG-1 ELC (ELC), the residual VSG-1 ELC (R-ELC) and the 3′ end of the VSG-1 basic copy (BC) are shown. Molecular weight standards are from a *Bgl*II digest of coliphage T4 DNA.

or -78. Restriction analysis showed that the 5′ genomic environment of one of these VSG-1 copies was identical to that of the BoTat-1 ELC and the VSG-1 R-ELCs in BoTats-2 and -4 (Fig. 2*a*). The DNA flanking the 3′ end of this VSG-1 gene was associated with an apparent chromosome telomere. We conclude, therefore, that this VSG-1 gene represents the VSG-1 R-ELC and that it was not deleted during the processes that produced BoTat-1(4).

The second supplementary VSG-1 sequence in BoTat-1(4) was newly produced in this variant. The 5′ flanking regions of this VSG-1 gene resembled the 5′ flanking region of the previously described VSG-1 ELC in BoTat-1(78) (Fig. 2*b*). However, it contained a 7–8-kb restriction endonuclease-insensitive sequence that is not present in the corresponding region of the BoTat-1(78) ELC. This was not surprising in view of previous reports that the 5′ environments of different ELCs in the same genomic site differ by the presence of variable length DNA sequences that are insensitive to restriction enzymes[9,10,13,19–22]. The 3′ end of the newly produced VSG-1 gene in BoTat-1(4) was again proximal to a chromosome telomere and thus cannot be closely linked to the telomeric VSG-1 R-ELC in this variant.

To determine which of the VSG-1 gene copies in BoTat-1(4) were active, chromatin was isolated and analysed with DNase I as described above. With a 5′-specific VSG-1 cDNA probe, only the 22-kb *Sac*I fragment containing the 5′ end of the newly produced VSG-1 gene was preferentially DNase I-sensitive; the 8.6-kb fragment containing the 5′ end of the VSG-1 R-ELC was stable (Fig. 4*a*). Similar experiments using 3′-specific VSG-1 cDNA plasmid as probe showed that the fragments containing the 3′ ends of the VSG-1 BC and R-ELC were slightly sensitive to DNase I treatment, but that the fragment containing the 3′ end of the new VSG-1 gene was very sensitive to such treatment (Fig. 4*b*). We have shown previously that the telomeric DNA associated with silent VSG genes is slightly sensitive to DNase I treatment[23]. These results suggested that only the new VSG-1 copy in BoTat-1(4) is transcribed. Thus, in BoTat-1(4), the VSG-1 R-ELC was not reactivated and a new, active VSG-1 ELC was created.

Our observations show that the VSG-1 ELC in BoTat-1 was inactivated *in situ* during the antigenic variations which produced BoTats-2, -4 and -1(4). This demonstrates that the simple presence of a VSG-1 ELC in a potential expression site is not sufficient for its expression. The new, active VSG-1 ELC and the inactive R-ELC in BoTat-1(4) were shown to be linked to different chromosome telomeres. Similary, the ELCs of the VSG-2 and VSG-4 genes were not closely linked to the VSG-1 R-ELCs in BoTats-2 and -4 (unpublished data). These results reinforce our previous assertion that there are multiple telomeric sites which can accept VSG-1 ELCs.

Overall, our results with *T. equiperdum* argue that VSG genes are activated by duplication and transposition into one of several telomeric acceptor sites which are subject to secondary control mechanisms. Thus a VSG gene could be activated either by direct replacement of the previously active ELC or by transposition into an inactive acceptor site which can subsequently be activated. This model explains how some VSG genes are apparently activated by a simple duplicative transposition (direct replacement of an active ELC) and how other VSG genes are activated without obvious duplication (activation of an inactive acceptor site containing a previously duplicated VSG gene). Loss of an ELC, as observed for the VSG-1 ELC after variation from BoTat-1 to BoTats-20, -28 and -78, would occur by its replacement in the acceptor site with a newly duplicated VSG gene copy. Conclusions similar to these have been reached by Young *et al.*[24].

The mechanisms which activate the duplicated VSG genes in the various acceptor sites are not yet understood. Translocation of a VSG gene in an acceptor site to a position *cis* to an 'expression element' via a reciprocal recombination 10–30 kb upstream from the VSG gene would explain our observations of multiple acceptor sites and the maintenance of the VSG-1 R-ELCs. Similarly, an 'expression element' could be recombined into a position 3′ to the VSG gene-containing acceptor site. Other mechanisms such as DNA modification or a system of repressors and inducers are also possible factors controlling VSG genes in a finite number of telomeric acceptor sites.

We thank Dr Charles Roth for careful reading of this manuscript. This work was supported by grants from the CNRS and the Institut National de la Santé et de la Recherche Medicale. G.A.B. received support from NIH grant AI06661.

Received 24 June; accepted 14 November 1983.

1. Vickerman, K. *Nature* **273**, 613–617 (1978).
2. Cross, G. A. M. *Proc. R. Soc.* **B202**, 55–72 (1978).
3. Englund, P. T., Hajduk, S. L. & Marini, J. C. *A. Rev. Biochem.* **51**, 695–726 (1982).
4. Capbern, A., Giroud, C., Baltz, T. & Mattern, P. *Expl Parasit.* **42**, 6–13 (1977).
5. Hoeijmakers, J. H. J., Frash, A. C. C., Bernards, A., Borst, P. & Cross, G. A. M. *Nature* **284**, 78–80 (1980).
6. Pays, E., Van Meirvenne, N., LeRay, D. & Steinert, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2673–2677 (1981).
7. Pays, E., Lheureux, M. & Steinert, M. *Nature* **292**, 265–267 (1981).

8. Majiwa, P. A. O., Young, J. R., England, P. T., Shapiro, S. Z. & Williams, R. O. *Nature* **297,** 514–516 (1982).
9. Longacre, S. *et al. Molec. cell. Biol.* **3,** 399–409 (1983).
10. Borst, P. & Cross, G. A. M. *Cell* **29,** 291–303 (1982).
11. Williams, R. O., Young, J. R. & Majiwa, P. A. O. *Nature* **299,** 417–421 (1982).
12. De Lange, T. & Borst, P. *Nature* **299,** 451–453 (1982).
13. Pays, E., Lheureux, M. & Steinert, M. *Nucleic Acids Res.* **10,** 3149–3163 (1982).
14. Raibaud, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 4306–4310 (1983).
15. Williams, R. O., Young, J. R. & Majiwa, P. A. O. *Nature* **282,** 847–849 (1979).
16. Young, J. R., Shah, J. S., Matthyssens, G. & Williams, R. O. *Cell* **32,** 1149–1159 (1983).
17. Young, J. R., Donelson, J. E., Majiwa, P. A. O., Shapiro, S. Z. & Williams, R. O. *Nucleic Acids Res.* **10,** 803–819 (1982).
18. Donelson, J. E., Young, J. R., Dorfman, D., Majiwa, P. A. O. & Williams, R. O. *Nucleic Acids Res.* **10,** 6581–6595 (1982).
19. Michels, P. A. M., Bernards, A., Van Der Ploeg, L. H. T. & Borst, P. *Nucleic Acids Res.* **10,** 2353–2366 (1982).
20. Borst, P. *et al.* in *Proc. Cetus–UCLA Meet. on Molec. Biol. of Host Parasite Interactions*, Park City, Utah (eds Agabian, N. & Eisen, H.) (in the press).
21. Michels, P. A. M. *et al. J. molec. Biol.* (in the press).
22. Laurent, M. *et al. Nature* **302,** 262–266 (1983).
23. Longacre, S. *et al. Molec. cell. Biol.* **3,** 410–414 (1983).
24. Young, J. R., Turner, M. J. & Williams, R. O. in *Proc. Cetus–UCLA Meet. on Molec. Biol. of Host Parasite Interactions*, Park City, Utah (eds Agabian, N. & Eisen, H.) (in the press).

Three-dimensional reconstruction from tilted sections of fish muscle M-band

P. K. Luther

Biophysics Section, Physics Department, Imperial College, London SW7 2BZ, UK

R. A. Crowther

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Fish muscle provides a particularly suitable specimen for studying the organization of thick filaments in vertebrate striated muscle because the filaments are arranged with a single orientation on a well ordered hexagonal lattice[1]. The M-band consists of sets of cross-links, which join the myosin filaments in the middle of the A-band. Previous work[1] concluded that the fish muscle M-band had the local symmetry of the dihedral point group *32*. We present here a quantitative analysis of transverse sections of the M-band, using general methods[2] developed for crystalline layers, to combine various tilted views. The three-dimensional map computed to a resolution of about 70 Å confirms previous findings[1]; it shows new features of the thick filament structure in the M-band region and provides new information on the use of three-dimensional reconstruction from sectioned biological material. The accompanying paper[3] describes a novel technique of three-dimensional reconstruction of the fish M-band from a single view of a slightly oblique plastic section.

In fish muscle the thick filaments are arranged in rotational register[1], making the M-band crystalline. There are three main axial levels of M-bridges within this M-band[4]: M1 in the middle, with M4 and M4′ about 220 Å on either side (Fig. 1). The bands at M6 and M6′, about 220 Å outside M4 and M4′, are very weak in the plaice fin muscle studied here. From a study[1] of very thin slightly oblique transverse sections, three distinct structures were identified, which were thought to be associated with the M1, M4 and M6 bands seen in longitudinal sections (Fig. 1). At M1 there were straight M-bridges while at M4 and M4′ the thick filament backbone comprised three subunits with bean-shaped M-bridges. The structure showed a clear hand which reversed from M4 to M4′ and the symmetry of the M-band corresponded to the dihedral point group *32*.

The micrograph in Fig. 2 shows an example of an M-band cross-section used for three-dimensional reconstruction. The thick filament spacing was about 350 Å and this value was used throughout, although the value in the native muscle would be somewhat larger. The section, estimated as about 600 Å thick from the interference colour during microtomy, should include the whole of a centrally located M-band from M4 to M4′ (440 Å). Micrographs were taken at tilt angles from −60° to +60° in steps of 5° about two mutually perpendicular axes. The total exposure was consequently large but it appeared that the section was relatively stable after the initial high dose exposure.

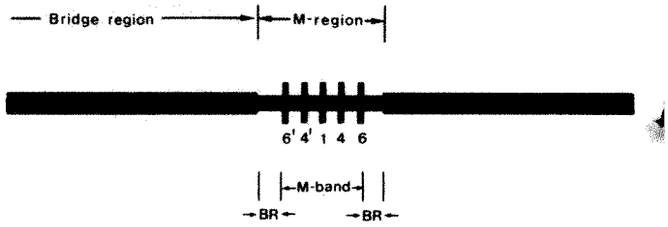


Fig. 1 Schematic drawing of part of a thick filament showing details of the myosin crossbridge-free M-region as it is seen in longitudinal sections of the A-band[4]. The M-region comprises the M-band and the bare region (BR). The three main levels of M-bridges in the M-band are M1 in the middle with the M4′ and M4 bridges about 220 Å to either side. Additional density is seen in some muscles at M6 and M6′, about 220 Å outside M4 and M4′.

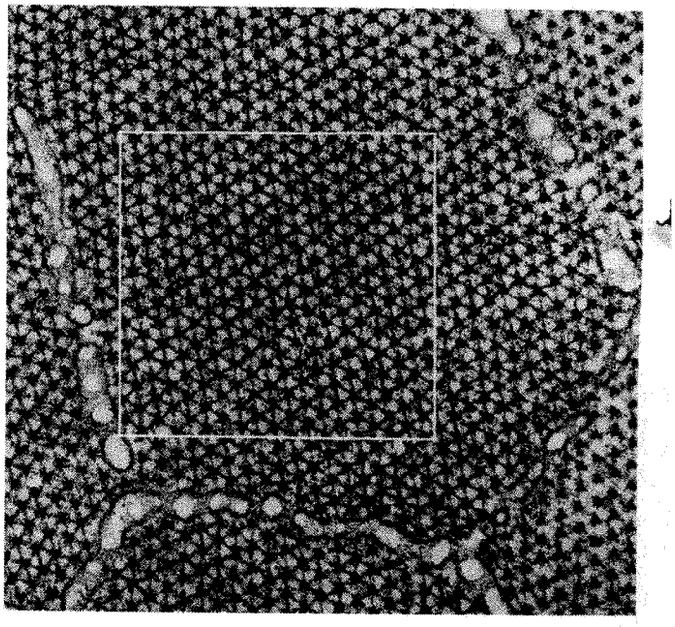


Fig. 2 Transverse section of the plaice fin muscle M-band (×80,000). The boxed area was used for reconstruction. The muscle was processed and embedded in Araldite as described previously[1]. Transverse sections of silver-grey interference colour were cut with a diamond knife which, from past experience using section folds, corresponds to a thickness of about 600 Å. The sections were stained with uranyl acetate and Reynolds lead citrate. For electron microscopy, a JEOL 200CX fitted with a rotation goniometer was used. Micrographs at a magnification of ×20,000, were obtained by tilting the section in steps of 5° from −60° to +60°, covering the low tilt angles first in order to stabilise the section. The section was then rotated by 90° and another tilt series obtained. The best areas were selected by optical diffraction.

Fourier transforms were computed of each view and a three-dimensional set of data was constructed using standard programs[2], assuming minimal P1 symmetry in the specimen. Figure 3 shows data for some of the stronger lattice lines. The transform of the untilted view showed strong 3-fold rotational symmetry when the appropriate phase origin was found on the thick filament, although the lattice was distorted by compression of about 10% in the direction of sectioning. The three-dimensional reconstruction computed with no assumption about symmetry also showed a similarly striking 3-fold rotation axis, though again with compression of the lattice. After correcting for the compression, the data were therefore 3-fold averaged and a further reconstruction computed. It is this 3-fold averaged map which will be described.

The computed thickness of the section in the reconstruction was only about 300 Å, a value much lower than the initial

0028–0836/84/060566—03$01.00

© 1984 Macmillan Journals Ltd

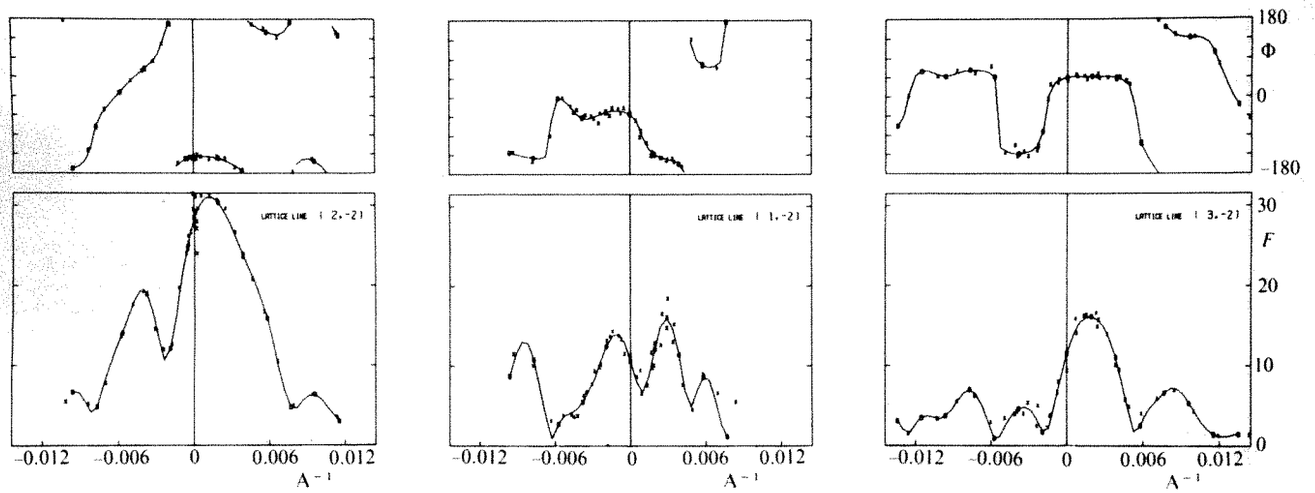
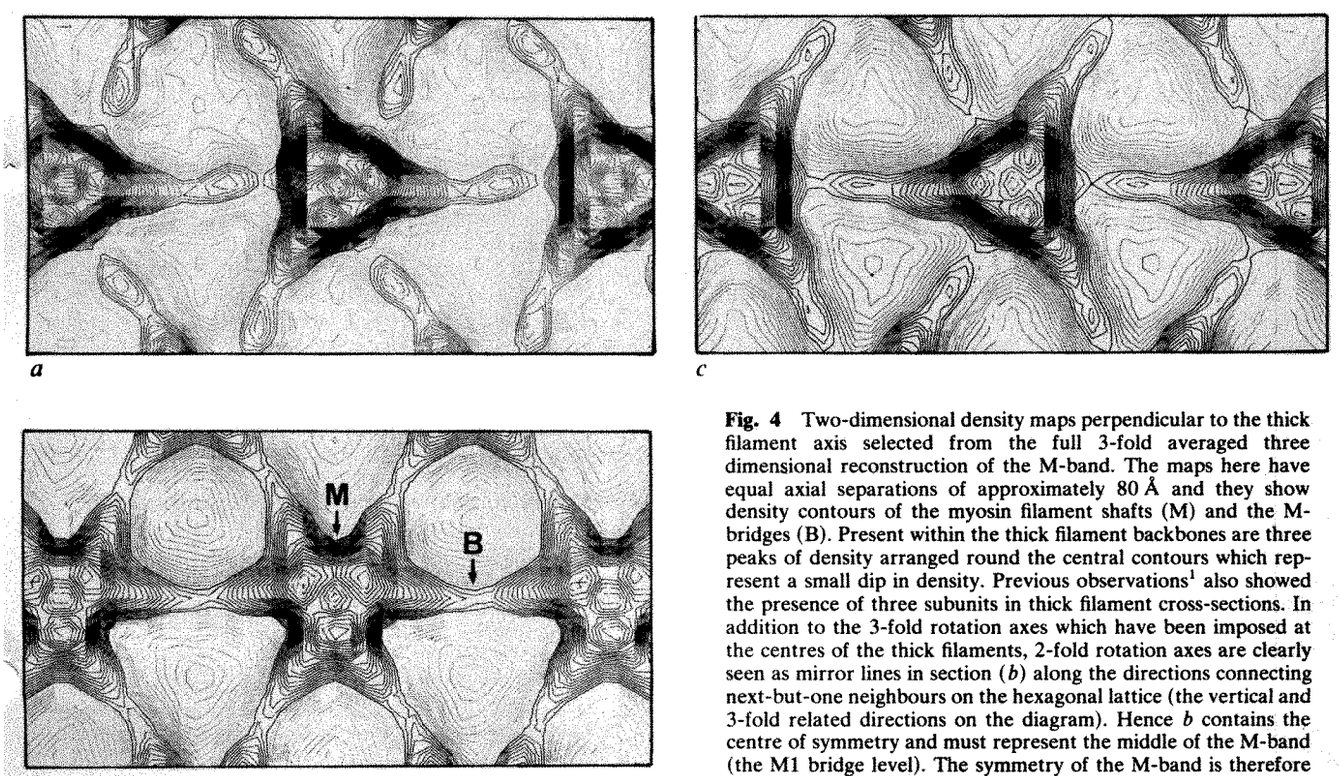


Fig. 3 Plots of observed Fourier transform data along three of the stronger lattice lines. In each pair of plots the lower one represents the amplitude and the upper one the phase. Observed points are plotted as crosses (×) and the equally spaced points interpolated by computer are plotted as circles (○) with continuous curves drawn between them.

thickness of about 600 Å estimated from the interference colour. This suggests that the section has shrunk considerably in the electron beam. Shrinkage of plastic sections during electron microscopy has been noted before[5,6] and a detailed study[6] showed that Araldite sections reduced in thickness by about 50%. Shrinkage of the M-band sections would result in the closing up of the M-bridge levels. A comparison of features common to the reconstruction from the oblique section[3] with this map also suggests a shrinkage to 50% of the true value.

The final three-dimensional reconstruction was built up from two-dimensional density maps computed at axial spacings of 20 Å nominal, which would correspond to about 40 Å in the original muscle. One such map (Fig. 4*b*) not located at the centre of the reconstruction, contains clear 2-fold rotation axes seen as mirror lines along directions of the hexagonal lattice connecting next-but-one neighbouring thick filaments. The bipolar nature of the thick filament[7] implies that this represents the centre of the M-band (that is, the M1 bridge level). Examples of maps at equal separations (about 80 Å in the muscle) on either side of the M1 plane are shown in Fig. 4*a* and *c*. The same 2-fold symmetry relating these maps (and others not shown) is striking; there can be little doubt that the local symmetry of the M-band is described by the dihedral point group *32*, and that the layer as a whole has space group P*321*.

Figure 5 shows two stereo pairs of the M-band reconstruction viewed from opposite sides. The M-band was not centrally located in the plastic section, and M1 lies deep in the model viewed as in Fig. 5*a* with M4 and the M6 region above it. In



a *c* *b*

Fig. 4 Two-dimensional density maps perpendicular to the thick filament axis selected from the full 3-fold averaged three dimensional reconstruction of the M-band. The maps here have equal axial separations of approximately 80 Å and they show density contours of the myosin filament shafts (M) and the M-bridges (B). Present within the thick filament backbones are three peaks of density arranged round the central contours which represent a small dip in density. Previous observations[1] also showed the presence of three subunits in thick filament cross-sections. In addition to the 3-fold rotation axes which have been imposed at the centres of the thick filaments, 2-fold rotation axes are clearly seen as mirror lines in section (*b*) along the directions connecting next-but-one neighbours on the hexagonal lattice (the vertical and 3-fold related directions on the diagram). Hence *b* contains the centre of symmetry and must represent the middle of the M-band (the M1 bridge level). The symmetry of the M-band is therefore that of the dihedral point group *32* as noted previously[1]. (Note that the density map in *b* was not located at the middle of the plastic section or of the three-dimensional reconstruction.)

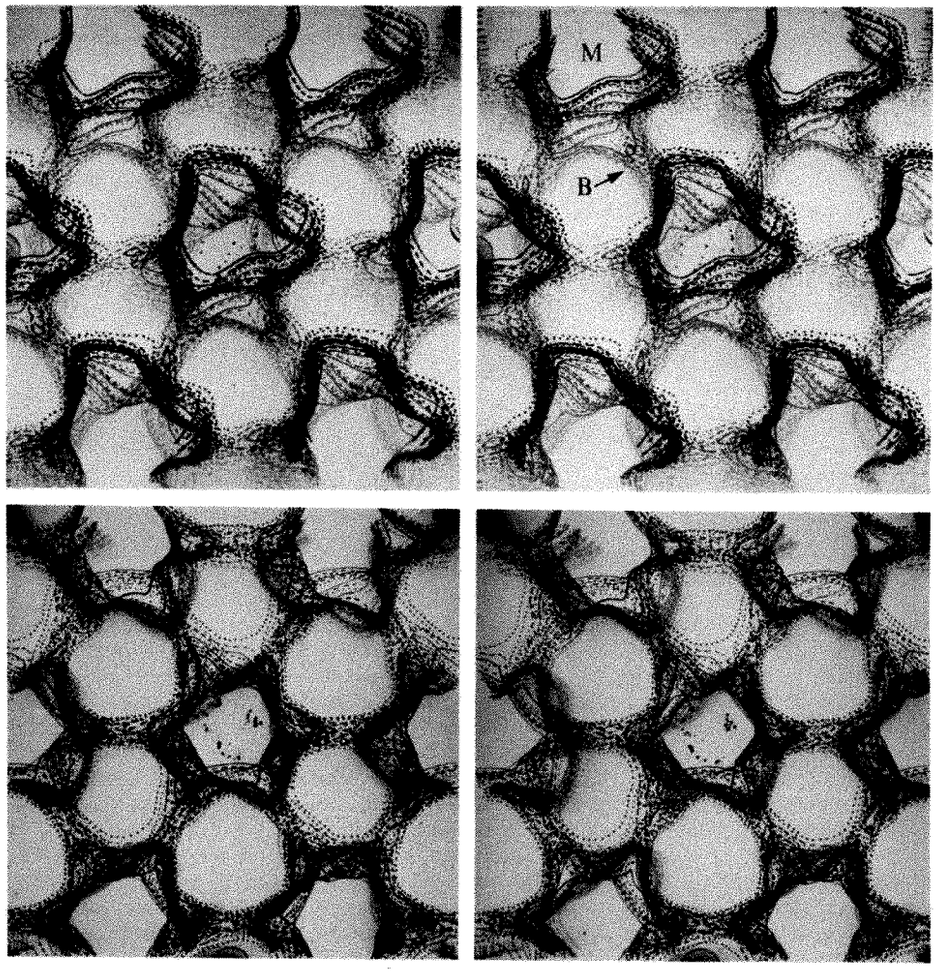


Fig. 5 Two stereo pairs of a Perspex model of the full three-dimensional reconstruction of the M-band viewed from 'above' (*a*) and 'below' (*b*). For clarity only three contour levels are shown. The dotted lines represent the lowest density, and the dashed and continuous lines represent respectively higher densities. Located in the centre of the images is a myosin thick filament (M). The lower part of the stereo view *a* shows the M-bridges (B) emanating from the thick filaments while the upper part shows the M6 region. The three points marked in the core of the central thick filament represent peaks of density. In *b* the peaks are seen to spiral through the M-band in an anticlockwise fashion viewed from the centre of the M-band. The stereo views clearly show 2-fold rotation axes in the M1 plane through next nearest thick filaments.

the opposite view shown in Fig. 5*b* M4′ lies at the top surface. The *32* symmetry of the M-band is clear in both stereo pairs. In Fig. 5*b*, three dots marked in the core of the central thick filament represent peaks of density and their mean separation is about 75 Å relative to the 350 Å filament spacing. The peaks spiral through the M-band in an anticlockwise manner as viewed from the centre of the M-band. However, at the low resolution of the reconstruction, this spiralling is also consistent with parallel rods of density overlapping at M1 as suggested earlier[1]. The hand of the whole M-band structure was confirmed independently using serial sections.

The outer profile of the thick filament is strongly modulated, giving the effect of ridges running along the filament. There are two sets of three ridges, one set on each side of the M1 level. The two sets are azimuthally out of register and overlap at the M1 level, giving the profile there a strongly six-lobed appearance (Fig. 4*b*). Moving from M1, one or other set quickly terminates, producing an overall triangular shape (Fig. 4*a*, *c*). These ridges run with a slight twist right up through the M6 region (Fig. 5*a*) and appear to terminate there[3]. The M-bridge density links to these ridges. It is not clear at this resolution whether to ascribe the density in the ridges to myosin tails, to M-bridges, to other M-band proteins or to some combination of these.

The three-dimensional reconstruction shows M-bridge density clearly but does not resolve the different axial levels of the M-band expected from the M-bridge stripes seen in longitudinal sections (Fig. 1). This may be because the plane of sectioning was not exactly transverse or may have wandered. Also the M-band itself may not have been exactly planar, since examination of longitudinal sections shows that the M-band may be slightly curved within a myofibril. These effects would lead to axial smearing of the M-bridge density and consequent lack of axial resolution. Additionally, shrinkage normal to the section must close up the three M-bridge levels and make them less easy to resolve. A study of the shrinkage behaviour of plastic sections in the electron microscope is under way and the results will be reported elsewhere.

The application of three-dimensional reconstruction methods to plastic sections has produced a more quantitative and objective picture of the M-band than was previously available. The fish muscle M-band is established to have *32* symmetry and it is likely the thick filament as a whole has the same symmetry. Despite the factors limiting the resolution of the M-bridges, the axial details in the thick filaments are displayed more clearly here than could be seen directly, even in the ultrathin (200 Å) sections used previously[1]. Further interpretation of the M-band must depend on specific labelling techniques. However, taken in conjunction with results from oblique reconstruction[3], these maps provide constraints which future models of the M-band must satisfy.

We thank Dr John Squire for helpful discussions. The electron microscopy was carried out in the Biophysics Department, King's College, London. This work was supported by the MRC.

Received 2 August; accepted 4 November 1983.

1. Luther, P. K., Munro, P. M. G. & Squire, J. M. *J. molec. Biol.* **151,** 703–730 (1981).
2. Amos, L. A., Henderson, R. & Unwin, P. N. T. *Prog. Biophys. molec. Biol.* **39,** 183–231 (1982).
3. Crowther, R. A. & Luther, P. K. *Nature* **307,** 569–570 (1984).
4. Sjostrom, M. & Squire, J. M. *J. molec. Biol.* **109,** 49–68 (1977).
5. Cosslett, A. *J. R. microsc. Soc.* **79,** 263 (1960).
6. Bennett, P. M. *J. Cell Sci.* **15,** 693–701 (1974).
7. Huxley, H. E. *J. molec. Biol.* **7,** 281–308 (1963).

Three-dimensional reconstruction from a single oblique section of fish muscle M-band

R. A. Crowther

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

P. K. Luther

Biophysics Section, Physics Department, Imperial College, London SW7 2BZ, UK

A new method of three-dimensional image reconstruction from electron micrographs is presented here, which enables a three-dimensional image to be produced from a single oblique section of a two- or three-dimensional crystal. The method, which is most powerful when the section is thin and the angle of obliquity small, has several potential advantages over the conventional method of reconstruction[1] using tilted views of the section. In particular the accumulated electron dose on the specimen is much smaller and the reconstruction is not affected by changes in the thickness of the section during exposure to the electron beam. The method involves the solution of a generally almost singular set of linear projection equations[2], relating through the sectioning geometry the three-dimensional crystal density to the two-dimensional projected image density. This can be achieved most conveniently by linear least squares filtering[3]. An application of the method to determine the structure of the M-band of fish muscle is described. The resulting map agrees well with that produced by the more conventional approach involving tilted views[4].

It is easiest both to visualize and to apply the method when the crystal is two-dimensional and when the direction of maximum slope of the section is normal to one of the cell edges of the crystal. Accordingly it is that situation which will be described and used here, although there should be no difficulty in working with a general oblique section of a three-dimensional crystal, except that the computation would be more complicated. With the above assumptions, the section contains rows of equivalent unit cells of the crystal (Fig. 1*a*), which can be averaged normal to the direction of maximum slope to give a 'strip image' one cell wide (Fig. 1*b*). Each cell of the strip image gives a projected superposition of a different set of levels from the full unit cell of the crystal (Fig. 2). As a consequence of the restrictive assumptions made about the sectioning geometry, the two-dimensional problem has been factorized, allowing one-dimensional averaging to give an improved signal-to-noise ratio in the strip image, followed by a simple one-dimensional reconstruction. For a general sectioning geometry this factorization would not be possible and the averaging and reconstruction could not be so separated.

The matrix of coefficients P_{ij} of the projection equations, $\sigma_i = \sum_j P_{ij}\rho_j$, (see Fig. 2) depends on the geometry of the crystal and of the section. In particular it depends on the rise per cell of the section and its thickness and these parameters must be known before the equations can be set up and solved. A choice must also be made of the pixel thickness and of the overall thickness of the specimen to be reconstructed. In general the projection equations comprise a rectangular set, with more observations σ_i than unknowns ρ_j, so that the system is most easily solved by least squares. It is nevertheless likely that the equations will be poorly conditioned, in that certain combinations of pixel weights ρ_j will be represented hardly at all in the projection data σ_i and will not therefore be recoverable without unacceptable noise amplification, as has been previously discussed for reconstruction from tilted views[2,5]. It is therefore necessary to solve the projection equations by a least-squares filtering procedure[3], in which only the best determined combinations of pixel weights ρ_j are recovered. This can be achieved by computing the eigensystem of the normal equations and recovering only those eigenvectors with eigenvalue λ such that λ/λ_{max} is greater than some specified filter level. The choice of filter level depends on the noise in the data and here a value of 0.01 was found to be appropriate.

For the M-band the sectioning geometry relative to the native muscle can be established approximately by inspection of the micrograph, since the separation of M9–M9′ along the thick

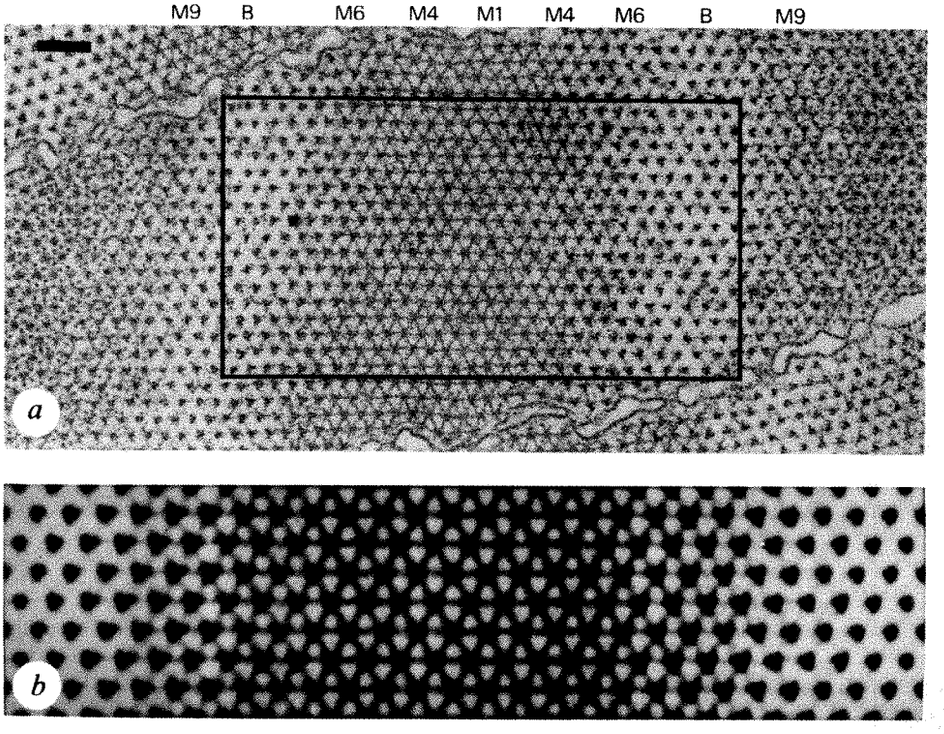


Fig. 1 *a*, Electron micrograph of a thin oblique, though approximately transverse, section of roach muscle M-band, prepared as described in ref. 7. The positively stained protein appears black and the thick filament profiles form an approximately hexagonal lattice. The direction of maximum slope lies left–right so that starting from the centre of the image one sees in both directions successively the M1, M4, M6, bare (B) and M9 regions of the M-band[6]. Scale bar, 100 nm. *b*, Strip image formed by one-dimensional averaging of the boxed area in *a*, normal to the direction of slope of the oblique section. The averaging by computer is performed directly on the image density, using bilinear interpolation[9] to correct for small distortions in the thick filament lattice and correlation to get the best alignment of rows of unit cells before averaging. The result is a strip image one cell wide, which has here been extended by repetition to an area four cells wide, so that the pattern can be more clearly seen. The strip image is sampled every 2.2 nm at equivalent points within each cell, so simplifying the subsequent reconstruction. The distorted hexagonal lattice of thick filament profiles is thus made perfectly hexagonal and is represented most conveniently in the computer by a centred rectangular cell of size $a \times \sqrt{3}a$, where $a = 35$ nm is the mean separation of thick filaments. Every rectangular cell therefore has a thick filament at each corner and one in the centre. The features around each thick filament profile show 3-fold symmetry and the strip image as a whole has a vertical mirror plane at its centre.

0028-0836/84/060569—02$01.00

© 1984 Macmillan Journals Ltd

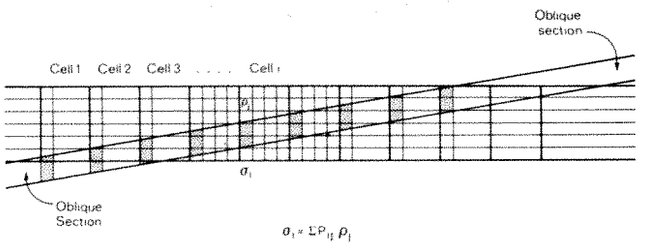


Fig. 2 Geometry of oblique sectioning of a two-dimensional crystalline layer one unit cell thick. If it is assumed that the direction of maximum slope is normal to one of the cell edges, then the description of the geometry is simplified (see text). Each unit cell is considered to be divided up into a three-dimensional array of pixels of density ρ_j. The oblique section then cuts out a gradually changing set of pixels from equivalent columns of density in the various unit cells, as indicated by the stippled areas. When the oblique section is viewed in the electron microscope the resulting image will represent the projected densities σ_i, corresponding to summing the subsets of pixels cut out by the section. The unknown densities ρ_j in the specimen are thus related to the measured density values σ_i by linear projection equations of the form $\sigma_i = \sum_j P_{ij}\rho_{ij}$, where $P_{ij} = 0$ if pixel ρ_j does not lie within the section in column i, $P_{ij} = 1$ if it does and P_{ij} will be a positive fraction if the pixel lies across the bounding surface of the section. For the simplified geometry considered, the σ_i will arise from equivalent points in each cell of the strip image and the different columns of density through the specimen can be reconstructed independently of each other. There will be one set of projection equations for each set of equivalent columns of density. Strictly speaking the oblique section ought to be tilted in the microscope to an angle equal to the sectioning angle before taking the picture. However, for small sectioning angles and thin sections the effect is not important, as can be seen in the strip image in Fig. 1*b* in which the thick filament profiles show no discernible elongation in the sectioning area.

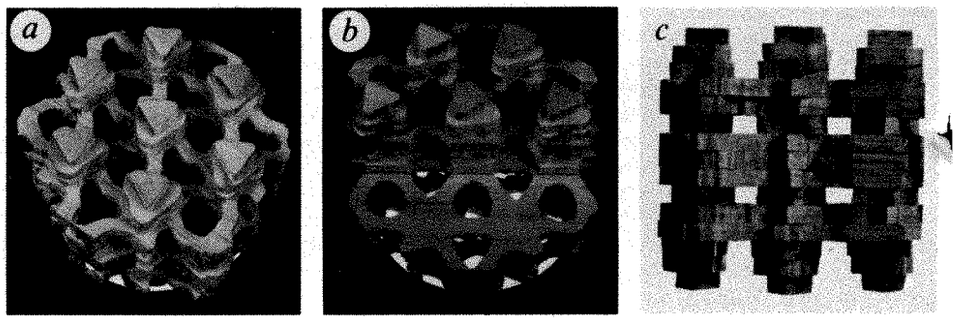


Fig. 3 A balsa wood model of the averaged reconstruction made from the strip image in Fig. 1*b*. Each section represents 6 nm and a total thickness of 90 nm is shown. *a*, The whole model consisting of a central thick filament surrounded by its six nearest neighbours. *b*, One-quarter of the model has been cut away to show the internal details. *c*, A side view along the direction of the hexagonal cell edge in which the filaments overlap. The 2-fold axis normal to the thick filament lies in the section revealed in the cut-away map *b*, which can be seen to have mirror lines through next nearest filaments. The absolute hand of the structure cannot be established from a single oblique section, so the handedness of the model was chosen to agree with that established for the reconstruction from tilted views[4].

filament is known[6] to be about 138 nm and in the oblique section (Fig. 1*a*) this rise takes place in about 38 thick filament spacings. Thus the section rise per filament or per unit cell in the strip image (Fig. 1*b*) is about 3.6 nm. The bare region between M6 and M9 is clearly visible but would not be seen if the section were thicker than about 30 nm. With these values of rise per cell and thickness, features should persist in the strip image for about six or seven cells, which appears to be the case here. The values of rise per cell and section thickness can be refined by setting up and solving the projection equations for a range of different values of the two parameters and taking those that give the smallest least squares residual as the best estimates. In this case the best values of rise per cell and section thickness were 3.62 nm and 27 nm respectively. This corresponds to a section angle of 5.9° to the strictly transverse for a thick filament spacing of 35 nm. A pixel depth of 6 nm was used and a total thickness of 102 nm reconstructed, giving 17 levels in all. However, only 12 of the 17 possible eigenfunctions satisfied the recoverability criterion $\lambda/\lambda_{max} > 0.01$ (see above), giving an effective independent pixel depth in the reconstruction of $102/12 = 8.5$ nm. This is rather less than one-third of the section thickness, so that the reconstruction procedure has produced a map with markedly better depth discrimination than can be obtained simply by looking at the strip image.

The M-band has been shown[4,7] to have *32* symmetry and it is clear that the strip image (Fig. 1*b*) has an overall vertical mirror plane at its centre and that the features around each thick filament profile are strongly 3-fold related. The map produced by the reconstruction procedures was therefore 3-fold and 2-fold averaged along appropriate directions to improve its signal-to-noise ratio. A model of this averaged map is shown in Fig. 3.

The reconstruction shows that the thick filaments are connected by strong fairly straight bridges (M1) at the centre of the M-band. About 22 nm away on either side the filaments are connected by curved bridges (M4, M4'). The thick filaments have a rather uniform diameter throughout the M-region but become much thinner about 40 nm from the centre of the M-band as is also clearly seen in the strip image (Fig. 1*b*). This appearance could be due to ridges of extra material running along the thick filament, giving the filaments a strongly three-lobed cross-section throughout the M4–M6 region. These ridges may well be formed partly or wholly by myomesin, which seems to run throughout the central M-region but which probably does not contribute to the M1 or M4 bridges[8]. The curved bridges at M4, which may be formed by creatine kinase[8], appear to start and end on these ridges.

The features seen in this oblique reconstruction agree well with those seen in the reconstruction from tilted views[4]. In particular, the shapes of the holes outlined by the thick filaments and M-bridges vary from section to section in the same way in the two maps. The principal differences are that in the oblique reconstruction the M4 bridges are more clearly visualized and the spacings along the filament direction are not affected by section collapse, because the computed geometry of the section is based on spacings close to those of the native muscle. Thus although this method of oblique reconstruction is intrinsically less powerful than that based on tilted views, it does nevertheless have some advantages and certainly gives improved depth discrimination compared with that obtained by direct viewing of oblique sections.

Received 2 August; accepted 4 November 1983.

1. Amos, L. A., Henderson, R. & Unwin, P. N. T. *Prog. Biophys. molec. Biol.* **39**, 183–231 (1982).
2. Crowther, R. A., DeRosier, D. J. & Klug, A. *Proc. R. Soc.* A**317**, 319–340 (1970).
3. Diamond, R. *Acta crystallogr.* **11**, 129–138 (1958).
4. Luther, P. K. & Crowther, R. A. *Nature* **307**, 566–568 (1984).
5. Klug, A. & Crowther, R. A. *Nature* **238**, 435–440 (1972).
6. Squire, J. M. in *The Structural Basis of Muscular Contraction* 332–343 (Plenum, New York, 1981).
7. Luther, P. K., Munro, P. M. G. & Squire, J. M. *J. molec. Biol.* **151**, 703–730 (1981).
8. Strehler, E. E., Carlsson, E., Eppenberger, H. M. & Thornell, L-E. *J. molec. Biol.* **166**, 141–158 (1983).
9. Crowther, R. A. & Sleytr, U. B. *J. ultrastruct. Res.* **58**, 41–49 (1977).



IUPHAR 9TH INTERNATIONAL CONGRESS OF PHARMACOLOGY

29 JULY – 3 AUGUST 1984

LOCATION

Barbican Centre for Arts and Conferences, City of London

SCIENTIFIC PROGRAMME

The scientific programme will extend over five days and consist of symposia, invited lectures, pharmacological debates, an educational meeting and free communications, the latter as both oral and poster sessions. The symposia will consist of three simultaneous sessions morning and afternoon and each day there will be four invited lectures. A new feature of the International Conference is the pharmacological debates. In these the intention is to select topics on which there are clear differences in evidence or its interpretation and have the issue debated between protagonists of each view with sufficient opportunity for the audience to participate. In the educational meeting it is hoped to concentrate on the place of various forms of programmed learning in Pharmacology and the instrumentation available. Finally, there will be free communications and to the fullest extent possible authors will be allowed to select whether to give these as oral communications or as a poster.

PROCEEDINGS

The Congress Proceedings are being published by Macmillan Press and may be ordered in advance of the registration form or at the Congress.

SOCIAL EVENTS

A full social programme is being arranged for delegates and accompanying guests. Details are given in the advance programme.

ACCOMMODATION

Accommodation has been reserved at advantageous rates in a number of hotels in various price categories and in student halls of residence. All accommodation is within easy travelling distance of the Barbican Centre.

DATES TO REMEMBER

31 January 1984	Deadline for receipt of abstracts for both invited papers and free communications.
1 May 1984	Deadline for college accommodation reservations.
18 May 1984	Deadline for receipt of registration at the standard fee (£150). There is a late fee after this date of a further £40. Deadline for receipt of papers for Proceedings volumes.

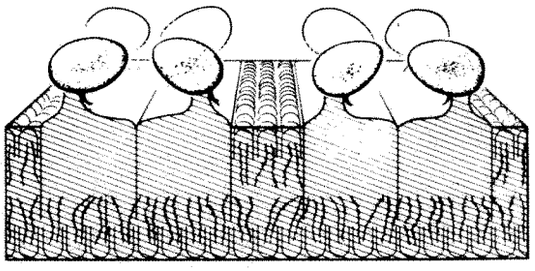
CONGRESS SECRETARIAT

Advance programmes, registration forms and abstract forms can be obtained from the Congress Secretariat:–

Congress Associates ICP
34 Stanford Road
London W8 5PZ
Telephone: 01 937 3163

Attention all biophysicists, molecular biologists and biochemists!

Biomembrane Structure and Function



Edited by
DENNIS CHAPMAN
Professor of Biophysical Chemistry
Royal Free Hospital
School of Medicine, London

Volume 4 in the TOPICS IN MOLECULAR AND STRUCTURAL BIOLOGY Series
Series editors:
Professor Watson Fuller, Dr Stephen Neidle and Professor Tony Atkinson

The application of physical techniques – such as X-ray diffraction and Raman spectroscopy – has revolutionised much of our thinking on the structure and function of membranes. In this important new study, contributors of international standing review a topical selection of approaches to the elucidation of **biomembrane structure and function.** The emphasis, throughout, is on the in-depth analysis of recent experimental work.

Published January 1984 Price £50.00 432pp
ISBN 0 333 31869 2

Contents

Calorimetric studies of model and natural biomembranes – *Diana Bach,* The Weizman Institute of Science, Israel; X-ray diffraction and electron microscope studies of the molecular structure of biological membranes – *Lee Makowski and Jade Li,* Columbia University, USA; Raman spectroscopy of lipids and biomembranes – *Surendra P. Verma and Donald F.H. Wallach,* Tufts-New England Medical Centre, USA; Infrared spectroscopic studies of model and natural biomembranes – *Ralph L. Amey,* Occidental College, USA and *Dennis Chapman*; Rotational and lateral diffusion of membrane proteins as determined by laser techniques – *Winfried Hoffman,* NUKEM GmbH, E.Germany, and *Colin J. Restall,* Royal Free Hospital, London; Theoretical models of monolayers, bilayers and biological membranes – *David A. Pink,* St Francis Xavier University, Canada; The Ca^{2+} dependent ATPase of sarcoplasmic reticulum – *G. Inesi and M. Kurzmack,* University of Maryland, USA.

Please send orders and requests for information to:
Carolyn Leuw, Product Manager,
Scientific and Medical Division,
The Macmillan Press Ltd.,
Houndmills, Basingstoke, Hants RG21 2XS, England

SCIENTIFIC & MEDICAL | M | MACMILLAN PRESS LTD

Circle No.67 on Reader Service Card.

verlag chemie

Methods of Seawater Analysis

Edited by (the late) K. Grasshoff, M. Ehrhardt, K. Kremling

Second, revised and extended edition 1983. Hardcover. XXVIII, 419 pages with 108 figures and 26 tables. DM 140,–. ISBN 3-527-25998-8

This second edition of a most successful book includes several new procedures: the use of capillary gas chromatography for the analysis and quantification of chlorinated hydrocarbons, the use of macroreticular resins for concentrating dissolved organic material from large volumes of seawater, and the use of HPLC for amino acid and carbohydrate determinations.

Electrophoresis

Official Journal of the International Electrophoresis Society

1984. Volume 5. Published bimonthly. Annual subscription rate DM 208,– plus postage and handling fee.

Electrophoresis is the name of an international journal that provides a unique forum for the publication of contributions that are meant to catch the attention of those engaged in the development and use of electrophoretic methods.

Electrophoresis publishes original papers, short communications and review articles. Research reports are written with particular emphasis on advanced methods of electrophoresis. Topics include new and improved analytical and preparative methods and theoretical aspects. Other features include book reviews, important conference dates and miscellaneous information.

In preparation:

A. Chrambach

Quantitative Gel Electrophoresis

verlag chemie

To obtain this book please write to:
Verlag Chemie GmbH
P.O. Box 1280/1290
D-6940 Weinheim
Federal Republic of Germany

Customers in U.S.A., Canada, Central and South America please write to:
Verlag Chemie International Inc.,
303 N.W. 12th Avenue,
Deerfield Beach, FL 33441, U.S.A.

Circle No.26 on Reader Service Card.

Customers and contractors

Philip Gummett

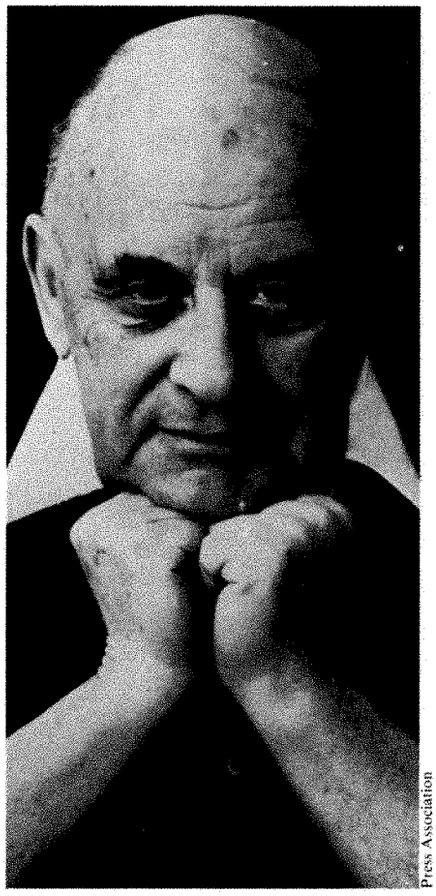
Government and Research: The Rothschild Experiment in a Government Department. By Maurice Kogan and Mary Henkel.
Heinemann Educational: 1983. Pp.196. £18.50, $37.50.

THE Rothschild Report of 1971 presaged one of the biggest shake-ups ever administered to the organization of science in Britain. It may in time be seen as the high point of the sectoral (or departmentally centred) approach to British science policy, especially if the Government adopts the reforms proposed by Sir Ronald Mason* in November 1983; these would strengthen the Advisory Board for the Research Councils and require all government research (civil and defence) to be included in the debate over the Science Budget of the Department of Education and Science, thus increasing central coordination.

Under Rothschild, government departments were to assume greater responsibility for formulating policy for applied research, which would then be implemented by their contractors. These might be in in-house research establishments, research councils, or elsewhere. By the same token, these contractors were to lose control over their applied research programmes, a particularly painful blow for the three bodies involved, namely, the Agricultural (ARC), Medical (MRC) and Natural Environment (NERC) Research Councils. The implications of the new regime were, however, greater in some departments than others, being modelled on the then current practice of the Ministry of Defence, and not involving much change in organizational reforms already in train at the Department of Trade and Industry.

The only attempt so far at overall evaluation of these reforms has been the one conducted by the government itself (*Review of the Framework for Government Research and Development (Cmnd 5046);* HMSO, 1979, Cmnd 7499); not surprisingly, it was short on analysis though useful as a summary of organizational developments. Kogan and Henkel's book, in contrast, is a detailed, thoroughly researched and methodologically path-breaking examination of how one part of the science–government system, the Department of Health and Social Security (DHSS) and the MRC, responded to Rothschild.

From 1974 the senior author worked as a consultant to DHSS on the operation of the post-Rothschild research management system. He and his research team enjoyed access to internal papers, attended over 200 departmental meetings, conducted over 200 interviews with key individuals and accompanied the Chief Scientist on some of his visits to external research units. The initiative of the DHSS in thus engaging Professor Kogan, and the encouragement given to him to publish, are to be applauded. The high quality of the work should encourage more departments to engage researchers in "on-line" evaluation of organizational reforms and, in an ideal world, it would also mark the end of bland official reviews like Cmnd 7499.



Press Association

Lord Rothschild — the report of 1971 "presaged one of the biggest shake-ups ever administered to the organization of science in Britain".

The price for this exceptional degree of access might well have been a story lacking in colour. And, indeed, most of the characters are not fully identified. But in general this does not matter: it is usually enough to know that we are dealing with an under-secretary in a policy division, or a member of the Chief Medical Officer's staff. Thanks, however, to the encouragement of Professor Arthur Buller, we do learn a lot about his own role when Chief Scientist at the DHSS, and this is all the more creditable because the authors make plain their disagreement with his view of science and its relationship with policy. We learn, for example, that he was less interested in cultivating the system for enunciating biomedical research policy (and thereby maintaining control over the funds transferred from the MRC) than in raising the quality of health services research (p.73); and that on his appointment in 1978 he determined to build up the scientific strength of the Department's research base, in part by strengthening the role of his own office in the commissioning of research at the expense of other parts of the machinery in which policymakers from DHSS divisions took the lead (p.86).

One cannot help wondering, though, what else could be said about how, in 1981, the MRC recaptured the funds lost to DHSS in 1972. For example, in addition to the point made above about the Chief Scientist's priorities, we also read, on p.73, that the MRC, in contrast to the Social Science Research Council (with which the Department also dealt), "enjoyed the confidence of a single, and supremely self-confident, profession strongly represented within" the DHSS. This sentence clearly alludes, in conformity with a theme well-documented in the book, to the institutional power of the biomedical research community and to its ability to impose its view that scientific development should be driven more by the internal norms of science than by social considerations. But does it also allude to the role of the Chief Medical Officer and his staff? One recalls here Sir Alec Merrison's tentative reference, before the House of Lords Select Committee on Science and Technology in April 1981, to the problems of fitting a Chief Scientist into a department which already had a powerful Chief Medical Officer. The role of the politicians in the DHSS is also largely passed over: it is unclear how far the attempt made from 1979 to cut spending on DHSS-funded research units derived from the political level (there being a General Election that year); and, again with reference to the recapture by the MRC of the transferred funds, what precisely lay behind the observation that MRC scientists "knew that they could beat the bureaucrats by appeals to the political system above their heads" (p.75)?

One other cavil: the organizational arrangements within the DHSS were extremely complex and perhaps required more careful description. In particular, the Research Liaison Groups (committees which brought together the policy divisions and scientific advisers to work out research programmes for specific policy areas) are introduced on p.12 as though they covered all aspects of the Department's work; on the next page we learn that many areas were

*Advisory Board for the Research Councils, *A Study of Commissioned Research: Report by Sir Ronald Mason.* Available from the Department of Education and Science, London.

not covered; only on p.77 are we told which areas were covered, having in passing (p.59) learnt that the rationale underlying these committees was to stimulate important but under-researched zones of action. It is clear enough when pieced together, but the task could have been easier.

These are trivial reservations, however, in comparison with the authors' achievements. For the book is not only a detailed review of post-Rothschild developments in the DHSS — a notable feat in itself, if one of limited appeal. It is also an original exercise in the combination of the approaches of the sociology of science and of public administration to the study of science policy. As such, it is a rich quarry of ideas on how to conduct such studies, and on the sheer complexity of the subject. It should be read not only by academic students of science policy and public administration, but by all who are concerned, in whatever way, with relations between science and government.

The book examines theoretically the extent to which science is steerable, or, in a different metaphor, permeable by social values. Drawing on the "finalization" school of sociology of science, the authors distinguish between different phases in the development of disciplines, arguing that disciplines are more steerable (though in different ways) when in the pre- and post-paradigmatic phases, but that in between, where work is beginning to crystallize around key theoretical models, the research programme is much more dictated by "internal" needs and is less susceptible to outside influence. They show how epistemological ideas must be linked with analyses of the institutional power of different sciences (contrast, for example, most medical research with most social science) in order to grasp the heterogeneity of science and the complexity of its relationships with policy. One might add, in the context of increasing interest in the evaluation of research, that these same considerations should urge caution in trying to apply blanket measures of research performance: the form, as well as the substance, of the products of different disciplines varies and demands great sensitivity in cross-disciplinary evaluations.

On the customer side of the picture, the authors show the variety of roles that exist in government, and the range of expectations that can be held of science. Officials concerned with policy-making, scientific advice, research management and research liaison have markedly different perspectives on science–government relations. Moreover, policymakers themselves are heterogeneous: some take a purely instrumental and short-term view of research; others, especially those with remits concerned more with policy analysis, are more interested in how research might help develop policy. Much emphasis is given to the need for, and complexity of, "brokerage" between departments and research councils, and between policy and science. We also learn a good deal about the need for sustained, long-term commitment to the development of the customer role, a process not aided by the relatively rapid turnover of staff on that side of the customer–contractor relationship.

Not surprisingly, the authors conclude that no single or straightforward model of science policy is appropriate, and that the metaphors of customer and contractor are oversimple. The customer metaphor implies that government can be clear and authoritative in stating its objectives "before entering the marketplace to purchase knowledge" (p.164). The contractor metaphor likewise assumes a coherent, identifiable and independent scientific community, able to negotiate from strength. In practice, both sides of the relationship exhibit a multiplicity of goals, functions, epistemologies and power bases. The social steerability of science depends, therefore, in a complex way on the effort put in by the customer, on the developmental state and institutional power of the scientific discipline concerned and on the level in the system at which one is working: micro-policy questions are easier to resolve than macro ones. Hence, finally, the conclusion that, at least in the broad field of social welfare, government should apply its energies to interaction with science rather than steerage of science: it should be concerned with the impact and implementation of research, and with the slow, steady and sustained development of strong communities of researchers and policy makers, rather than with rationalistic or imperative planning. □

Philip Gummett is Senior Lecturer in the Department of Science and Technology Policy, University of Manchester. He is author of Scientists in Whitehall *(Manchester University Press, 1980).*

East–West pairing

R.J. Elliott

Excitons.
Edited by E.I. Rashba and M.D. Sturge.
North-Holland: 1983: Pp.860. Dfl.425, $181.

Excitons is one of the first (actually Volume 2) of a series of books entitled *Modern Problems in Condensed Matter Sciences,* to be edited by V. M. Agranovich and A. A. Maradudin, and published jointly in English by North-Holland and subsequently in Russian by the publishing house Nauka. Each volume has editors and contributors who, like the general editors, are drawn from the Soviet Union and from the West.

As we all know, communication with Soviet colleagues is not as easy as we would like, partly because of language differences and partly because of the restrictions on travel to and from the Soviet Union. It is therefore particularly useful to have a series of up-to-date reviews which come in this form. It will make it less easy for scientists in the West to overlook Russian work in related fields.

Excitons are particularly appropriate for such a joint review. One leg of the theoretical foundation of the subject is firmly planted in Russia, on the work of Frenkel, Pekar and others, and relates largely to ionic and organic materials. The other is based in the West on the work of Wannier, Mott and others, and refers most directly to semiconductors. Experimental work in both areas has been carried out in parallel in all countries; for example, the rich exciton spectrum of cuprous oxide was evaluated in detail by Gross in Leningrad and by Nikitine in Strasbourg, and similar parallels exist in the work on polariton effects and on high-density excitons such as electron-hole droplets.

The book contains 18 chapters devoted to recent developments in this vigorous area of solid state physics and chemistry, their diversity displaying the remarkable wealth of the subject. Several refer to polariton (excitaron) effects, which lead to spatial dispersion and non-local effects, which appear remarkable and unusual in classical optics. Another group of papers deals with the effects of perturbations on exciton spectra, either arising from applied electric and magnetic fields, or from impurities. Isolated impurities cause trapping and in a semiconductor alloy the large number of impurities can give rise to different and important effects. Multi-exciton complexes are also considered, ranging from the relatively simple bi-excitons in CuCl to the many particle electron-hole complexes found in some semiconductors. Finally, the book's coverage has been extended to cover excitons in more specialized materials, including strained molecular crystals, magnetic insulators, and photosynthetic and other biological systems. Vibrational excitons (bound phonon pairs due to molecular anharmonicity) are also included.

The quality of the articles is uniformly high. There is naturally some variation in approach, but this is inevitable in the treatment of such a diverse field. The editors have brought together so many aspects of exciton physics that all those with an interest in the subject are bound to find not only useful reviews of their own speciality, but interesting insights into related fields. This volume augurs well for the rest of the series — I hope that at least some libraries will be able to afford it. □

R.J. Elliott is Wykeham Professor of Physics in the Department of Theoretical Physics, University of Oxford.

Lead in history and history in lead

Josef Eisinger

Lead and Lead Poisoning in Antiquity.
By Jerome O. Nriagu.
Wiley: 1983. Pp.437. £52.20, $73.10.

AMONG the technological arts discovered by our ancestors, none has played a larger role in shaping our civilization than metallurgy; and because the common minerals of lead melt at low temperatures, lead was the first metal to be refined. Lead may also have given rise to our oldest chemical industry, the production of white lead (ceruse), and is responsible for our oldest occupational disease.

The oldest lead object found by archaeologists is a string of beads worn in Anatolia some 8,000 years ago, and its use in jewellery suggests that this was a time when lead was still new and rare. Because of lead's malleability, corrosion resistance and other desirable qualities, human ingenuity devised many other uses for the metal and it attained great commercial importance in the classical world. The lead mines of Cartagena in southern Spain were exploited by Carthage before the area was conquered by Rome, and were worked by 40,000 miners who left a legacy of more than 10^7 tons of slag. In antiquity, lead was employed in coinage, glass, medicines, warfare (as sling bullets and in molten form during sieges), ship building, cosmetics (various oxides as eye shadows and so on), glazes for pottery, pigments, masonry (as mortar) and, of course, in "plumbing".



Smelting lead in the sixteenth century. The illustration comes from one of the works of Georgius Agricola (1494–1555), the German mineralogist who was the first to attempt to impose scientific order on the hitherto unsystematized knowledge of miners. (Reproduced from *Gmelins Handbuch der anorganischen Chemie*, Vol.47, Pt A1; Verlag Chemie, 1973.)

These are just a few of the myriad facts which Dr Nriagu has collected in his book, along with poetic and literary references to lead, arcane lead lore and the role of lead in alchemical theory. As a geologist and geochemist, the author is on familiar ground when dealing with the lead resources of the ancient world, including China and India, and with the contemporary methods of prospecting, mining and production. But he covers virtually all aspects of the metal's ancient history and has assembled an impressive bibliography from diverse disciplines which will be of great value.

The presentation of this encyclopaedic work is unfortunately not commensurate with its scope, however. The problem of organizing so much information into a readable text has not always been solved successfully: too many facts and quotations are offered without the necessary background or critical comment, and an unusually sloppy proof-reading job has left an abundance of annoying misspellings.

No aspect of the history of lead discussed by Dr Nriagu is likely to provoke as much interest and controversy as the prevalence of chronic lead disease in antiquity and his suggestion that it played an important part in the decline of the Roman Empire. This thesis was first advanced by Gilfillan in 1965 and has, ever since, irritated historians and classicists who are familiar with the complexities of that event. Gilfillan's historical model nevertheless has great popular appeal, and questions about it are raised in almost every discussion of the history of lead poisoning.

There exists, indeed, a great deal of literary, experimental and, lately, an increasing amount of archaeological evidence that many Romans were exposed to dangerous levels of lead. The most important exposure resulted from the unhappy custom of preserving and sweetening wine by the addition of a syrup *(sapa)*, which was prepared by simmering grape juice (must) in a lead kettle. It has been shown that *sapa* prepared according to any of several first-century recipes contained about $1 g Pb\ l^{-1}$, and when added to wine in the recommended proportion would produce chronic lead disease. Without *sapa*, or other preservatives such as resin which was, and is, preferred by the Greeks, the extensive wine trade of the Roman Empire would have been impossible.

Even those Romans who eschewed leaded wines ingested *sapa*, for it was a common cooking ingredient, and saturnine cosmetics and medicines were widely used. Dr Nriagu estimates that the rate of lead absorption ranged from $15 \mu g d^{-1}$ for slaves to $250 \mu g d^{-1}$ for aristocrats, compared to $2 \mu g d^{-1}$ in pre-technological societies and 30–50 $\mu g d^{-1}$ for the modern American.

Dr Nriagu raises the question why, in a society in which occupational plumbism, particularly among miners, was rampant, the connection between lead ingestion and disease remained undiscovered. Quite apart from the fact that miners were generally slaves or prisoners of war, beyond humanitarian concern or the care of physicians, the notion of a specific cause for a chronic disease, which we take for granted, was quite foreign to Graeco-Roman medicine. Galen and other contemporary physicians saw disease, including lead colic, as the result of an imbalance of the humours, and this view prevailed until some 1,500 years later when Paracelsus and his followers insisted that disease had specific chemical causes. The aetiology of the colic epidemics which were caused by the consumption of leaded wines and which persisted for many centuries was, in fact, not identified until 1696 (see *Med. Hist.* **26**, 279; 1982).

But while a strong case can be made for widespread lead disease in the Roman Empire and excessive drinking at the courts of some emperors, does it justify Gilfillan's hypothesis?

Dr Nriagu considers that the gluttony and other excesses of the Julio-Claudian and Flavian emperors were lead-related, although this is difficult to reconcile with the loss of appetite and constipation which are among the prominent symptoms of chronic plumbism. And did not the Empire attain its greatest wealth, power and extent under Trajan and other sober and effective emperors, who succeeded these sodden miscreants? It has on the other hand been suggested that gradual depopulation contributed to the failure of the Western Empire to defend itself against the invasions of the fifth century, and lead-induced infecundity may indeed have played a part in this. But the disappearance of the patriachal families in the first century can and has been explained otherwise, and may indeed have strengthened the state by introducing new blood into the most enduring political system which the West has evolved.

In the final analysis the evidence which has been marshalled in support of Gilfillan's hypothesis is, at best, anecdotal and occasionally spurious. There is little doubt that countless Roman citizens suffered and died from plumbism, but the link between that health disaster and the fall of the Roman Empire not only remains unproven but is probably unprovable. □

Josef Eisinger is at AT&T Bell Laboratories, Murray Hill, New Jersey.

Chandrasekhar on Eddington

Fred Hoyle

Eddington: The Most Distinguished Astrophysicist of his Time.
By S. Chandrasekhar
Cambridge University Press: 1983. Pp.64. £7.50, $12.50.

ARTHUR Stanley Eddington was born on 28 December, 1882. Two centenary lectures were given in his memory, and the Master and Council of Trinity College, Cambridge, made the happy decision of asking Professor Chandrasekhar to deliver them. To this assignment he brought to bear all the care and scholarship which has attended his work down the years. The first of his lectures bears the same name as the book itself; the second is entitled "Eddington: The Expositor and the Exponent of General Relativity".

The first lecture has five sections: parts I and II are biographical, III is concerned with Eddington's early researches on stellar motions, IV is on stellar structure, and V is an amusing summary of Eddington's relations with his contemporaries, relations both friendly and stormy. The work on stellar structure, the internal constitution of the stars as Eddington preferred to call it, was of such massive proportions that I must pass it by except for the following brief sketch. Of the factors on which the luminous output of a star depends, Eddington showed that by far the most important is its mass. Given the mass it was possible to predict, without any knowledge of the source of a star's energy, what the luminous output must be, and to do so with a degree of accuracy that was considered remarkable in the 1920s.

Even within the short space available in a lecture, Professor Chandrasekhar brings out the remarkable degree to which Eddington was self-contained in his work, less influenced by others immediately around him than is usual for a scientist and reminding us of Peer Gynt's motto: To thyself be sufficient.

The second lecture has nine sections. Part I is a brief description of the origins of Einstein's theory, II is concerned with Eddington's early enthusiasm for the theory and his part in organizing the eclipse expeditions to test the predicted bending of light past the Sun, and III contains Professor Chandrasekhar's own recollections about this episode in Eddington's life. I was amused to find this problem: "A affirms that B denies that C declares that D is a liar" rearing its head again, a problem which last tormented me in the year 1936 when I attended Eddington's lecture course, "The Combination of Observations". There were turbulent arguments among us students about it, and frankly speaking I never could see that a determination of the probability that D spoke the truth had a solution. I am glad even after these many years to find Professor Chandrasekhar slipping in the extra information that D really made a statement. Possibly Eddington put in this condition himself, but it was hard to judge from his lectures; unlike the beautiful clarity of his writing, Eddington's lectures were a quagmire through which we heaved ourselves as best we could.

Part IV of the second lecture is an appraisal in glowing terms of Eddington's splendid book, *Mathematical Theory of Relativity*. Like Professor Chandrasekhar, I too continue to use it. In Part V we have a brief exposition of Eddington's attempt at a unified theory of gravitation and electromagnetism. Then after a light relief in VI, with much quoting of poetry, we come to what Professor Chandrasekhar calls the unhappy development of Eddington's ideas, to what he himself referred to as fundamental theory, an attempted fusion of physics and cosmology, which Professor Chandrasekhar describes with plenty of supporting quotations as a transition from sureness to cocksureness.

Where, one can ask, does praiseworthy sureness end and unpraiseworthy cocksureness begin? Since there are plenty of examples of important work being ignored for many years, it is hardly a satisfactory answer that the judgement lies in the reactions of a man's contemporaries. The issue is better related to fact. Where facts are many or are strong, it is possible to be sure. Where they are few, and are subject to a variety of interpretations, sureness becomes cocksureness, which unfortunately one must agree was the situation in Eddington's later work.

Nevertheless, one word of defence! Is Eddington's cosmology really negated because of the discovery of primordial helium and the microwave background? With a non-zero cosmical constant, one can have an initial big-bang that is adjusted by a special choice of parameters to pass asymptotically close to the Einstein static state, and with this special choice Eddington could have defended himself, as indeed Lemaître did. Discovery of the existence of primordial helium and the microwave background date from 1965. Is it not a little ominous for those who are so cocksure about their ideas in cosmology today that the factual evidence for their position has remained nearly static over almost two decades? □

Sir Fred Hoyle was an undergraduate student of Arthur Eddington's at the University of Cambridge in 1935-1936.

Son of John Paul

G.D. Clarke

Culture of Animal Cells: A Manual of Basic Technique.
By R. Ian Freshney.
Alan R. Liss/Wiley: 1983. Pp.295. $49.50, £33.

THE washing of glassware, media making and cell production may seem generations away from gene cloning, monoclonal antibodies and fluorescent-activated cell sorting. They are not of course; but in large laboratories they are a central service and, if efficiently carried out, those who perform them may not be readily identified in corridors or canteens. In a decade of rapid advances, especially in molecular genetics, such services may be undervalued, but never by one used to a less-favoured environment.

The major part of Ian Freshney's book covers the setting up of a cell culture facility and the routine methods necessary to its function. The author then gives brief protocols for the more specialized techniques, referring always to the original publications. The bibliography is excellent and the cross-referenced list of equipment and reagents with addresses of manufacturers (in Europe and North America) will prove useful to many. The employment of trained staff, or advice from experienced colleagues, is preferable to book learning for those setting up a culture unit from scratch; but this book, with its large format, *Scientific American* lucidity, and generous use of diagrams, will be an essential adjunct.

The protocols, which have been taken direct from the Methods Book of a versatile laboratory, must be almost foolproof. Nevertheless, novices looking only for one particular procedure would be advised to spend some hours on a general browse. History records that many wrinkles and pitfalls are only obvious after expensive and time-consuming experience; Ian Freshney mentions them all somewhere but they could be constantly repeated.

Common sense suggests that the sections on organization and routine procedures will be of greatest use in developing countries, where budgets are tight and labour cheap. So a short appendix on alternative technology might have been included, while the absence of reference to probabilistic events in the sections on cell synchrony and physical cell separation could lead biochemists into an inappropriate use of cell populations. But these are small factors in a book invaluable in a wide range of rapidly expanding fields. The simple presentation of established routines, and introduction to the invaluable serum-free media of Ham and Sato, and to modern molecular techniques, make this a worthy competitor for Professor Paul's classic of 1959–1975. □

G.D. Clarke is in the Cell Proliferation Studies Laboratory of the Imperial Cancer Research Fund, Lincoln's Inn Fields, London.

Mathematics

Combinatorial Emuneration. By I.P. GOULDEN and D.M. JACKSON. *Wiley: 1983. Pp.569. ISBN 0-471-86654-7. £45, $63.*

q-Hypergeometric Functions and Applications. By H. EXTON. *Ellis Horwood/Halsted: 1983. Pp.341. ISBN 0-85312-491-4/0-470-27453-0. £22.50, $38.95.*

The Nature of Mathematical Knowledge. By P. KITCHER. *Oxford University Press: 1983. Pp.287. ISBN 0-19-503149-0. £15.*

Solitons. London Mathematical Society Lecture Notes, 85. By P.G. DRAZIN. *Cambridge University Press: 1983. Pp.136. Pbk ISBN 0-521-27422-2. £7.95, $14.95.*

Topology and Geometry for Physicists. By C. NASH and S. SEN. *Academic: 1983. Pp.320. ISBN 0-12-514080-0. £25, $39.50.*

Astronomy

ESA/COST 204: Phased-Array Workshop. *European Space Agency: 1983. Pp.66. No Pbk ISBN. 60 FF.*

In Quest of Telescopes. By M. COHEN. *Cambridge University Press/Sky Publishing: 1983. Pp.131. ISBN 0-521-24989-9/0-933346-25-5. £8.50.*

Material Sciences under Microgravity. Proceedings of the 4th European Symposium held April 1983, Spain. *European Space Agency: 1983. Pp.440. No Pbk ISBN. 140 FF.*

The Planet Mars. Proceedings of a Workshop held August 1982, Leeds. *European Space Agency: 1983. Pp.120. No Pbk ISBN. 90 FF.*

The Structure and Evolution of the Universe. Relativistic Astrophysics, Vol.2. By Ya.B. ZEL'DOVICH and I.D. NOVIKOV. *University of Chicago Press: 1983. Pp.718. ISBN 0-226-97957-1.*

Technology

Design and Installation of Solar Heating and Hot Water Systems. By J.R. WILLIAMS. *Ann Arbor: 1983. Pp.427. ISBN 0-250-40593-8. £37.*

The Global Race in Microelectronics: Innovation and Corporate Strategies in a Period of Crisis. By D. ERNST. *Campus-Verlag: 1983. Pp.290. Pbk ISBN 3-593-33137-3. DM 58.*

Manifolds, Tensor Analysis, and Applications. By R. ABRAHAM, J.E. MARSDEN and T. RATIU. *Addison-Wesley: 1983. Pp.582. ISBN 0-201-10168-8. Np.*

New Frontiers in Technology Application: Integration of Emerging and Traditional Technologies. E.U. von WEIZSÄCKER, M.S. SWAMINATHAN and A. LEMMA (eds). *Tycooly International, Dublin: 1983. Pp.271. Hbk ISBN 0-907567-66-5; pbk ISBN 0-907567-67-2. Hbk $50, £32.50; pbk $25, £16.25.*

Satellite Microwave Remote Sensing. T.D. ALLAN (ed.). *Ellis Horwood/Halsted: 1983. Pp.526. ISBN 0-85312-494-9/0-470-27397-6. £45, $77.85.*

Solar Energy '82: Resources-Technologies-Potential. *European Space Agency: 1983. No Pbk ISBN. 190 FF.*

Physics

Absorption and Emission by Atmospheric Gases: The Physical Processes. By E.J. McCARTNEY. *Wiley: 1983. Pp.320. ISBN 0-471-04817-8. £47.50, $66.50.*

Absorption and Scattering of Light by Small Particles. By C.F. BOHREN and D.R. HUFFMAN. *Wiley: 1983. Pp.530. ISBN 0-471-05772-X. £42.70, $59.80.*

Equilibrium Thermodynamics 3rd Edn. By C.J. ADKINS. *Cambridge University Press: 1983. Pp.285. Hbk ISBN 0-521-25445-0; pbk ISBN 0-521-27456-7. Hbk £22.50, $39.50; pbk £7.95, $14.95.*

Fast Nuclear Rotation. By A. SZYMANSKI. *Oxford University Press: 1983. Pp.241. ISBN 0-19-851463-8. £35.*

The Infancy of Atomic Physics: Hercules in his Cradle. By A. KELLER. *Oxford University Press: 1983. Pp.230. ISBN 0-19-853904-5. £12.50.*

Measurement of Suspended Particles by Quasi-Elastic Light Scattering. B.E. DAHNEKE (ed.). *Wiley: 1983. Pp.570. ISBN 0-471-87289-X. £37.95, $53.15.*

Multiparticle Dynamics 1982. 13th International Symposium. W. KITTEL, W. METZGER and A. STERGIOU (eds). *World Scientific: 1983. Pp.1018. ISBN 9971-950-53-7. £73.50, $104.*

Non-Linear Integrable Systems—Classical Theory and Quantum Theory. Proceedings of RIMS Symposium held May 1981, Japan. *World Scientific Publishing: 1983. Pp.289. ISBN 9971-950-32-4. £23.60, $33.*

Optical Waveguide Sciences. Proceedings of the International Symposium held June 1983, Republic of China. By H. HUNG-CHIA and A.W. SNYDER. *Martinus Nijhoff: 1983. Pp.360. ISBN 90-247-2848-7. Dfl.115, $49.50.*

Physics, 2nd Edn. By J.W. KANE and M.M. STERNHEIM. *Wiley: 1983. Pp.716. ISBN 0-471-08323-2. £20.50, $32.80.*

Physics Handbook: Elementary Constants and Units, Tables, Formulae and Diagrams and Mathematical Formulae. By C. NORDLING and J. ÖSTERMAN. *Studentlitteratur/Verlag Chemie International: 1983. Pp.431. ISBN 0-89573-138-X. $23.*

Principles of Acoustic Devices. By V.M. RISTIC. *Wiley: 1983. Pp.359. ISBN 0-471-09153-7. £35.75, $49.95.*

The Quantum Theory of Light, 2nd Edn. By R. LOUDON. *Oxford University Press: 1983. Pp.393. Hbk ISBN 0-19-851152-3; pbk ISBN 0-19-851155-8. Hbk £30; pbk £15.*

Radar Calibration. Proceedings of EARSeL Workshop held December 1982, Austria. *European Space Agency: 1983. Pp.180. No Pbk ISBN. 90 FF.*

Radiometry and the Detection of Optical Radiation. R.W. BOYD. *Wiley: 1983. Pp.254. ISBN 0-471-86188-X. £33.20, $46.50.*

Statistical Mechanics of Elasticity. By J.H. WEINER. Wiley: 1983. Pp.439. ISBN 0-471-09773-X. £47, $65.85.

Superconducting Magnets. By M.N. WILSON. *Oxford University Press: 1983. Pp.335. ISBN 0-19-854805-2. £17.50.*

Environmental Sciences

Ecotoxicology: The Study of Pollutants in Ecosystems. By F. MORIARTY. *Academic: 1983. Pp.230. ISBN 0-12-506760-7. £19.50, $32.50.*

Environment and Population: Problems of Adaptation. By J.B. CALHOUN. *Praeger: 1983. Pp.486. ISBN 0-03-063337-0. $60.*

The Fight to Save the Redwoods: A History of Environmental Reform 1917–78. By S.R. SCHREPFER. *University of Wisconsin: 1983. Pp.338. ISBN 0-299-08850-2. £16.90.*

Industrial Respiratory Protection. By M.F. TERESINSKI and P.N. CHEREMISINOFF. *Ann Arbor: 1983. Pp.349. ISBN 0-250-40587-3. £27.*

Wastes in the Ocean, Vol.1: Industrial and Sewage Wastes in the Ocean. By I.W. DUEDALL *et al. Wiley: 1983. Pp.431. ISBN 0-471-09772-1. £52.25, $73.*

Wastes in the Ocean, Vol.2: Dredged-Material Disposal in the Ocean. D.R. KESTER *et al.* (eds). *Wiley: 1983. Pp.299. ISBN 0-471-09771-3. £38, $53.25.*

Earth Sciences

An Outline of Soil and Rock Mechanics. By P. HABIB. *Cambridge University Press: 1983. Pp.149. Hbk ISBN 0-521-24461-7; pbk ISBN 0-521-28704-9. Hbk £15, $29.95; pbk £5.95, $11.95.*

Applied Geomorphology: Geomorphological Surveys for Environmental Development. By H.Th. VERSTAPPEN. *Elsevier Biomedical/North Holland: 1983. Pp.437. ISBN 0-444-42181-5. Np.*

Dance of the Continents: Adventures with Rocks and Time. By J.W. HARRINGTON. *J.P. Tarcher: 1983. Pp.254. Hbk ISBN 0-87477-168-4; pbk ISBN 0-87477-247-8. Hbk $15; pbk $9.50.*

Future Weather: Carbon Dioxide, Climate and the Greenhouse Effect. By J. GIBBIN. *Penguin: 1983. Pp.272. Pbk ISBN 0-1402-2459-9. £3.50.*

Mineralogy, 2nd Edn: Concepts, Descriptions, Determinations. By L.G. BERRY and B. MASON. *W.H. Freeman: 1983. Pp.561. ISBN 0-7167-1424-8. £25.95.*

Residual Deposits: Surface Related Weathering Processes and Materials. By R.C.L. WILSON. *Blackwell Scientific Publications: 1983. Pp.258. ISBN 0-632-01072-X. £25.*

Soil Biotechnology. By J.M. LYNCH. *Blackwell Scientific Publications: 1983. Pp.191. Pbk ISBN 0-632-00952-7. £10.80.*

Weather in Your Life. By L.J. BATTAN. *W.H. Freeman: 1983. Pp.230. Hbk ISBN 0-7167-1436-1; pbk ISBN 0-7167-1437-X. Hbk £17.95; pbk £8.95.*

Biological Sciences

Abortion and the Status of the Fetus. W.B. BONDESON *et al.* (eds). *Reidel: 1983. Pp.349. ISBN 90-277-1493-2. Dfl.75, $29.50.*

Analysis of Vertebrate Structure, 2nd Edn. By M. HILDERBRAND. *Wiley: 1983. Pp.654. ISBN 0-471-09058-1. £22.95, $33.*

Biosynthesis of Isoprenoid Compounds, Vol.2. J.W. PORTER and S.L. SPURGEON (eds). *Wiley: 1983. Pp.552. ISBN 0-471-09038-7. £114, $159.50.*

Born Too Soon: Preterm Birth and Early Development. By S. GOLDBERG and B.A. DiVITTO. *W.H. Freeman: 1983. Pp.201. Hbk ISBN 0-7167-1445-0; pbk ISBN 0-7167-1446-9. Hbk £14.50; pbk ISBN £5.80.*

Butterflies: A Colour Field Guide. By M. DEVARENNE. *David & Charles: 1983. Pp.180. ISBN 0-7153-8488-0. £6.95.*

California Serogroup Viruses. Proceedings of an International Symposium held November 1982, Ohio. C.H. CALISHER and W.H. THOMPSON (eds). *Alan R. Liss: 1983. Pp.428. ISBN 0-8541-0123-4. £32.*

Cellular Acclimatisation to Environmental Change. A.R. COSSINS and P. SHETERLINE (eds). *Cambridge University Press: 1983. Pp.254. ISBN 0-521-24384-X. £29.50, $59.50.*

Chromosome Mutation and Neoplasia. J. GERMAN (ed.). *Alan R. Liss: 1983. Pp.486. ISBN 0-8451-0220-6. £73.*

Current Topics in Hematology, Vol.4. S. PIOMELLI and S. YACHNIN (eds). *Alan R. Liss: 1983. Pp.212. ISBN 0-8451-0353-9. £32.*

Current Topics in Microbiology and Immunology, Vol.102. By M. COOPER *et al. Springer-Verlag: 1983. Pp.152. ISBN 3-540-12133-1/0-387-12133-1. DM 92, $39.70.*

Development and Evolution. The 6th Symposium of the British Society for Developmental Biology. B.C. GOODWIN, N. HOLDER and C.C. WYLIE (eds). *Cambridge University Press: 1983. Pp.437. ISBN 0-521-24949-X. £45, $89.50.*

Diving and Asphyxia: A Comparative Study of Animals and Man. By R. ELSNER and B. GOODEN. *Cambridge University Press: 1983. Pp.168. ISBN 0-521-25068-4. £25, $49.50.*

Écologie Générale. By R. BARBAULT. *Masson, Paris: 1983. Pp.224. Pbk ISBN 2-225-79497-9. 75 FF.*

The Ecology of Animal Movement. I.R. SWINGLAND and P.J. GREENWOOD (eds). *Oxford University Press: 1983. Pp.311. ISBN 0-19-857575-0. £27.50.*

Elements of Biological Science, 3rd Edn. By W.T. KEETON and C.H. McFADDEN. *Wiley: 1983. Pp.729. Pbk ISBN 0-393-95255-X. £10.95.*

Essentials of Human Anatomy, 7th Edn. By R.T. WOODBURNE. *Oxford University Press: 1983. Pp.632. Pbk ISBN 0-19-261435-5. £15.*

Genetic Rearrangement. Proceedings of the 5th John Innes Symposium. K.F. CHATER *et al.* (eds). *Croom Helm: 1983. Pp.296. ISBN 0-7099-0779-6. £16.95.*

Genetics. By G.J. BREWER and C.F. SING. *Addison-Wesley: 1983. Pp.729. ISBN 0-201-10138-6. £24.60.*

Growth and Maturation Factors, Vol.1. G. GUROFF (ed.). *Wiley: 1983. Pp.362. ISBN 0-471-09709-8. £51.75, $72.50.*

Immunogenetics. By M.B. ZALESKI *et al. Pitman: 1983. Pp.514. ISBN 0-273-01925-2. £18.75.*

Ion Exchange Membranes. D.S. FLETT (ed.). *Ellis Horwood/Halsted: 1983. Pp.210. ISBN 0-85312-640-2/0-470-27452-2. £19.50, $33.75.*

Isozymes. Current Topics in Biological and Medical Research, Vol.7: Molecular Structure and Regulation. The first of five volumes constituting the proceedings of the 4th International Congress held June 1982, Texas. By M.C. RATTAZZI, J.G. SCANDALIOS and G.S. WHITT. *Alan R. Liss: 1983. Pp.350. ISBN 0-8451-0256-7. £52.*

Les Arbres. By M. BECKER, J.-F. PICARD and J. TIMBAL. *Masson, Paris: 1983. Pp.144. Pbk ISBN 2-225-80020-0. 80 FF.*

The Lives of Plants. By D.M. STONE. *Charles Scribners: 1983. Pp.304. ISBN 0-684-17407-5. $15.95.*

Mammals of Britain and Europe. By J. POPE. *Pelham: 1983. Pp.176. ISBN 0-7207-1426-5. £14.95.*

Membrane Filtration: A User's Guide and Reference Manual. By T.D. BROCK. *Springer-Verlag: 1983. Pp.381. ISBN 3-540-12128-5/0-387-12128-5. DM 87, $37.50.*

Monotremes and Marsupials: The Other Mammals. The Institute of Biology's Studies No.150. By T.J. DAWSON. *Edward Arnold: 1983. Pp.87. Pbk ISBN 0-7131-2853-4. £3.30.*

Multiple Choice Questions in Histology. By R. COLEMAN. *Pitman: 1983. Pp.285. Pbk ISBN 0-272-79733-2. £5.95.*

Muscles and Their Neural Control. By G. HOYLE. *Wiley: 1983. Pp.689. ISBN 0-471-87709-3. £57, $79.75.*

Mycorrhizal Symbiosis. By J.L. HARLEY and S.E. SMITH. *Academic: 1983. Pp.496. ISBN 0-12-325560-0. £35, $59.50.*

Natural Selection and Its Constraints. By G. MAYO. *Academic: 1983. Pp.170. Hbk ISBN 0-12-481450-6; pbk ISBN 0-12-481452-2. Hbk np; pbk £5.95, $9.50.*

Neural Basis of Elementary Behaviour in Stick Insects. By U. BASSLER. *Springer-Verlag: 1983. Pp.169. ISBN 3-540-11918-3/0-387-11918-3. DM 79, $34.10.*

Neural Transmission, Learning, and Memory. International Brain Research Organization Monograph Series, Vol.10. By R. CAPUTTO and C.A. MARSAN. *Raven; 1983. Pp.290. ISBN 0-89004-860-6. $78.*

The New Frontiers in Plant Biochemistry. By T. AKAZAWA, T. ASAHI and H. IMASEKI. *Martinus Nijhoff: 1983. Pp.270. ISBN 90-247-2829-0. Dfl.110, $48.*

Organic Photochemistry, Vol.6. A. PADWA (ed.). *Dekker: 1983. Pp.438. ISBN 0-8247-7003-X. SwFr.198.*

Orthopteran Mating Systems: Sexual Competition in a Diverse Group of Insects. D.T. GWYNNE and G.K. MORRIS. *Westview Press: 1983. Pp.376. Pbk ISBN 0-86531-391-1. £26.*

Phytotelmata: Terrestrial Plants as Hosts for Aquatic Insect Communities. J.H. FRANK and L.P. LOUNIBOS (eds). *Plexus, New Jersey: 1983. Pp.293. Pbk ISBN 0-937548-05-7. $24.95.*

Plant Reproductive Ecology. By M.F. WILLSON. *Wiley: 1983. Pp.282. ISBN 0-471-08362-3. £33.25, $46.50.*

Retroviruses 1. Current Topics in Microbiology and Immunology, Vol.103. By P.K. VOGT and H. KOPROWSKI. *Springer-Verlag: 1983. Pp.146. ISBN 3-540-12167-6/0-387-12167-6. DM 96, $41.40.*

A Revised Handbook to the Flora of Ceylon, Vol.4. By M.D. DASSANAYAKE and F.R. FOSBERG. *Amerind, New Delhi: 1983. Pp.532. No ISBN. Np.*

Species at Risk: Research in Australia. Proceedings of a Symposium held November 1981, Canberra. By R.H. GROVES and W.D.L. RIDE. *Springer-Verlag: 1982. Pp.216. ISBN 3-540-11416-5/0-387-11416-5. DM 79, $34.10.*

Structure and Function of Fc Receptors. A. FROESE and F. PARASKEVAS (eds). *Dekker: 1983. Pp.312. ISBN 0-8247-1814-3. SwFr. 120.*

Systems Ecology: An Introduction. By H.T. ODUM. *Wiley: 1983. Pp.644. ISBN 0-471-65277-6. £47.50, $66.50.*

Techniques in Molecular Biology. By J.M. WALKER and W. GAASTRA. *Croom Helm: 1983. Pp.333. Hbk ISBN 0-7099-2747-9; pbk ISBN 0-7099-2755-X. Hbk £19.95; pbk £9.95.*

Traite de Zoologie Tome XVI, Fascicule 7. By P.P. GRASSE. *Masson, Paris: 1983. Pp.1121. No ISBN. 1100 FF.*

Unusual Microorganisms: Gram-Negative Fastidious Species. E.J. BOTTONE (ed.). *Dekker: 1983. Pp.126. ISBN 0-8247-1911-5. SwFr. 79.*

Viruses Associated with Human Cancer. By L.A. PHILLIPS. *Dekker: 1983. Pp.696. ISBN 0-8247-1738-4. SwFr. 252.*

Applied Biological Sciences

Advances in Blood Substitute Research. Proceedings of an International Symposium held September–October 1982, California. R.B. BOLIN, R.P. GEYER and G.J. NEMO (eds). *Alan R. Liss: 1983. Pp.500. ISBN 0-8451-0122-6. £43.*

Agricultural Insect Pests of the Tropics and Their Control, 2nd Edn. By D.S. HILL. *Cambridge University Press: 1983. Pp.746. ISBN 0-521-24638-5. £37.50, $69.50.*

Biological Methods of Prospecting for Minerals. By R.R. BROOKS. *Wiley: 1983. Pp.322. ISBN 0-471-87400-0. £40.80, $57.15.*

Calcium Antagonism in Heart and Smooth Muscle: Experimental Facts and Therapeutic Prospects. By A. FLECKENSTEIN. *Wiley: 1983. Pp.399. ISBN 0-471-05435-6. £57, $80.*

Cellular Systems for Toxicity Testing. Annals of the New York Academy of Sciences, Vol.407. G.M. WILLIAMS, V.C. DUNKEL and V.A. RAY (eds). *New York Academy of Sciences: 1983. Pp.484. Hbk ISBN 0-89766-206-7; pbk ISBN 0-89766-207-5. Hbk np; pbk $95.*

Chronic Toxicity of Antiepileptic Drugs. By J. OXLEY, D. JANZ and H. MEINARDI. *Raven: 1983. Pp.318. ISBN 0-89004-847-5. $43.*

DNA Repair: A Laboratory Manual of Research Procedures, Vol.2. E.C. FRIEDBERG and P.C. HANAWALT (eds). *Dekker: 1983. Pp.392. ISBN 0-8247-1805-4. SwFr. 132.*

Drogenanalyse: Dünnschichtchromatographische Analyse von Arzneidrogen. By H. WAGNER, S. BLADT and E.M. ZGAINSKI. *Springer-Verlag: 1983. Pp.321. ISBN 3-540-11867-5/0-387-11867-5. DM 148, $63.80.*

Drug Fate and Metabolism: Methods and Techniques, Vol.4. E.R. GARRETT and J.L. HIRTZ (eds). *Dekker: 1983. Pp.485. ISBN 0-8247-1849-6. SwFr. 198.*

Epithelial Ions and Transport: Application of Biophysical Techniques, Vol.4. By M.M. CIVAN. *Wiley: 1983. Pp.204. ISBN 0-471-04869-0. £56.95, $79.75.*

Foundations of Biochemical Engineering: Kinetics and Thermodynamics in Biological Systems. H.W. BLANCH, E.T. PAPOUTSAKIS and G. STEPHANOPOULOS (eds). *American Chemical Society: 1983. Pp.522. ISBN 0-8412-0752-6. £50.*

Methods of Enzymatic Analysis, 3rd Edn., Vol.1: Fundamentals. By J. BERGMEYER and M. GARBI. *Verlag Chemie/Verlag Chemie International: 1983. ISBN 3-527-26041-2/0-89573-231-9. DM 170 (Subscribers), DM 230.*

Modelling Data Analysis in Biotechnology and Medical Engineering. G.C. VANSTEENKISTE and P.C. YOUNG (eds). *Elsevier Biomedical/North Holland: 1983. Pp.376. ISBN 0-444-86596-9. £34.09.*

Modern Trends in Human Leukemia. V.R. NETH *et al.* (eds). *Springer-Verlag: 1983. Pp.518. Pbk ISBN 3-540-11858-6/0-387-11858-6. DM 196, $84.50.*

Modified Nucleosides and Cancer. Recent Results in Cancer Research, Vol.84. G. NASS (ed.). *Springer-Verlag: 1983. Pp.432. ISBN 3-540-12024-6/0-387-12024-6. DM 128, $55.20.*

Multiple Dopamine Receptors: Receptor Binding Studies in Dopamine Pharmacology. By M. TITELER. *Dekker: 1983. Pp.173. ISBN 0-8247-1735-X. SwFr. 92.*

Noninvasive Methods in Atherosclerosis Research. Atherosclerosis Reviews, Vol.10. By R. JOHNSON HEGYELI. *Raven: 1983. Pp.214. ISBN 0-89004-873-8. $49.*

Pain, Analgesia, and Addiction: The Pharmacologic Treatment of Pain. By B. STIMMEL. *Raven: 1983. Pp.326. ISBN 0-89004-605-0. $56.*

The Regulation of DNA Replication and Transcription: The Role of Trigger Molecules in Normal and Malignant Gene Expression. Experimental Biology and Medicine, Vol.8. By M. BELJANSKI. *Karger: 1983. Pp.190. ISBN 3-8055-3631-3. SwFr. 124, DM 148, $74.25.*

Statistical Analysis of DNA Sequence Data WEIR (ed.). *Dekker: 1983. Pp.272. ISBN 0 7032-3. SwFr. 120.*

Targeted Drugs. E.P. GOLDBERG (ed.). *1983. Pp.296. ISBN 0-471-04884-4. £52.25.*

Therapeutic Apheresis and Plasma Perfu Proceedings of the 3rd National Conference held 1982, Dallas. By R.S.A. TINDALL. *Alan R. 1983. Pp.492. ISBN 0-8451-0106-4. £37.*

Urbanization and Cancer Mortality: The U States Experience 1950–75. By M.R. GREENBE *Oxford University Press: 1983. Pp.276. ISBN 503173-3. £30.*

Psychology

Basic Psychology. By H. GLEITMAN. *W Norton: 1983. Pp.505. Pbk ISBN 0-393-95254-1. £9*

Historical Origins of the Concept of Neurosis. J.M.L. PINERO. *Cambridge University Press: 19 Pp.107. ISBN 0-521-24972-4. £17.50, $34.50.*

Introduction to Clinical Psychology: Perspecti Issues and Contributions to Human Service. By N SUNDBERG, J.R. TAPLIN and L.E. TYLI *Prentice-Hall: 1983. Pp.496. ISBN 0-13-47945. £22.45.*

The Psychology of Reading and Spelling Disabilit By A.F. JORM. *Routledge & Kegan Paul: 19 Pp.134. Pbk ISBN 0-7100-9344-6. Np.*

Psychology's Sanction for Selfishness: The Error Egoism in Theory and Therapy. By M.A. WALLAC and L. WALLACH. *W.H. Freeman: 1983. Pp.3 Hbk ISBN 0-7167-1465-5; pbk ISBN 0-7167-1466 Hbk £17.95; pbk £8.30.*

Thinking, Problem Solving, Cognition. By R. MAYER. *W.H. Freeman: 1983. Pp.426. Hbk ISB 0-7167-1440-X; pbk ISBN 0-7167-1441-8. Hbk £21.9 pbk £11.95.*

General

A Cry for Health: Poverty and Disability in the Thir World. G. SHIRLEY (ed.). *The Third World Grou for Disabled People/Ahrtag: 1983. Pp.96. Pbk ISB 0-946279-00-4/0-907320-04-X. £2.50.*

Asbestos, Vol.2: Properties, Applications, an Hazards. S.S. CHISSICK and R. DERRICOTT (eds) *Wiley: 1983. Pp.652. ISBN 0-471-10489-2. £39.75 $79.95.*

Briefwechsel 1945–68. By K. JASPERS and K.H BAUER. *Springer-Verlag: 1983. Pp.119. Pbk ISBN 3-540-12102-1/0-387-12102-1. DM 28, $11.60.*

By Candlelight: The Life of Dr Arthur Hill Hassal 1817–94. By E.A. GRAY. *Robert Hale: 1983. Pp.192. ISBN 0-7090-0922-4. £10.95.*

Bypasses: A Simple Approach to Complexity. By Z.A. MELZAK. *Wiley: 1983. Pp.245. ISBN 0-471-86854-X. £33.20, $46.50.*

Comparative Social Recognition. By P. COLGAN. *Wiley: 1983. Pp.281. ISBN 0-471-09350-5. £35.75, $50.*

Forest Water Ecosystems. Developments in Hydrobiology, Vol.13. Nordic Symposium held September–October 1981, Sweden. By C. FORSBERG and J-A. JOHANSSON. *Dr. W. Junk: 1983. Pp.163. ISBN 90-6193-764-7. Dfl.125, $54.50.*

The Human Environment: Action or Disaster? By R. CLARKE and J. PALMER. *Tycooly International, Dublin: 1983. Pp.73. Pbk ISBN 0-907567-65-7. Np.*

Izaak Walton: The Compleat Angler 1653–76. *Oxford University Press: 1983. Pp.435. ISBN 0-19-812313-2. £35.*

Lead and Lead Poisoning in Antiquity. By J.C. NRIAGU. *Wiley: 1983. Pp.437. ISBN 0-471-08767-X. £47.50, $66.50.*

The Logic of Decision, 2nd Edn. By R.C. JEFFREY. *University of Chicago Press: 1983. Pp.231. ISBN 0-226-39581-2. £16.*

Man on Earth. The Marks of Man: A Survey from Space. By C. SHEFFIELD. *Sidgwick & Jackson: 1983. ISBN 0-283-98956-4. £12.95.*

Moral Problems in Medicine, 2nd Edn. S. GOROVITZ *et al.* (eds). *Prentice-Hall: 1983. Pp.655. ISBN 0-13-600742-2. £22.45.*

The Nature of Explanation. By P. ACHINSTEIN. *Oxford University Press: 1983. Pp.385. ISBN 0-19-503215-2. Np.*

CHROMOSCAN 3

A new computing, scanning densitometer, so versatile it will sample anything...



...well, almost anything!

Perhaps a Picasso is a slight exaggeration! But it's no exaggeration to say that no other scanning densitometer can match the versatility of CHROMOSCAN 3.

All substrates up to 210mm square including gel strips, rods or slabs, TLC and HPTLC plates, acetate and paper can be scanned, using reflectance or transmittance in visible or ultra-violet, plus fluorescence and quench fluorescence modes.

And, CHROMOSCAN 3 is easily programmed via its microprocessor so that, even with the most complex test, the operator need only insert fresh samples.

Results are rapidly and clearly presented by the integral printer/plotter in an immediately usable form.

Peaks and troughs are automatically detected with per cent and total integral printed out as well as fraction concentrations and A/G ratio when appropriate.

Ease-of-use, speed of operation, accuracy and above all versatility make CHROMOSCAN 3 the most advanced computing scanning densitometer available.

Write now for further information or contact:

Joyce-Loebl
A Vickers company

Marquisway, Team Valley, Gateshead, Tyne & Wear NE11 OQW. Tel: (0632) 822111. Telex: 53257

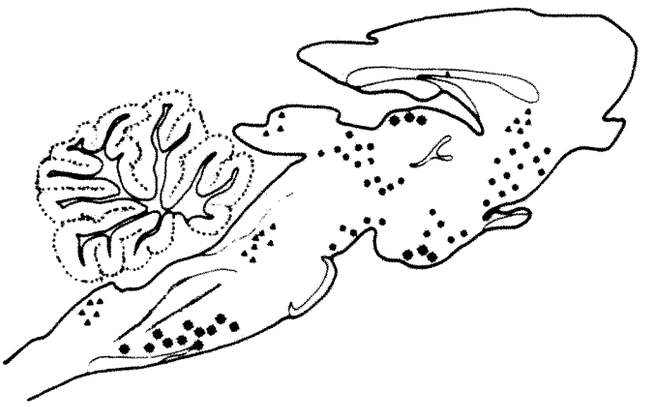
U.S.A. Vickers Instruments Inc. P.O. Box 99, Riverview Business Park No. 27, 300 Commercial Street, Malden, Massachusetts 02148 U.S.A. Telephone: (617) 324-0350. Telex: 949413.

W. Germany Joyce Loebl GmbH. Emanuel-Leutz-Strasse 1, 4000 Dusseldorf 11, W. Germany. Telephone: (211) 594091-94. Telex: 858 1998.

Circle No.24 on Reader Service Card.

Fronti rs in Neuropsychiatric Research

Edited by
Earl Usdin,
Department of Psychiatry and Human Behavior, University of California, Irvine, USA
Menek Goldstein,
Department of Psychiatry, New York University School of Medicine, USA
Arnold J.Friedhoff,
Department of Psychiatry, New York University School of Medicine, USA



August 1983; £40.00; 380pp; ISBN 0 333 347447

Based upon the proceedings of a CINP satellite symposium held in Corfu in June 1982, this book brings together contributions from the many fields involved in psychiatric research

Contents

Preface – Arnold J.Friedhoff; **Section I – Alterations of Neurotransmitter Systems in Affective Disorders –** Noradrenergic signal transfer as a target of antidepressant therapy – *Fridolin Sulser, Fumihiko Okada, D.Hal Manier, David D.Gillespie, Aaron Janowsky and Radhakanta Mishra;* Trans-synaptic mechanisms in the action of imipramine – *M.L.Barbaccia, De-Maw Chuang, O.Gandolfi and E.Costa;* Regional variations in 5-HT receptor populations and in ^{3}H-imipramine binding sites in their responses to chronic antidepressant treatment – *K.Fuxe, S-O.Ögren, L.F.Agnati, F.Benfenati, L.Cavicchioli, B.Fredholm, K.Andersson, C.Farabegoli and P.Eneroth;* The possible role of central epinephrine, α_2-adrenoreceptors and presynaptic dopamine receptors in affective disorders– *Menek Goldstein, Jorgen Engel, Isaac Regev and Shogo Mino;* Molecular aspects of regulation of α_2-adrenergic receptors – *D.C.U'Prichard, B.D.Perry, C.H.Wang, J.C.Mitrius and D.J.Kahn;* **Section II – Mechanisms of Action of Antidepressant Drugs –** Serotonin selective reuptake inhibitors - relationship between chemical structure and pharmacological effects *S.Agurell, H.Hall, T.Högberg, U.H.Lindberg, T.de Paulis, S.B.Ross and S-O.Ögren;* Effects of chronic administration of antidepressant drugs on central serotonergic receptor mechanisms – *S-O.Ögren, K.Fuxe, O.G.Berge and L.F.Agnati;* Neurochemical effects of the atypical antidepressant diclofensine in the rat – *W.P.Burkard, H.H.Keller, M.Da Prada and W.Haefely;* Oxaprotiline and its enantiomers: do they open new avenues in the research on the mode of action of antidepressants? – *A.Delini-Stula, A.Vassout, K.Hauser, H.Bittiger, U.Buech and H-R.Olpe;* Effects of single and multiple doses of antidepressant drugs on the ^{3}H-spiperone-labeled serotonin receptors in the frontal cortex of the rat – *Claes Rüdeberg;* Relationship between adaptive changes in brain α_2- and ß-adrenergic receptors following repeated imipramine, yohimbine and electroconvulsive shock treatment to rats – *A.I.Salama, A.Garcia, C.H.Wang, L.Lin and D.C.U'Prichard;* A review of the properties of a new specific 5-HT reuptake inhibitor - fluvoxamine maleate – *Jennifer S.Wakelin;* Biochemical and physiological mechanisms of action of carbamazepine in affective illness – *Robert M.Post and Thomas W.Uhde;* **Section III – Atypical Antidepressants and Mechanisms of Action –** Bupropion: an empirical pharmacological approach to drug development – *Loren Miller and Warren Stern;* Antidepressant and potential antimanic actions of GABA receptor agonists – *G.Bartholini, B.Scatton, B.Zivkovic, P.Worms and K.G.Lloyd;* Fluoxetine: a clinically effective non-tricyclic antidepressant – *Louis Lemberger, Ray Fuller, David Wong and Paul Stark;* The dexamethasone suppression test as a research variable – *John F.Greden;* **Section IV – Correlation of Biological and Clinical Factors in Affective Disorders –** Progress in pharmacotherapy of depression: research implications – *Anastasios Georgotas, Daniel Bush, William E.Hapworth, Mary Kim and Samuel Gershon;* Relationship of anxiety disorders to affective disorders – *David L.Dunner;* Application of sleep investigations in understanding antidepressant drug–clinical drug interactions – *David J.Kupfer and James Perel;* **Section V – Clinical and Basic Aspects of Affective Disorders –** Biochemical aspects in striatal dopamine receptor desensitization – *Ingeborg Hanbauer and Maurizio Memo;* Receptor–receptor interactions: possible new mechanisms for the action of some antidepressant drugs – *L.F.Agnati, K.Fuxe, F.Benfenati, L.Calza, N.Battistini and S-O.Ögren;* A double-blind comparative trial of oxaprotiline with amitriptyline and placebo in outpatients with moderate depression: relationship of urinary MHPG levels – *The Oxaprotiline Study Group;* Platelet α_2-adrenergic receptor activity in clinical studies of depression – *R.H.Lenox, J.E.Ellis, D.A.Van Riper, Y.H.Ehrlich, J.M.Peyser, J.E.Shipley and L.A.Weaver;* The olfactory bulbectomized rat as a model of depression – *Suzanne Jancsár and B.E.Leonard;* Closing Remarks – *Peter Hartocollis;* Index.

Please send orders and requests for information to:
Felicity Parker, Product Manager, Scientific and Medical Division,
The Macmillan Press Ltd, Houndmills, Basingstoke, Hampshire RG21 2XS, UK

SCIENTIFIC & MEDICAL | M | MACMILLAN PRESS LTD

Circle No.60 on Reader Service Card.

Computers in chromatography

Richard Patience gives a personal view of how to tackle the growing array of computers being used in chromatography.

How many chemists and biochemists, when faced with a computerized chromatography system, know which button to press and when to press it, but have no idea how the system works? My own first encounter with such a system — a DEC PDPB/e laboratory computer attached to a capillary gas chromatograph/mass spectrometer (GC/MS) — only served to give me a firm conviction that it was all far too complex and that I would never learn to use it. Of course, in time, I did and, what is more, became quite at home with it. But it is one thing to know what to do with a computer keyboard and quite another to know why you do it!

In the mid 1970s, when I first started working with computerized chromatography, laboratory computers were quite rare — and quite large — and generally found only on more sophisticated and expensive equipment, like GC/MS. Temperature and solvent gradients in GC and liquid chromatography (LC) respectively were set up manually. The Pye 104, the Perkin Elmer F17 and the Varian 2700 GCs were all operated in this way, whilst the major LC company, Waters, had the M660 gradient analysis programmer, which has only recently been superceded by the microprocessor-driven M680. 'Data collection' was by chart recorder.

Shortly after I embarked on my research career, there were two major developments. First, in gradient formation, microprocessors began to replace manual control, particularly in GC. Microprocessor-controlled gradients were set up from a small keyboard and parameters such as gradient slope were stored in the memory. Automatic cooling at the end of the run meant that the whole cycle could be preprogrammed and initiated by a single key, but unfortunately you still had to inject the sample.

The second improvement came with the arrival of integrators to calculate peak areas for quantitation. This was a real advantage — both in terms of time and accuracy — even though some early models produced only a numerical strip print out and no chromatogram.

One of the main advantages claimed for microprocessors was an improvement in reproducibility of repeat analyses, in other words an increase in retention times. I have no reason to dispute that, but in my opinion there were, and still are, two disadvantages to microprocessors. The problem with many systems was that, unlike manual units, once started the programme could not be altered without aborting the whole run. This gives a degree of inflexibility that is fine for routine analyses but can frequently be inconvenient when dealing with less well defined samples. What is more, gradient controllers and integrators originally had to be programmed and started separately. The poor user needed three or four hands — one to inject (two for the less steady-handed), one to initiate the gradient programmer and one to start the integrator.

Now, of course, all this has changed and often the whole analysis sequence is completely automatic. The computer boom has reached even HPLC — slower to benefit than GC but rapidly catching up. So, faced with the present array of chromatographic systems, how does the practical biochemist or chemist decide which best suits his or her needs? The mechanical aspects are relatively easy; from practical experience of dismantling pumps and setting up equipment it is possible to assess the mechanics of an HPLC system. But computers — particularly software — are much harder to evaluate without actually using them. If this be the case, then where does one start?

Choosing a system

One tactic is to begin with an assessment of the facilities available from the present generation of computers. Fig. 1 shows a stylized configuration of a hypothetical HPLC system. The controller can be programmed to initiate and control a number of events at preselected times. Furthermore when remote computers or terminals are linked up, the system can be controlled remotely and data can be generated and sent to distant units.

The range of functions offered by individual manufacturers varies considerably, of course. More sophisticated systems include the Varian Vista 402, the Spectra Physics Labnet, Waters' M721 and associated units, the LDC CCM system, Perkin Elmer's Series 4 + Chromatographics 2, Pye Unicam's 4850, Hewlett Packard's 1090 + HP85, Dupont's 8800 and Sentinel, and Gilsen and ACS, which use Apple IIe computers.

Why, though, should such sophistication interest a typical chromatographer? One advantage of microprocessor-based systems has already been mentioned — increased reproducibility of retention times of repeat analyses. However, to me the main advantage lies in the ability to coordinate different units and events from a single keyboard. Gone is the need for three hands to start a run; with fully automated instruments one finger may be enough for dozens of analyses. Of course there are always disadvantages. One of these is cost — around £7,000 to £10,000 at present on top of the HPLC cost. Another is simply the level of complexity of the system itself, which can be confusing for non-specialists, and inevitably introduces a degree of redundancy to the system. Nevertheless, I am sure that the use of computers in chromatography will continue to increase, particularly as they become cheaper. □

Richard Patience is in the department of chemical endocrinology at Saint Bartholomew's Hospital, London.

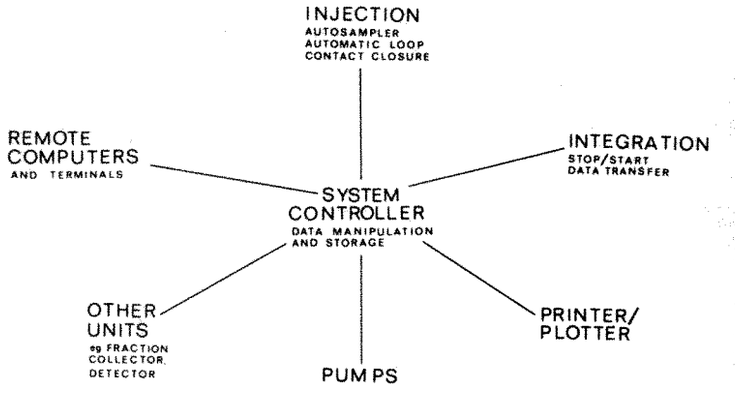


Fig. 1. Stylized configuration of a computerized HPLC system. The controller regulates; (1) injection, which may be by autosampler, automatic loop (user loads loop, controller injects it) or contact closure (user loads and injects, which closes electrical contact and starts controller program); (2) pumps: gradient formation preprogrammed; (3) integration: stop/start or integration and transfer of data to the controller for manipulation, storage on disk or tape, or plotting; (4) printer/plotter; (5) other units, such as a fraction collector or detector; (6) remote computers or terminals.

High performance liquid chromatography

● HPLC Technology has launched a range of HPLC column block heaters. The heaters operate in the temperature range ambient to 99°C and reproduce temperatures with an accuracy of ± 0.5°C; their temperature rise time from ambient to 60°C is 10 minutes. Columns of various lengths (with or without pre-columns) can be used. Heaters are available for larger diameter columns as well as for heating the injection system.
Circle No. 100 on Reader Service Card.

● Perkin-Elmer has introduced several new HPLC columns. The 3×3 columns, so-called because they are 3 cm long and use 3-μm packing materials, offer reduced analysis times and lower solvent consumption. The short column length eliminates high back-pressures. The columns can be used with most liquid chromatography systems, but operate best with Perkin-Elmer equipment. The combination of the Perkin-Elmer series 4 solvent delivery system, the LC-85B detector with a 1.4-μl flowcell and 3×3 columns provides the fastest analyses.
Circle No. 101 on Reader Service Card.

● Bio-Rad's HPLC column heater will produce temperatures between ambient +5°C and 150°C with accuracy of ± 2°C, while operating in environments with temperatures between −5°C and 50°C. A thermostat set to turn off power automatically at 180°C provides over-temperature protection. After a standard warm up period, the column heater will maintain temperatures with a stability of ± 0.1°C. The heater has a dual column capacity, and can accommodate columns from 5 to 30 cm in length and from ¼ to ½ inch in diameter. A 'solvent preheat block' is also available as an accessory.
Circle No. 102 on Reader Service Card.

● A series of prepacked HPLC columns of high-pressure hydroxyapatite has been introduced by Bio-Rad. The Bio-Gel HPHT system has a standard column, which is 100 + 7.8 mm and has a capacity of 100 mg for protein or 5 mg for DNA. Separations can be concluded within 30 minutes. Any technique using standard hydroxyapatite protocol can be applied to the high-pressure system. The columns can be used to purify monoclonal antibodies from mouse ascites fluid and to purify plasmids. They are either available individually or as a system which incorporates the use of a sacrificial guard column.
Circle No. 103 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● Gelman Sciences has introduced a miniature disposable membrane filter unit designed for HPLC users. The Acro LC13 has a 0.2, 0.45 or 1.0-μm, 13-mm diameter fluoropolymer membrane filter sealed into a polypropylene housing. This reduces liquid sample loss to 0.014 ml. The new fluoropolymer membrane is naturally wetted by aqueous samples whilst retaining chemical compatibility with solvents such as methanol, chloroform, *N*-heptane, methylene chloride and acetonitrile.
Circle No. 104 on Reader Service Card.

● A new alkylphenone HPLC internal standard kit is now available from Pierce. Alkylphenones are a family of neutral compounds. Neither solvent modifiers nor acidic nor basic buffers will affect their retention volume. Due to their wide solubility range, both polar and non-polar solvent systems can be used. Nanogram quantities are detectable with a common UV detector. Each kit contains one gram each of nine alkylphenones.
Circle No. 105 on Reader Service Card.

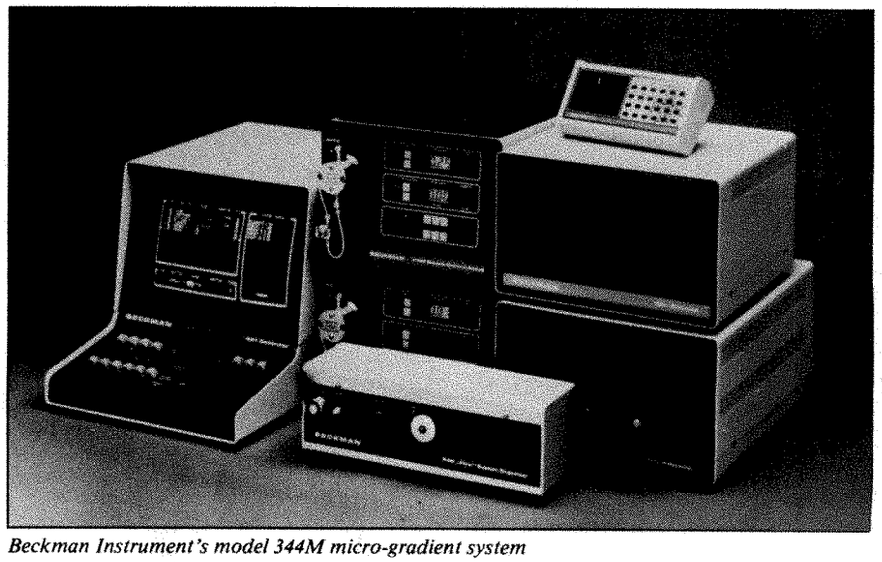
● A new HPLC pumping system has been introduced by Knauer. It has an electronically-controlled motor, two sapphire pistons and two cartridge-type ruby ball check valves. Its unusual cam design gives correct precompression and will compensate the pressure feedback electronically for liquid compressibility. Changing from micro- to macro-chromatography is achieved by exchanging pump heads. The unit can be interfaced with most commercial controllers or with its companion microprocessor programmer models 50A and B.
Circle No. 106 on Reader Service Card.

● A new carbohydrate HPLC column is now available from IBM Instruments. The new column is packed with a 5-μm stationary phase. Analyses can be performed at room temperature using a mobile phase of acetonitrile and water. Typical applications of the new column include baseline separation of critical sugars, separation of saccharides from polysaccharides, separation of polysaccharides and maltose/isomaltose ratio determination. The carbohydrate column can be supplied in a 4.5×250 mm size.
Circle No. 107 on Reader Service Card.

● Distilled in glass and filtered through 0.2-μm Teflon membranes, Pierce HPLC/spectro grade solvents are intended to have both HPLC and spectrophotometric applications. Label information includes: formula, formula weight, safety data, flash point, first-aid procedures, UV absorbance data, gas chromatography assay, evaporation residue, and water content.
Circle No. 108 on Reader Service Card.

● Two types of guard columns have been introduced by IBM Instruments to prevent liquid chromatography samples with non-eluting materials contaminating HPLC columns. The guard columns are compatible with HPLC instrumentation. One of the columns has normal (silica) pellicular packings while the second has reversed-phase (octadecyl) packings. Each guard column kit contains 4.5 × 50 mm repackable hardware and 6 g of packing material. Replacement packing is available in 10-g units.
Circle No. 109 on Reader Service Card.

● Beckman Instruments has introduced the model 344M micro-gradient system which is designed to work with 2-mm HPLC columns. The system includes the model 114M pump which features flow ranges of 0.001 to 1.00 ml min^{-1} or 0.01 to 9.99 ml min^{-1}. A new system organizer incorporates a low column dynamic mixer which is designed to blend solvent combinations at low flow rates.
Circle No. 110 on Reader Service Card.



Beckman Instrument's model 344M micro-gradient system

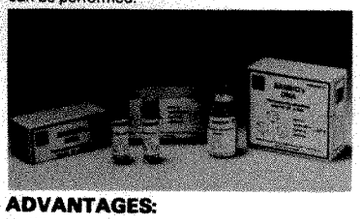
DO YOU WORK WITH CULTURED CELLS OR BIOPSY SPECIMENS?

THEN YOU KNOW THAT DNA IS THE BEST REFERENCE VARIABLE

SANBIO INTRODUCES: NUCLESAN 100

QUICK ANALYSIS:

By means of the test combination kit double stranded DNA can be measured adequately in a very short time. With one package 60-120 tests (depending on the DNA-range of the sample), including the calibration curve samples, can be performed.



DNA-RANGE:

The method* allows to measure small quantities of DNA *(5-30 µg)* in cultured cells or biopsy specimens. It opens the possibility of relating biochemical parameters to the exact number of living cells.

ADVANTAGES:

Up till now methods for measuring double stranded DNA were limited in their application because of low sensitivity or because of complex extraction procedures (Burton, K. Biochemical Journal (1956) 62, 315-322). The actual method liberates DNA in one single step from cells or biopsy specimens. Then the DNA is complexed stochiometrically to a substance with high affinity to DNA. The complex formed can be determined fluorimetrically. All ingredients are available in one package in balanced quantities. The clear user's information in four languages enables a simple quantitative determination for every scientist.

*Hukkelhoven, M.W.A.C., Vromans, E., Markslag, A.M.G. and Vermorken, A.J.M. *Anticancer Research* (1981) 1, 341-344.
Research Unit for Cellular Differentiation and Transformation, University of Nijmegen (NL).

SANBIO BV Life Science Products

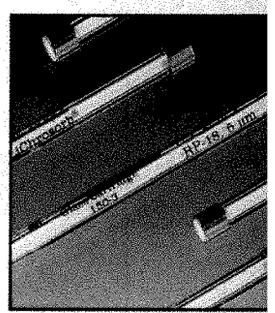
P.O. Box 45, 5388 ZG NISTELRODE, Telex 37493 SANBO NL
Telephone: 04124 - 2102 The Netherlands.

Circle No.03 on Reader Service Card.

CGC Glass Cartridges

A new system for HPLC in glass-bodied columns

- chemical inertness as well as unequalled surface smoothness of the internal tube wall
- usability under high pressures due to a special hardening procedure for the glass tube
- easy connection to an optional HPLC unit
- advantageous price compared with steel columns



Please ask for our brochure

Reagents
MERCK

E. Merck, Frankfurter Straße 250, D-6100 Darmstadt 1
Federal Republic of Germany

Circle No.23 on Reader Service Card.

British Journal of PHARMACOLOGY

The most respected journal for those professionally involved in the pharmacological sciences.

Pharmacologists, like other scientists, have to cope with ever-increasing amounts of information. A publication which only includes papers of the highest standard and relevant to current pharmacological research is obviously required. The *British Journal of Pharmacology* is just such a journal.

Original papers, submitted from all parts of the world, examine every aspect of drug action. Chemotherapy, systemic pharmacology, drug mechanics, pharmacokinetics, psychopharmacology, autopharmacology are all given extensive coverage. *British Journal of Pharmacology* is thus a vital source of information for physiologists, clinicians, immunologists, molecular biologists and biochemists as well as pharmacologists.

Keep up-to-date, subscribe now!

ANNUAL SUBSCRIPTION

UK £145.00
USA/ Canada $360 Surface/ $440.00 Airmail
Rest of the World £160.00 Surface/ £210 Airmail

Orders should be accompanied by remittance, cheques made payable to Macmillan Journals Limited: Subscription Department, Brunel Road, Basingstoke, Hampshire, RG21 2XS, England.

Sample copies may also be obtained from the same address.

Circle No.68 on Reader Service Card.

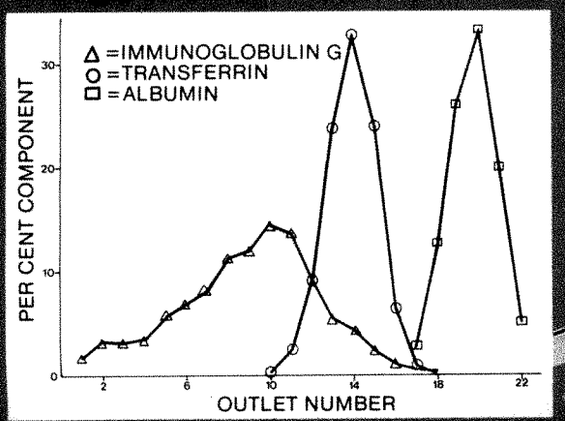
BIOSTREAM

[illegible] Reveals For [illegible]

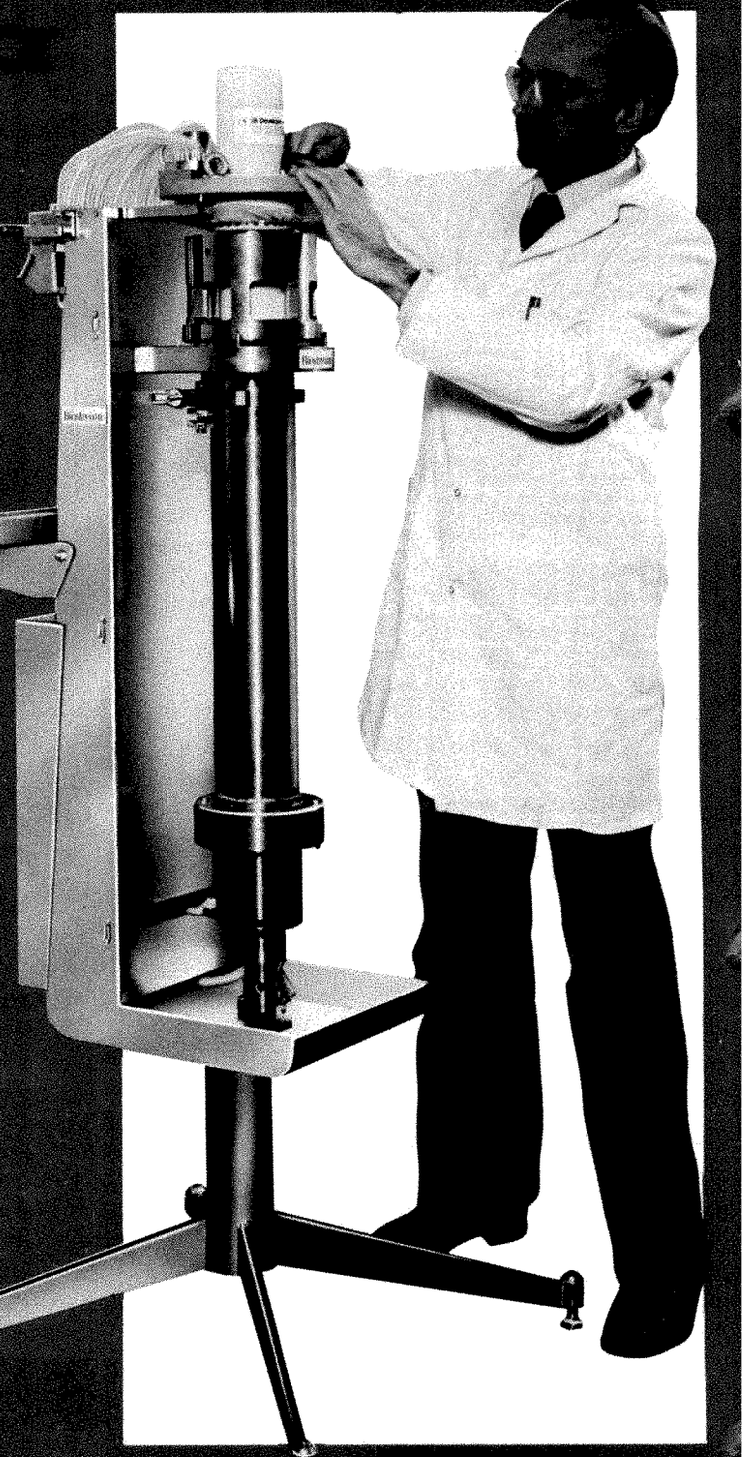
The BIOSTREAM separator provides continuous, free flow electrophoresis on a scale not previously available. Designed for production plant operation it has a throughput up to 1000 times that of other commercial electrophoresis systems.

BIOSTREAM separates rapidly and reproducibly with excellent retention of biological activity. This enables better product yields and purities to be achieved. A wide range of biological materials may be processed including simple molecules, macromolecules and whole cells.

Although intended for large scale applications BIOSTREAM can also process volumes of less than one litre Thus identical separations can be performed at laboratory, pilot plant and production scales.

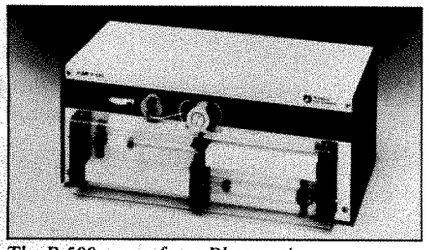


THE FRACTIONATION OF DESALTED HUMAN PLASMA
DATA BY COURTESY OF THE HARWELL [illegible] BORATORY.

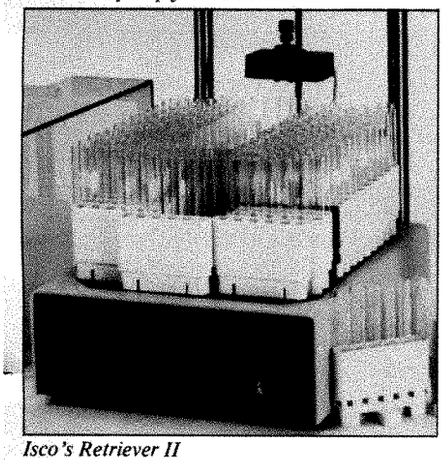


[illegible] Limited

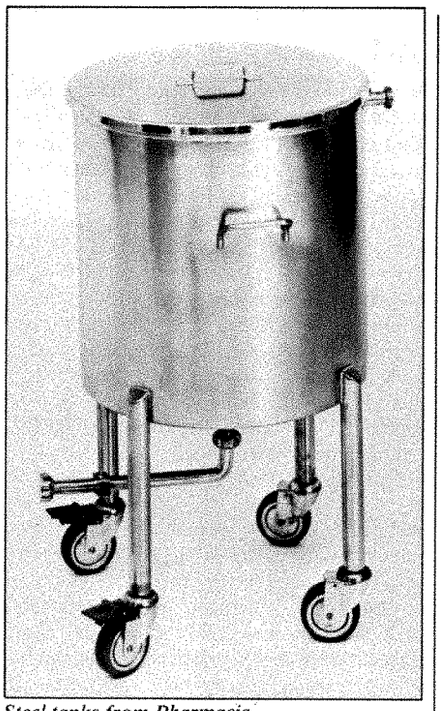
[illegible]



The P-500 pump from Pharmacia



Isco's Retriever II



Steel tanks from Pharmacia

Chromatographic accessories

● The new Retriever II linear fraction collector from Isco can handle up to 174 tubes, yet it takes up just a square foot of bench space. Racks are available to hold either 174 of the 12 and 13-mm diameter test tubes, 116 of the 10 to 18-mm tubes, or 42 scintillation vials. The top of the collector can be lifted off. Retriever II can be programmed to collect by time, drop, discharges from a siphon or volumeter, or pulses from a pump.
Circle No. 111 on Reader Service Card.

● Pharmacia Fine Chemicals has introduced a new high precision pump, the P-500. This solvent delivery system is for use when a constant, precise flow is required, as in general liquid chromatography. It is designed for use with Pharmacia's new FPLC high performance chromatography media but is also compatible with traditional separation methods. The pump can be used at atmospheric pressure for columns of Sephadex and Sephacryl. Piston movement is controlled. All materials in contact with the eluent are compatible with aqueous buffers and the borosilicate glass used in the P-500 is impervious to solvents.
Circle No. 112 on Reader Service Card.

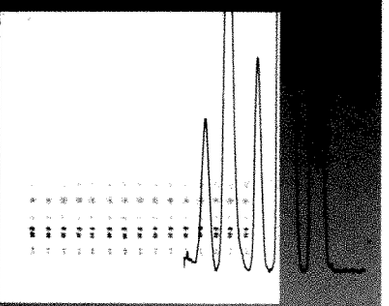
● A range of power supplies is now available from Uniscience which produce from 250–4,000 V. It includes the EC300 with the isoelectric focusing end point detector. Also on offer is a family of gradient formers for producing linear, convex, concave and exponential gradients. Two microdialysis models (4 and 24 chambers) are also offered.
Circle No. 113 on Reader Service Card.

● Pharmacia Fine Chemicals has a range of stainless steel tanks that facilitate the preparation, filtration, delivery and collection of buffers and samples used in large scale chromatography. The range is comprised of a buffer preparation tank (capacity 300 litres) fitted with a circulation pump and filter, flat-bottomed buffer tanks (25–300 litres) and conical sample tanks (50–300 litres).
Circle No. 114 on Reader Service Card.

● A dedicated software package has been developed for ion chromatography by Nelson Analytical. This software is offered as part of the standard chromatography software package for Nelson Analytical's 4400 series multi-instrument data systems, which are based on the HP 200 series desktop computers. Data from up to 10 ion chromatographs (20 detectors), or from conventional gas and liquid chromatographs can be processed on the same system simultaneously. Data sampling rates for this system range from one point every 10 seconds to 100 points every second.
Circle No. 115 on Reader Service Card.

● Scientific Glass Engineering is now producing the items required to combine on-column injection with bonded phase capillary columns. These include on-column injectors with special vitreous silica needle syringes, a range of bonded phase columns in vitreous silica and low dead-volume connectors with graphite, vespel or kalrez ferrules.
Circle No. 116 on Reader Service Card.

A chromatography laboratory is incomplete unless it includes instrumental thin-layer chromatography.



While other chromatography techniques are often employed, (instrumental) TLC has been found to be the method of choice for many applications.

However, this is only possible if appropriate instrumentation is applied to the task!

COMPLETE TLC SYSTEMS – everything from the simplest tool to fully automatic quantitative scanning equipment – all from one source – is only available from CAMAG.

Consult your CAMAG specialist.

But first request CAMAG's latest TLC catalog.

CAMAG

CAMAG · Sonnenmattstr. 11
CH-4132 Muttenz/Switzerland

US-Distributor:
APPLIED ANALYTICAL INDUSTRIES, INC,
Route 6, Box 55
New Hanover County Industrial Airpark
Wilmington, North Carolina 28405
(919) 763-4536

CAMAG has agents in almost all countries.

Circle No.04 on Reader Service Card.

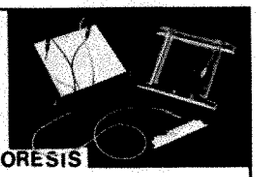
Columns and packing materials

● IBM Instruments has added a column for molecular weight analysis to its line of gel permeation chromatography/size exclusion chromatography (GPC/SEC) columns. The new GPC/SEC Scout column is available in a cross-linked divinylbenzene gel. This means that many organic solvents can be used with it, including dimethylformamide. The Scout column simplifies the use of column sets and can be used for molecular weight distribution analysis of polymers with a range of 500 to 4,000,000.
Circle No. 117 on Reader Service Card.

● Pharmacia Fine Chemicals has introduced its Monobeads (see *Nature* **305** 95) into prepacked ion exchange columns. An integral part of their fast protein liquid chromatography (FPLC) system, Monobeads represent a new generation of pressure and *p*H stable chromatographic absorbents. They have been designed for rapid protein, peptide and polynucleotide separation. The Monobeads are available in 3 dimensions — Mono Q for anion exchange, Mono S for cation exchange and Mono P, a chromatofocusing matrix which separates proteins according to their isoelectric points. Their monodispersity allows high efficiency packing and medium pressure operation. They feature a particle size distribution of 9.8 μm + 2%. The prepacked columns are made of borosilicate glass, which enables the gel bed to be seen.
Circle No. 118 on Reader Service Card.

ADVERTISEMENTS

SUBMERGED AGAROSE GEL ELECTROPHORESIS



$89 carriage extra

Pt electrodes
10 x 7cm gel area
UV transparent

TEL. 0734 788000

LABQUIP READING AND MANCHESTER UK

Circle No.07 on Reader Service Card.

Liposomes

A **new** laboratory instrument (LIPOPREP®-GD-1) is available for the **reproducible** preparation of extremely homogeneous, unilamellar liposomes of controllable size. The instrument permits the **simultaneous use of five different lipid mixtures** and provides extensive versatility. Applications range from membrane reconstitution, remodelling of cellular functions, liposome drug delivery systems, microanalysis of drugs and metabolites, to antibody targeting, clinical diagnostic assays, and genetic engineering.
For details write to:
DIANORM-Geräte, P.O. Box 126, D-8 München 65, FRG.

Circle No.11 on Reader Service Card.

● A series of wide-pore silica-based materials has been produced by HPLC Technology for the separation of proteins, peptides and enzymes. The TechoGel range comprises 200 and 300 Å pore-size silicas, in both 5-μm and 10-μm particle sizes. The TechoGel range consists of a GP phase for gel permeation up to MW 250,000; ion exchange, which covers weak anions, weak cations and strong cations; reverse phase — C4, C6, C8, C18, phenyl and diphenyl and preparative phases, including ion exchange and reverse phase. TechoGel is available in prepacked HPLC columns or as loose media.
Circle No. 119 on Reader Service Card.

● Du Pont has introduced a Zorbax phenyl column for HPLC with reversed phase selectivity for moderately polar compounds, including many pharmaceuticals. The new phenyl column offers an alternative selectivity to other Zorbax alkyl group reversed phase packings, such as ODS, C8 and TMS. This means that chromatograms of two photoreactive chemicals (cyclohexylacrylate and benzophenone) eluted from Zorbax phenyl and C8 columns under identical conditions will show reversed elution order. The Zorbax phenyl column also gives a small shoulder on the dimethoxyacetophenone peak. The phenyl column contains 5-μm Zorbax silica packing modified with a dimethylaryl silane, using procedures to maximize monolayer surface coverage. Post capping completes surface coverage. The columns have a 4.6-mm internal diameter and are available in 15 and 25 cm lengths. Performance characteristics described by typical theoretical plates (N) are 9,000 for the 15-cm column and 15,000 for the 25-cm column. The compound used in the characterization was toluene.
Circle No. 120 on Reader Service Card.

● The new liquid chromatography chambers from Revco have four temperature controls, display temperature in all four quadrants continuously and have an alarm system that tests itself automatically every 24 h. They also have computer connections for collecting, storing and monitoring temperature data automatically. The chambers also have an optional auto-dialer alarm system whereby they will call any of 10 numbers, a 240-day electronic temperature recorder and a remote audio alarm.
Circle No. 121 on Reader Service Card.

● Two new chiral columns are now being produced by J.T. Baker Chemicals that are designed to separate racemic mixtures and stereoisomers. Two types of column have been produced; the DNBPG ionically bound to 5-μm aminopropyl silica (60-Å) for lipophilic samples and a covalently-bound DNBPG which is suitable for pharmaceutical applications.
Circle No. 122 on Reader Service Card.

● J.T. Baker has introduced a range of HPLC columns containing Bakerbond materials bonded to 5-μm spherical silica gel with a pore diameter of 60 Å. The range includes octadecyl (C18), octyl (CS), quartenary amine, aliphatic sulphonic acid, diol, aliphatic carboxyl, diphenyl and cyano. The columns are available in the standard size of 4.6 mm (internal diameter) × 25 cm with SSI fittings. In addition a number of wide-pore columns has been introduced for the separation and purification of proteins, peptides and oligonucleotides. The wide-pore columns have a pore diameter of 330 Å. The range of columns includes octadecyl, octyl, diphenyl, cyano and polyethyleneimine. The Bakerbond bonded phases are also available separately with particle sizes of 5 μm (spherical), 10 μm (irregular) and 40 μm (irregular).
Circle No. 123 on Reader Service Card.

Electrophoresis

● The Dionex IonPac membrane reactor uses a new method of reagent introduction which aids in the determination of metals, sequestering agents, silicates and amino acids. The membrane reactor is for use with the Dionex post column reactor and a series 2000i ion chromatograph. The membrane reactor's fibre membrane is permeable to a variety of reagents. During operation, pressurized reagent from the pulseless post column reactor is introduced into the column effluent stream along the length of the fibre membrane. This means that reagents are added at a pulse-free flow-dependent rate. The volume of reagent introduced in this method is minimal, reducing analyte dilution effects. Flow-dependent noise and dilution effects are also reduced.
Circle No. 124 on Reader Service Card.

● A casting tray is being marketed by Bio-Rad that can be used to produce ultra-thin gels for thin layer electrofocusing. The tray allows 0.4-mm polyacrylamide gels to be cast on glass plates or, when used in conjunction with Bio-Rad's new gel support film for polyacrylamide, allows 0.2-mm polyacrylamide gels to be produced.
Circle No. 125 on Reader Service Card.

● The Gelman electrophoresis system, which uses cellulose acetate membranes as support medium, is based upon the established semi-micro II electrophoresis chamber. This chamber has been designed to reduce running costs. It requires 200 ml of buffer to effect separation of up to 96 samples, including standards and controls. Up to 24 samples may be separated simultaneously. Sample application is by either a four- or an eight-place push button
Circle No. 126 on Reader Service Card.

LONDON OFFICE Keith Walesby 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024)
AMERICAN OFFICES NEW YORK Miss Gene Fein 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900
SAN FRANCISCO JJHS (415) 392 6794 ●**PASADENA** JJHS (213) 796 9200
CHICAGO Didier & Broderick (312) 498 4520 ● **BOSTON** CEL Assoc (617) 848 9306
CANADIAN OFFICE TORONTO Peter Drake 17 Pine Crescent, Toronto, Ontario M4E 1L1 (416) 690 2423

RATES UK — Rest of world — £11.00 per centimetre. Minimum 3 cm. £2 is charged for the re-direction of replies to advertisements with a box number.

CONDITIONS. The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.

UNIVERSITY OF CHICAGO

ASSISTANT OR ASSOCIATE PROFESSOR IN THE RESEARCH ASSOCIATE TRACK

o assume major responsibility in a ellular electrophysiology laboratory tudying arrhythmogenesis and the ctions of antiarrhythmic drugs. xperience in voltage clamping, able analysis, ion sensitive micro- lectrodes, and computer pro- ramming preferable. Excellent cientific environment.

Renewable term appointment with ull fringe benefits. Immediate vailability.

Please send curriculum vitae, epresentative reprints, and names of hree references to: Morton F rnsdorf, MD, Chief, Section of ardiology, University of Chicago lospitals, Box 423, 950 E 59th treet, Chicago, Ill 60637.

(NW285)A

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF ORAL AND MAXILLO-FACIAL SURGERY

RESEARCH ASSOCIATES (TWO)

Applications are invited for these two posts for an initial period of three years, to undertake research in laboratories within the Department of Pharmacology.

Applicants should have experience in either electrophysiology or microvascular pharmacology. Initial salary range pa: £7,190 — £8,530. Superannuation.

Further details and informal visits by contacting Professor J P Rood, Department of Oral and Maxillofacial Surgery, Turner Dental School, Higher Cambridge Street, Manchester M15 6FH (061-273 5252).

Applications, with a curriculum vitae and the names of two referees, to be submitted by March 5th.

(1149)A

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

MICROBIOLOGY DEPARTMENT

A research scientist is required to devise and develop new rapid methods for estimating the bacteriological quality of milk, milk products and other foods. The further development and automation of the direct epifluorescent filtration technique (DEFT) is envisaged. Additionally, the application of radioimmune, ELISA, and allied techniques for food analysis will be explored.

Appointment will be as Scientific Officer (£5,682 — £7,765) or Higher Scientific Officer (£7,149 — £9,561). Non-contributory superannuation.

Candidates should have a First or Upper Second Class Honours degree in biological or physical sciences with at least two years' relevant post-qualifying experience for appointment as HSO.

Applications to the Secretary, N.I.R.D. Shinfield, Reading RG2 9AT.

Please quote reference 83/19.

(1168)A

UNIVERSITY OF LEEDS

DEPARTMENT OF EARTH SCIENCES

LECTURER

Applications are invited for the above post available from 1 October 1984. The duties of the post will include teaching Engineering Geology students at undergraduate and MSc level; applicants should have suitable qualifications and preferably some research or industrial experience in this area.

Salary on the academic scale for Lecturers (£7190 — £14125) according to age, qualifications and experience.

Informal enquiries may be made to Professor J C Briden, telephone (0532) 431751, ext 6415.

Application forms and further particulars may be obtained from the Registrar, the University, Leeds LS2 9JT, quoting reference number 49/26. Closing date for applications 27 February 1984. Overseas applicants may apply by telegram or telex (556473) in the first instance.

(1165)A

University of Massachusetts Medical School
Interdepartmental Program in Environmental Health Sciences
Two Senior Tenure Track Positions Available
SPONSORING DEPARTMENTS: Family & Community Medicine, Pathology & Pharmacology

PREFERRED AREAS OF GENERAL RESEARCH INTEREST include Mutagenesis/Carcingenesis, and Inhalation/Pulmonary or Reproductive Toxicology.

The University of Massachusetts Medical School seeks to recruit two Senior faculty members to join several of its faculty in developing the above interdepartmental program. Appointments may begin in the fall of the 1984-85 academic year. The requirements of each participating department are described further below. Multidepartmental, joint appointments will be considered, and applications and inquiries from established teams of investigators working in one or more of the above areas are encouraged. Each faculty member will be expected to pursue an independent research program and to participate, in the context of an interdepartmental program, in service, medical education, and/or the education of graduate students in an institutional Ph.D. Program in Medical Sciences. General inquiries or letters of application (including a curriculum vitae and a list of three references) should be sent before 31 March 1984 to:

Neal C. Brown, Professor & Chairman
Department of Pharmacology, University of Massachusetts Medical School, 55 Lake Avenue North,
Worcester, MA 01605; (617) 856-2151.

POSITION REQUIREMENTS

FAMILY & COMMUNITY MEDICINE POSITION

Associate Professor or Professor with M.D. and/or Ph.D. and an established research program in one of the areas cited above. **Preferred Research Interests** include the study of the relationship of environmental/occupational exposure to effects on human health. Other duties would include the performance of health/exposure assessment of environmental/occupational hazards and participation in the formulation of related public policy.

For further specific information contact Dr. Barry Levy, Department of Family and Community Medicine, UMMC, Worcester, MA 01605. (617) 856-2151.

PATHOLOGY-PHARMACOLOGY POSITION

Associate Professor or Professor with M.D. and/or Ph.D. and an established program in one of the areas cited above. **Preferred Research Interests,** regardless of the appointee's departmental affiliation and the specific areas of interest, his/her research should incorporate the use of analytical chemistry and related techniques. The specific research interests should fall within the context of the areas cited above and be relevant to the broad discipline of environmental-toxicologic pathology or pharmaco-toxicology – including the pharmacokinetics and metabolism and mutagenic/carcinogenic effects of drugs and environmental chemicals.

For further specific information contact as appropriate – Dr. Neal Brown (Pharmacology, see above) or Dr. Guido Majno, Chairman, Department of Pathology, UMMC, Worcester, MA 01605; (617) 856-2115.

University of Massachusetts Medical Center

55 Lake Avenue North Worcester, MA 01605

An affirmative action equal opportunity employer (NW277)A

Biotechnology Australia

Career Opportunities in Biotechnology

Biotechnology Australia Pty Ltd, Australia's leading biotechnology research company, is in the process of expanding its research and development activities. There are immediate opportunities for the research and development positions listed below.

The company provides a first class research environment for over 70 staff in modern laboratories on Sydney's North Shore. The laboratories are equipped with a full range of instruments and fermenters necessary for projects requiring the skills of molecular biologists, microbiologists and biological chemists. Topics under investigation include studies of new methods for vaccine production with recombinant DNA techniques, biological control of insect pests with genetically modified bacteria and parasitic nematodes, and the microbiological leaching of minerals. Most projects involve collaboration with leading groups in universities, CSIRO and research institutions.

The research group is supported by experts in planning and evaluation and there are extensive facilities for scale-up and process development.

Applications are now invited for the following seven positions.

Molecular Biologists

Position No. 1001 is for a candidate with Post-Doctoral experience in molecular biology or allied fields of biochemistry or microbiology, for appointment as a Research Scientist.

Position No. 1002 is for a candidate with an Honours Degree in similar subjects or in immunology, for appointment as an Experimental Officer.

Position No. 1003 is for a Post-Doctoral Fellow funded for 3 years in the first instance. Training in recombinant DNA and allied immunological techniques is required.

Position No. 1004 is for a Post-Doctoral Fellow funded for 2 years in the first instance for the development of vaccines against enteric infections using recombinant DNA technology.

Fermentation Scientist

Position No. 1005 is for a candidate with an Honours Degree in microbiology or fermentation with knowledge of chemical or biochemical engineering. Appointment will be at the Experimental Officer level to work on solid and liquid fermentations and product recovery.

Entomologist

Position No. 1006 is for a candidate with an Honours or higher degree in entomology for appointment as an Experimental Officer. The position would be for 2 years in the first instance.

Biotechnologist

Position No. 1007 is for a candidate with an Honours Degree in microbiology, biochemical engineering or allied discipline for appointment as an Experimental Officer. The position would be for 2 years in the first instance.

All positions carry salaries and conditions of employment competitive with other leading research organizations.

Applications including a c.v. and names of two referees should be sent to:

Professor N. S. Willetts
Director of Research
Biotechnology Australia Pty. Ltd.
P.O. Box 20
Roseville N.S.W. 2069 Australia

(W712)A

BEATSON INSTITUTE FOR CANCER RESEARCH
POST-DOCTORAL SCIENTIST

There is a vacancy for a scientist with training and experience in nucleic acid biochemistry and/or molecular biology and/or cell biology to join a group studying human colorectal cancer and metastasis. Appointment will be to University Research Staff Range IA (£8,530 to £11,615).

Applications with names and addresses of two academic referees and complete cv to Dr. G. D. Birnie, Beatson Institute, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD Scotland. (1171)A

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
PO BOX 30772, NAIROBI, KENYA

Applications are invited from suitably qualified candidates for the position of

ACADEMIC COORDINATOR

for the African Regional Postgraduate Programme in Insect Science (ARPPIS). ARPPIS is a collaborative high-level research training programme between the International Centre of Insect Physiology and Ecology (ICIPE) and eleven Participating Universities in Africa. The programme, which is limited to PHD training, is based at the ICIPE, which acts its executing agency. Coursework is offered as advanced refresher courses in insect ecology, biostatistics, physiology and biochemistry, pathology, taxonomy and morphology by an international teaching staff. Participating Universities are responsible for nomination of Postgraduate students, who are selected by an Academic Board comprising representatives of the participating Universities; the latter offer the degrees. ARPPIS became functional in March 1983, with student body of eight PhD candidates from five African countries.

ACADEMIC COORDINATOR (Ref: SCI/84/1)

QUALIFICATIONS: The candidate should be an experienced academic lecturer level upwards, with research and teaching experience in the biological sciences. Experience in project administration, particularly in a setting with tradition on Postgraduate training will be a distinct advantage.

RESPONSIBILITY: The selected candidate will assist the ICIPE in the planning of the academic programme, its implementation and coordination, and act as Secretary to the Academic Board of ARPPIS. The board has responsibility in the recruitment of the teaching staff, the selection of students, and the reviewing of curricula and examinations. In addition to his other duties, the appointee is expected to participate in the teaching and supervision of the Postgraduate students.

SALARY AND TERMS: Negotiable, and include attractive benefits. The successful candidate will be offered an initial two-year appointment, with the possibility of extension.

Applications, giving detailed information on general education, professional qualifications, experience, marital status, age, present terms of employment (including salary), names and addresses of four referees (including one personal referee), and photocopies of relevant certificates (which once submitted will not be returned) should be addressed to:

The Administrative Manager,
ICIPE Research Centre,
PO Box 30772,
NAIROBI, Kenya

CLOSING DATE: 21st March 1984. (W736)A

UNIVERSITY OF LANCASTER
Department of Environmental Sciences
RESEARCH ASSOCIATE

Applications are invited for the post of postdoctoral Research Associate to work with Dr. R. M. Harrison, Dr. M. Kelly and Dr. S. R. Aston on a two year study of transuranic radionuclides in the estuarine environment. Applicants should possess a PhD degree, and research experience in environmental chemistry or geochemistry would be advantageous. Salary is in the range £7,190 – £8,530 p.a.

Further particulars may be obtained (quoting reference LO11/R) from the Establishment Office, University House, Bailrigg, Lancaster, LA1 4YW, where applications (seven copies) naming three referees, should be sent NOT LATER THAN 24 FEBRUARY 1984. (1167)A

THE HELLENIC ANTICANCER INSTITUTE

invites applications for the post of

DIRECTOR

of its Research Center coprising Biochemistry, Biology, Cell Physiology and Immunology Departments.

Candidates must have proven experience in Cancer Research and ability to develop their own research projects.

Applications should be accompanied by a detailed curriculum vitae, a summary of proposed research projects and the names of three referees and should reach: The Hellenic Anticancer Institute, 171 Alexandras Ave., Athens, Greece, not later than February 29th 1984. The appointment originally for three years will be renewable and will carry a Greek University proffessorial salary. (W737)A

Universiteit van Amsterdam

professor of biochemistry (m/f)

Applications are invited for a full-time position as Professor in the Interfaculty Department of Biochemistry, University of Amsterdam. There are seven other full-time professors in the Department.

The successful applicant should undertake, co-ordinate and stimulate research in biochemistry, preferably with regard to integrated biological systems, and will be required to teach biochemistry to undergraduate and postgraduate students. In addition, the successful applicant will have certain administrative duties connected with the organisation of the Department and the Faculty.

He or she will be expected to speak Dutch or to master Dutch within two years of appointment.

Gross salary depends on age and experience and ranges from Dfl. 6.561,- to Dfl. 9.283,- per month. (Dutch Civil Servants Code).

Further particulars can be obtained from the Secretary of the Appointment Committee, Prof. Dr. K. van Dam, B.C.P. Jansen Instituut, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands, telephone 020-5222125.

Applications, quoting number 7525, accompanied by an extensive curriculum vitae, a list of publications, a description of present research interests and the names of at least three referees, should be sent within four weeks to: The Chairman of the Appointment Committee, Professor Th.J. de Boer, Laboratory of Organic Chemistry, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands.

Suggestions for possible candidates may be sent to the Committee.

Women in particular are invited to apply.

(W734)A

KING FAISAL UNIVERSITY
Al-Hasa, Saudi Arabia

College of Veterinary Medicine and Animal Resources

invites applications for faculty positions in the following disciplines:

- **Vet. Deficiency Diseases and Clinical Lab. Diagnosis**
- **Endocrinology**
- **Vet. Microbiology**
- **Vet. Gynaecology/Obst.**
- **Food Hygiene and Meat Inspection**
- **Vet. Public Health/ Food Hygiene and Zoonosis (milk products hygiene)**
- **Vet. Pathology**

Requirements: Doctorate for Professors, Associate and Assistant Professors. MSc degree for Lecturers. Publications in professional journals, minimum of 2 years teaching experience for Asst. Professors and 4 years for Associate Professors and Professors. **Applicants must be fluent in both Arabic and English.** All faculty members are expected to conduct research in their area of interest.

Salaries are highly competitive. Contracts are for one year renewable.

Benefits include free furnished housing or housing allowance, annual return air tickets for a family of four, 60 days paid annual leave, generous luggage overweight allowance, educational allowance for children. No Saudi tax.

Closing date for applications: 9 March 1984. (W745)A

Application forms can be obtained from either of the following addresses, stating clearly the position applied for:

Dean, College of Veterinary Medicine and Animal Resources

or

c/o UK Recruiting Office
King Faisal University
29 Belgrave Square
London SW1X 8QB

King Faisal University
PO Box 1757
Al-Hasa
Saudi Arabia 31982

BIOMEDICAL RESEARCH OPPORTUNITIES
NATIONAL INSTITUTES OF HEALTH

The Intramural Research Division of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK), has openings for highly qualified applicants in many exciting areas of biomedical research. NIADDK, one of 11 Institutes comprising the National Institutes of Health (NIH), located 20 minutes from Washington, D.C., in suburban Maryland, offers a unique research opportunity for scientists from both the U.S. and abroad. The NIH commitment to full-time research and its academic atmosphere together with state-of-the-art instrumentation and extensive interaction among the 2500 doctoral-level scientists at the NIH constitute a biomedical research environment that is unequalled elsewhere.

The majority of the 350 positions at NIADDK are filled by postdoctoral and sabbatical-leave scientists for periods of up to several years. For further information about specific research in areas listed below, send inquiries to: Recruitment Program, Dr. Jesse Roth, Director of Intramural Research, NIADDK, National Institutes of Health, Bldg. 10, Rm. 9N-222, Bethesda, MD 20205.

- Gene expression
- Nucleic acid and chromatin structure
- Molecular genetics and recombinant DNA
- DNA and RNA replication
- Membrane biology/cell organelles
- Transport phenomena
- Hormones
- Enzymology
- Receptors
- Development and differentiation
- Protein structure and function
- Biophysics
- Mathematical biology
- Chemistry (all branches)
- Pharmacology
- Immunology
- Molecular mechanisms of disease
- Intercellular communication
- Neurobiology

NIH is an Equal Opportunity Employer

(NW276)A

SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH

SCIENCE GRADUATE: MICROBIOLOGY

We require the services of a Science Graduate (MSc or PhD) in the field of medical microbiology, with a special interest in antibiotics. The applicant should preferably be experienced in the techniques of susceptibility tests and antibiotic assays, but any suitably qualified person will be considered. The appointee will be expected to supervise an antibiotics reference laboratory and to participate in its research programmes..

We offer a competitive salary plus annual service bonus, housing subsidy, subsidised medical aid, Pension Fund and 30 days leave per annum.

Address applications, including curriculum vitae, to: Head Personnel Services, SAIMR, PO Box 1038, Johannesburg, 2000, Republic of South Africa.

(W742)A

TRANSLATOR/EDITOR

We are leading translation company with over 25 years' experience in the pharmaco-medical field. We need to increase our output capacity and are looking for a person with suitable qualifications and/or experience, English mother tongue and sound knowledge of German to join our team, working in an informal and stimulating environment. The work involves processing translations in all branches of medicine and pharmacology, including research reports, manufacturing specifications and published literature.

Salary according to qualifications and experience, subject to annual review, plus sundry benefits.

Apply in writing to: (1188)A

Dr A.M.A. Mincer, Chief Editor
AD — EX (TRANSLATIONS) LTD
P.O. Box 428, 296 Kingston Road, London SW20 8LZ

SHEFFIELD HEALTH AUTHORITY
MEDICAL LABORATORY SCIENTIFIC OFFICER

To be based in the Department of Haematology at the Royal Hallamshire Hospital. Candidates must be qualified to HNC level preferably in Haematology. This research post is tenable for 1 year commencing as soon as possible. The successful applicant will be involved in a study of the effects of fish-oils on red cell deformability.

Salary Scale: £5,491 £5,696, £6,144, £6,263, £6,436, £6,626, £6,851, £7,098, £7,306, £7,510, £7,747.

For further information contact Dr. F. E. Preston/Dr. M. Greaves, Telephone Sheffield (0742) 26484, extension 2122. Application forms are available from the Personnel Department, Westbrook House, Sharrow Vale Road, Sheffield S11 8EU. Telephone 686911 extension 145. Completed application forms should be returned no later than 23rd February 1984. (1173)A

UNIVERSITY OF BRISTOL

POST-DOCTORAL RESEARCH ON THEORY OF MOLECULAR PHOTODISSOCIATION PROCESSES

The experimental and theoretical study of molecular photo-dissociation processes forms one of the fastest developing research areas of chemical physics. Applications are invited for a post-doctoral research position to work on the theory of these processes. The research project will involve the development of fundamental theory as well as the running and modification of large computer programs. Experience in molecular collision theory, spectroscopy or large scale computational work will be an advantage.

Applications for the position should be sent to: Dr G G Balint-Kurti, School of Chemistry, Bristol University, Bristol BS8 1TS.

Applications should include the names of two referees. Further information about the position will be sent upon request. (1146)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following position:

RESEARCH SCHOOL OF PHYSICAL SCIENCES RESEARCH FELLOW IN ASTROPHYSICS

The Mount Stromlo and Sidin Spring Observatories (Director Professor D S Mathewson) operat seven telescopes on Mount Stromlo and on Siding Spring Mountain with a wide variety of advanced in struments for spectroscopy, photo metry, polarimetry and direc imaging. A 2.3 metre advance technology telescope will come int operation at Siding Spring in 1984 The main lines of research are o evolutionary problems in stars, th interstellar medium, globula clusters, galactic structure, th Magellanic Clouds, external galaxie and radio sources. Universit astronomers have access to th Anglo-Australian Telescope, the 6 metre radio telescopes at Parke NSW and Tidbinbilla, and th synthesis telescopes of the Universit of Sydney. A VAS 11/780 compute and a PDS micro-photometer ar available for data reduction an image processing: there is also a lin with the University's UNIVAC facility.

Appointment is available fron mid-1984. Closing date: 15 Marc 1984.

Conditions of apointment: Salar will be in accordance wit qualifications and experience withi the range: Research Fellow $A23,39 — $A30,734 pa. Current exchang rate is $A1 = UK63p.

Appointment as Research Fello normally up to three years with th possibility of extension to maximu of five years.

Grants are provided towards trav and removal. Assistance towar housing is given to an appointee fro outside Canberra. Eligible appoi tees will be required to join the Supe annuation Scheme for Australia Universities. The University reserv the right not to make an appointmer or to make an appointment b invitation at any time.

Prospective applicants shoul write to the Registrar of the Unive sity, GPO Box 4, Canberra, AC 2601, Australia, or to the Associatio of Commonwealth Universitie (Appts), 36 Gordon Square, Londo WC1H 0PF, for further particula before submitting applications.

(W744)A

POSTDOCTORAL Fellow — Endo crinology — Autoimmune endocrin disease group requires fellow wit experience in basic immunology cell biology to work in areas of in vitro antibody control, monoclon antibody production and antige purification. opportunity for inde pendent work and career develop ment. Two year position availabl Summer '84. Send cv to Dr T Davies, Division of Endocrinology The Mount Sinai School of Medicine One Gustave L Levy Place, Nev York, NY 10029. An Equal Opport unity Employer. (NW268)A

UNIVERSITY OF HONG KONG

HOSPITAL BIOCHEMIST
(Re-advertisement)

pplications are invited for a post of ospital Biochemist in the Clinical iochemistry Unit of the Hospital athology Services of the University.

Candidates should posses ppropriate professional qualifica-ons and have experience of hospital ochemistry and a background of search in related problems. The ccessful candidate will work in the linical Biochemistry Unit which ovides a chemical pathology rvice to the Queen Mary Hospital ıder the supervision of a Senior onsultant.

Annual salary (superannuable) is ı an 11 point scale: HK$135,780 — 7,100 (£12,344 — 20,645) (Sterling uivalent as at January 23, 1984). arting salary will depend on quali-cations and experience.

At current rate, salaries tax will not ceed 15% of gross income. ousing benefits at a rental of 7½% salary, children's education lowances, leave and medical nefits are provided.

Further particulars and plication forms may be obtained om the Association of ommonwealth Universities ppts), 36 Gordon Square, London C1H 0PF, or from the ppointments Unit, Secretary's ffice, University of Hong Kong, ong Kong. Closing date: 31 March 84. (W739)A

THE UNIVERSITY OF MANCHESTER/ ARESBURY LABORATORY

SYNCHROTRON RADIATION POST DOCTORAL POSTS IN X-RAY SPECTROSCOPY
Re-advertisement

pplications are invited for two sitions funded by SERC, tenable om as soon as possible for two years the first instance. One post is ncerned with research at the nchrotron Radiation Source at aresbury and the successful ndidate would be responsible for e development and commissioning a fluorescence detector facility on e X-Ray Spectroscopy Station on e Wiggler beam line. The second st is in connection with the recently tablished EXAFS service at aresbury, which is designed to hance the applications of EXAFS chniques to the study of chemical d biochemical systems. Applicants e expected to hold a PhD in ysics, Chemistry, or a Biological ience. Experience in strumentation and/or computation ould be advantageous. Salary range £7,190 — £11,615 uperannuation).

Applications, with full CV and the mes of two referees, to either Dr D Garner, Chemistry Department, niversity of Manchester, Manchester 13 9PL or Dr S S Hasnain, aresbury Laboratory, Warrington A4 4AD by February 21st, 1984. (1180)A

SOUTH MANCHESTER AUTHORITY CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE PATERSON LABORATORIES

A vacancy exists at postdoctoral level within a group devoted to the study of the epithelial cells of a variety of tissues. The interests of the group centre on the cellular organisation of proliferation in a wide range of surface epithelia (skin, oral mucosa and gastrointerestinal tract), the mechanisms involved in the control of cell proliferative and differentiation and the malfunction of these controls that result in cancer and other proliferative disorders. We are particularly concerned in bringing new scientific interests into the group and add to the wide-ranging approaches concerned in bringing new scientific interests into the group and add to the wide-ranging approaches that are currently used. Fields of particular interest include epithelial cell culture, flow cytometry, monoclonal antibodies and molecular biology. The successful candidate would be able to develop his own area of study within the overall framework and under the general guidance of Dr C S Potten the group leader. Potential projects could include the use of epidermal cultures to analyse control factors, the indentification of early differentiation events in epithelial tissues and their relationship to carcinogen target cells.

The work is supported by grants from the Cancer Research Campaign and the position would be tenable for three years with the possibility of renewal.

Applications should be made to Dr C S Potten, The Paterson Laboratories, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, and include a curriculum vitae, the names of two academic referees and outline of interests in connection with the work described above. (1184)A

ASSISTANT PROFESSOR MOLECULAR BIOLOGY

The Department of Microbiology, Louisiana State University (LSU), Baton Rouge, LA invites applications for an anticipated tenure-track position at the Assistant Professor level.

Candidates must have post-doctoral experienced and strong credentials in molecular biology. Individuals with an interest in biotechnology are especially encouraged to apply. Preference will be given to those with experience in recombinant DNA techniques.

Send curriculum vitae, a description of future research interests, and a list of three references by April 1, 1984, to: Dr M D Socolofsky, Chairman, Department of Microbiology, 508 Life Sciences Building, Louisiana State University, Baton Rouge, LA 70803. LSU is an Equal Opportunity/ Affirmative Action Employer.

Anticipated Hire Date: Fall 1984. (NW284)A

Cranfield
Silsoe College

A new Chair in support of the food production industry

Applications are invited for a new Chair at Silsoe College, Cranfield Institute of Technology. This Chair will be the first appointment leading to the establishment of a strong, research-based group in support of the food industry in Britain.

Preference will be given to applicants who are recognised authorities in a particular field of science relevant to food production. The successful candidate will be capable of forming and leading a multidisciplinary team undertaking a balance of fundamental and applied research supported by industrial and other food sector sponsors.

The programme of work may be concerned with the development of a better understanding of the nature and properties of food raw materials and their inter-relationships. Work may also be undertaken on the synthesis of more cost-effective food manufacturing processes through improved engineering or food design.

For further particulars please contact: The Personnel Department, Cranfield Institute of Technology, Cranfield, Bedford MK43 0AL. Telephone: Bedford (0234) 750111 ext 3336.

Closing date for applications: 12 March 1984. (1153)A

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory appointment:

PROFESSORSHIP OF AGRICULTURAL ZOOLOGY

Prior to application, further information (including application procedure) may be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries: 693244 ext 431.

The closing date for receipt of completed applications is Thursday, 8th March 1984. (1150)A

YOU'VE GOT A BRIGHT IDEA

for new biological or chemical compounds.

WE HAVE THE CAPITAL

to develop new drugs.

We are not a pharmaceutical firm but a Swiss-based, independant financial group which is prepared to let you participate in a capital venture for the exploitation of your project.

Our objective: the development of new biological or chemical products having previous pharmalogical screening up to the stage of clinical tolerance and efficacy required to permit licensing negotiations.

To obtain all the information required for initial selection, please write in English, French or German to:
DEBIOPHARM S.A. Petit-Chêne 38 - 1001 LAUSANNE (Switzerland). (W732)A

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
P.O. BOX 30772, NAIROBI, KENYA

REF: S7/ ADVERT/ 20/ 47920th January 1984

DEPUTY DIRECTOR

(REF: SCI/ 83/ 7): Readvertisement

The ICIPE is an international institution headquartered in Nairobi, Kenya. It undertakes advanced research in insect science and post management, and offers high-level training to insect scientists and technologists from Africa and other developing countries. It has 40 senior scientists; and is funded by a consortium of donors comprising United Nations and international bodies, governmental aid agencies, and private foundations.

Applications are invited from highly qualified scientists and widely experienced reserach managers for the senior research management position of DEPUTY DIRECTOR at the ICIPE.

The Deputy Director reports directly to the Director of the Centre, and in his absence, acts for him. He will, in addition, have overall responsibility for the Research Division of the ICIPE, which includes the research programmes, research support units, and technical support services (including field station facilities); and maintain close liaison with supportive Divisions within the ICIPE (on Administration, Finance, and Communication and Training). The successful candidate will be a person with a solid research background in insect science or pest management or plant protection, he will have had considerable experience in science policy matters and research management; and he will be a successful internationalist able to work in an African environment. A more detailed job description will be sent to qualified candidates.

The renumeration package will be competitive, and will reflect the vital importance of this position within the ICIPE. Applications, giving updated curriculum vitae and addresses of three referees should be addressed (in confidence) to:

The Director,
ICIPE,
P.O. Box 30772,
NAIROBI, Kenya

Closing Date: 31st March 1984. (W735)A

THE UNIVERSITY OF ADELAIDE

invites applications from both men and women for the following position:

RESEARCH ASSOCIATE IN MICROBIOLOGY AND IMMUNOLOGY

To work in a small group as a molecular biologist on specific surface components of E Coli and Salmonella. The project requires the cloning and analysis of genes relevant to outer membrane proteins and lipopolysaccharide followed by genetic and molecular analysis. Our interest is in using these techniques to analyse the function and regulation of surface proteins, and also to determine the molecular changes involved in the evolution of the extreme variability of Salmonella surface polysaccarides: the appointee could be involved in one or both of these areas. The Department has a strong base in molecular genetics including several other interacting projects involving cloning and sequencing techniques as tools, and is well equipped for this type of work.

Applicants should hold a PhD in the area of molecular genetics and preferably have one to two years relevant postdoctoral experience. The position which is funded by the ARGS is available from January 1984 for one year in the first instance. There is a commitment by the ARGS to provide funds for the position for two years.

Further information concerning the details of the position may be obtained from Dr. P. Reeves of the Department of Microbiology and Immunology (08) 228 5436.

Further information about the general conditions of all appointments may be obtained from the Senior Assistant Registrar (Personnel) at the University.

Salary per annum: $A20164 x 5 — $23100.

Applications, in duplicate, quote reference number X3930 and giving full personal particulars (including citizenship), details of academic qualifications and names and addresses of three referees, should reach the Senior Assistant Registrar (Personnel) at the University of Adelaide, GPO Box 498, Adelaide, South Australia 5001 (telex: UNIVAD AA89141), as soon as possible and not later than 9 March 1984.

The University reserves the right not to make an appointment or to appoint by invitation. (W738)A

MRC CELL BIOPHYSICS UNIT

Applications are invited for a three-year, short-term

POSTDOCTORAL RESEARCH POSITION

available from 1 October 1984, to work on the molecular biology of dedifferentiation and morphogenesis in amphibian limb regeneration. This position is part of a collaboration between Dr Jeremy Brockes and Dr Roger Patient involving the analysis of gene expression in relation to these phenomena. Recent studies have led to the development of monoclonal antibodies as markers for blastemal and differentiated cell types both in vivo and in vitro; further studies will also involve the application of nucleic acid methodology. Experience in recombinant DNA techniques would be an advantage.

The salary will be on the scale £8,376 — £11,896 inclusive per annum; MRC superannuation scheme.

Applications with the names and addresses of two referees should be sent by 29 February 1984 to Mr D M Drummie, MRC Cell Biophysics Unit, King's College London, 26-29 Drury Lane, London WC2B 5RL; telephone 01-836 8851. (1159)A

UNIVERSITY OF IOWA

DEPARTMENT OF ZOOLOGY

POSTDOCTORAL POSITIONS

available to study the molecular and developmental biology of cell motility and chemotaxis in the cellular slime mold Dictyostelium discoideum.

Please send curriculum vitae and names of three references to: Dr David R Soll, Department of Zoology, University of Iowa, Iowa City, IA 52242. The University of Iowa is an Equal Opportunity/Affirmative Action Employer. (NW283)A

UNIVERSITY OF WARWICK

POSTDOCTORAL RESEARCH FELLOW IN MOLECULAR VIROLOGY

In the Department of Biological Sciences to work on a project funded by the European Economic Community. The work will be concerned with the application of recombinant DNA techniques to the study of Rotaviruses. Salary will be on the Research IA scale: £7,190 to £11,615 pa. The position is available from 1st April 1984 for an initial period of two years. Further information can be obtained by telephoning Dr M A McCrae on Coventry (0203) 24011 ext 6024.

Applicants should send a CV with the names and addresses of two referees to the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, quoting reference MAM/NAR. Closing date 27th February, 1984. (1152)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

UNIVERSITY LECTURER OR UNIVERSITY DEMONSTRATOR IN IMMUNOLOGY

Applications are invited from Immunologists or from Molecular Biologists with an interest in Immunology for a University Lectureship or University Demonstratorship, the appointment to commence as soon as possible and preferably not later than 1 October 1984. Candidates should hold a PhD degree, or the equivalent, and have several years post-doctoral experience in Immunology. The apointment will be for three years, with the possibility, for a University Lecturer of re-appointment to the retiring age or, for a University Demonstrator, re-appointment for a further two years.

The pensionable scale of stipends, for persons not ordinarily resident in college, are for a University lecturer £9,875 a year, rising by eleven annual increments to £15,085 and for a University Demonstrator £7,630 a year, rising by four annual increments to £9,425. There is no grade of Senior lecturer.

Further information about the duties and conditions of appointment may be obtained from the Secretary of the Appointments Committee for the Faculty of Biology 'B', 19 Trumpington Street, Cambridge CB2 1QA, to whom applications (12 copies), including a curriculum vitae, list of publications and the names of not more than three referees, should be sent so as to reach him not later than 1 April 1984. (1157)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF ANATOMY

Applications are invited for the post of

LECTURER/SENIOR LECTURER

Applicants must have experience in both the teaching and examination of the anatomical sciences. The successful candidate will be required to develop the teaching of histology and will be encouraged to continue/develop his/her personal research programme. Departmental research interests include, Biomechanics, Embryology, Ergonomics, Microcirculation and the use of computers in teaching morphology.

Post available from 1 April 1984. Salary on scale £7,190 — £14,125 (Lecturer) or £13,515 — £16,925 (Senior Lecturer) plus £1,180 London Allowance, with superannuation benefits.

Further particulars available from the School Office, RFHSM, Rowland Hill Street, London NW3 2PF (01-794 0500 ext 4262) to which applications (7 copies of curriculum vitae, including the names and addresses of two referees) should be sent by 2 March 1984. Visits to the department can be arranged by appointment with the Departmental Secretary. Tel: 01-794 0500 ext 4355. (1183)A

COMMONWEALTH AGRICULTURAL BUREAUX

SCIENTIFIC INFORMATION OFFICER

at the COMMONWEALTH INSTITUTE OF PARASITOLOGY

Duties: Preparing abstracts in English for **Helminthological Abstracts** and **Protozoological Abstracts** which cover world scientific literature in these fields, indexing, and answering scientific inquiries.

Qualifications: A degree in zoological science or related subject, plus a reading knowledge of at least one European language (German preferred) and the ability to write good English. Applicants must be able to render the content of scientific publications into concise, accurate and readable abstracts. It is expected that the successful candidate will wish to make a career in the field of scientific information rather than research.

Salary: In the scales £5,682 – £7,765 pa, £7,149 – £9,561 pa. Starting salary according to qualifications and experience.

Applications forms and further particulars from: Commonwealth Institute of Parasitology, 395A, Hatfield Road, St. Albans Herts AL4 0XU. Tel: 0727 33151

Closing date for applications 1st March 1984. (1161)A

DRUG DESIGN AND DEVELOPMENT

METABOLISM AND KINETICS

Pfizer Central Research is part of a multi-national organisation engaged in the research, development, manufacture and marketing of a wide range of human and animal health products.

Currently over 500 scientists and supporting staff work at Sandwich in modern, well equipped laboratories. An additional 70,000 sq ft of laboratory and office space is currently being built and will be ready for occupation in the summer.

At that time we shall need an additional high calibre PhD to join our Drug Metabolism Department. We are seeking a chemist/biochemist or analytical chemist who is completing his/her PhD research project in metabolic biochemistry, pharmacokinetics or bioanalysis this year, or someone with up to 3 years' post-doctoral experience in these areas.

The function of drug metabolism is to provide information on the absorption, distribution, metabolism and excretion of drugs in laboratory animals and man. Firstly, so that a better understanding of the mode of action and toxicology can be achieved and, secondly, to aid in the design of drugs with a more desirable pharmacokinetic profile. The department has a major commitment to the application of drug metabolism to the drug discovery process and the person we seek will be self-motivated and enjoy the challenge of being responsible for the development of assay methods and suitable animal models for the study of the kinetics of potential new drugs.

Pfizer

This exciting new appointment offers a highly competitive basic salary plus a bonus related to personal performance. We have a thriving sports and social club with first-class facilities. We also offer generous help with relocation expenses.

If you are interested in this appointment, please send your cv, in confidence, to: D. W. Sells, Personnel Manager, Pfizer Central Research, Ramsgate Road, Sandwich Kent CT13 9NJ. (1194)A

1979

MRC CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow,
Middx HA1 3UJ

APPLIED IMMUNO-CHEMISTRY RESEARCH GROUP

Applications are invited from science or medical graduates for a 3 year postdoctoral position to participate in studies of the structure and function of glycoprotein and glycolipid markers of human tumours and leukemic cells detected by monoclonal antibodies. The work will be carried out in a research group (head of group Dr Ten Feizi) which is well equipped for immunochemistry and carbohydrate biochemistry. A strong background in biochemistry is desirable.

Salary scales are related to those for university non-clinical academic staff and the NHS for clinically qualified candidates. Salary on appointment will depend on age and experience.

Application forms and further details may be obtained by writing to Miss J Cottam, Personnel Dept, quoting ref 108/1/3784. Closing date for applications 2nd March 1984. (1162)A

PHYSICS

Research positions. Applications are invited for positions in the following areas: nuclear and accelerator based physics: nuclear structure, nuclear astrophysics. PIXE with a proton microprobe and applications of nuclear techniques to materials analysis. Solid state and applied physics: magnetic inspection techniques for gas pipelines, effects of stress on the magnetisation of steels, theory of ferromagnetic hysteresis, nuclear magnetic and quadrupole resonance, solid state theory, fast ion transport, surface diffusion, thin films and ferroelectrics, physics at millikelvin temperatures, phase transitions, positron and positronium interactions in solids, metals at high magnetic fields, ultrasonics. Astronomy and astrophysics: galactic structure and evolution, general relativity, relativistic astrophysics, radio astrophysics, stellar structure and evolution, star formation and the interstellar medium. Candidates of either sex are equally encouraged to apply. Salary dependent on qualifications.

Please apply, enclosing a curriculum vitae and the names of three referees, to: Dr M J Stott, Head, Department of Physics, Queen's University, Kingston, Ontario K7L 3N6. (NW281)A

UNIVERSITY OF LONDON

COURTAULD CHAIR OF ANIMAL HEALTH AND PRODUCTION AT THE ROYAL VETERINARY COLLEGE

The Senate invite applications for the above Chair which will become vacant from 1 October on the retirement of Professor J A Laing.

The Chair has previously been entitled the Courtauld Chair of Animal Husbandry and Veterinary Hygiene and the title has been changed to reflect a change of emphasis which has taken place.

Candidates should possess a registrable veterinary qualification and have a specialised knowledge and active research interest in one or more aspects of the subject.

Salary within the Professorial range. The current minimum is £18,461 per annum including London Allowance.

Intending applicants should obtain further particulars from the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, before submitting applications (10 copies). Closing Date for applications: 28 March 1984. (1177)A

INSTITUTE OF CANCER RESEARCH

Fulham Road, London SW3

A post-doctoral vacancy exists in the Chemical Carcinogenesis Section for a scientist with experience of recombinant DNA research to join a team working on the molecular basis of mutation and transformation of mammalian cells by carcinogens. The scientist appointed will have as close colleagues members of both the Chemical Carcinogenesis and Cell and Molecular Biology Sections of the Institute, and will be encouraged to develop his or her own ideas in relation to the overall problem. The appointment will be for a minimum of 3 years, with salary appropriate to age and experience within the scale £7,630 — £10,250 p.a., plus London Allowance of £1,186 p.a. and superannuation (USS).

Applications with full career details in duplicate and the names of two referees should be sent to the Personnel Officer, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 300/G/41. Further details of the post may be obtained by contacting Professor P. Brookes (Tel: 02404-2326). (1169)A

KING FAISAL UNIVERSITY
Al-Hasa, Saudi Arabia
College of Agriculture and Food Sciences

invites applications for faculty positions in the following disciplines:

MEN'S COLLEGE
- **Agricultural Climatology/Water Resources**
- **Farm Power and Machinery**
- **Agricultural Engineering**
- **Rural Sociology**
- **Horticulture, Crop Physiology and Genetics**
- **Poultry and Animal Breeding**
- **Meat Science and Technology**
- **Fish Sciences**
- **Analytical Chemistry (preferably Computer-Aided Chemistry)**

WOMEN'S SECTION
- **Child Development and Family Life**
- **Home Management and Family Finance**
- **General and Organic Chemistry**

APPLICANTS MUST BE FLUENT IN BOTH ARABIC AND ENGLISH.

Requirements: PhD for Assistant and Associate Professors. Master's degree for Lecturers. Publications and minimum of 2 years teaching experience for Asst. Professors and 4 years for Associate Professors.

Salaries are highly competitive. Contracts are for one year renewable.

Benefits include free furnished housing or housing allowance, annual return air tickets for a family of four, 60 days paid annual leave, generous luggage overweight allowance, educational allowance for children. No Saudi tax.

Closing date for applications: 9 March 1984. (W746)A

Application forms can be obtained from either of the following addresses, stating clearly the position applied for:

Dean, College of Agriculture and Food Sciences

c/o UK Recruiting Office
King Faisal University
29 Belgrave Square
London SW1X 8QB

or

King Faisal University
PO Box 420
Al-Hasa
Saudi Arabia 31982

MICROBIAL BIOCHEMIST/ PHYSIOLOGIST

A challenging career opportunity is available for a Microbial Biochemist/Physiologist in the biotechnical group at the Celanese Chemical Company Technical Center located in Corpus Christi, Texas.

The successful applicant will join a team of dedicated scientists engaged in developing biotechnology-based processes for the production of a variety of chemicals.

The position requirements are as follows:

- **PhD in Biochemistry or Microbiology**
- **Postdoctoral experience in the area of microbial metabolism or biochemistry**
- **Experience with the genetic manipulation of microorganisms is also desirable.**

This position requires participation in ongoing research programs as well as innovative thinking leading to new programs.

Interested candidates should forward a résumé with salary history in confidence to:

Personnel Supervisor
Celanese Chemical Company Technical Center
P.O. Box 9077
Corpus Christi, Texas 78469

equal opportunity employer m/f

(NW273)A

CELANESE®
CHEMICAL COMPANY, INC.

PROTEIN BIOCHEMIST

A senior biochemist is required to join a team working on scrapie and the related unconventional slow infections of the CNS. The appointee will be expected to develop and lead an innovative research programme to characterise (a) the infectious unit of scrapie (b) a gene product controlling the disease (c) cerebral amyloid and (d) scrapie-associated fibrils.

Applicants should have a good honours degree in chemistry or biochemistry and a PhD or the equivalent. A considerable proven research record in protein biochemistry is essential, proferably with experience in the analysis of hydrophobic proteins. The appointment will be as Principal Scientific Officer with salary in the range £11,343 – £14,931.

Applications giving full curriculum vitae and names of two referees to The Secretary, AFRC MRC Neuropathogenesis Unit, West Mains Rd., Edinburgh EH9 3JF. (1176)A

UNIVERSITY OF NOTTINGHAM

SCHOOL OF AGRICULTURE

RESEARCH ASSISTANT IN REPRODUCTIVE PHYSIOLOGY

A post-doctoral Research Assistant is required for a period of 18 months to study the use of milk temperature measurements as an aid to oestrus detection in dairy cattle. Applicants should have a higher degree i Animal Physiology, or a relate subject, and should have computin experience.

Application, including a full c and names and addresses of tw referees should be submitted to Pro G E Lamming, University o Nottingham School of Agriculture Sutton Bonington, Loughborough Leics LE12 5RD from whom furth written details of the post can b obtained. (1187)A

An der Formal – und Naturwissenschaftlichen Fakultät der Universität Wien ist die Planstelle eines Ordentlichen Universitätsprofessors für

BIOSTRATIGRAPHIE

zu besetzen.

Dar Ordinariat hat in Ergänzung der vorhandenen Forschungs – und Lehreinrichtungen die Biostratigraphie in Forschung und Lehre (im Ausmaß des Studienplanes der Studienrichtung Erdwissenschaften für das Diplom – und das Doktoratsstudium) zu vertreten.

Voraussetzungen für die Bewerbung sind: Habilitation oder eine andere, gleichwertige wissenschaftliche Qualifikation; Publikationstätigkeit in biostratigraphischer Richtung; internationale Erfahrung und Mitarbeit an fachspezifischen Projekten.

Die Bewerbungen mit den üblichen Unterlagen (Lebenslauf unter besonderer Berücksichtigung des wissenschaftlichen Werdeganges, Publikationsliste, Angaben zur bisherigen Lehr – und Forschungstätigkeit, sowie geplante zukünftige Forschungsrichtung) sind bis zum *15 April 1984* an das Dekanat der Formal – und Naturwissenschaftlichen Fakultät der Universität Wien, A-1010 Wien, Dr. Karl Lueger Ring 1 erbeten." (W743)A

DURHAM UNIVERSITY LECTURESHIP IN PHYSICS

Applications are invited for a Lectureship in Physics tenable from 1 October 1984 under the SERC Special Replacement Schme to enable the University to appoint an outstanding young researcher to a tenured post. The successful applicant will be expected to contribute to undergraduate and postgraduate teaching, particularly in the field of Solid State Physics, and to become actively involved in experimental research on Magnetism for which excellent facilities exist.

Salary on the scale £7,190 – £14,125 with superannuation.

Applications (3 copies) naming three referees should be sent by 29 February 1984 to the Registrar, Science Laboratories, South Road, Durham DH1 3 LE, from whom particulars may be obtained.

(1156)A

CHAIRMAN

DEPARTMENT OF ANATOMY

Applications are invited for th position of Professor and Chairman Department of Anatomy, Schools o Medicine and Dentistry, Universit of Alabama in Birmingham. We ar seeking candidates with research an teaching experience, leadershi qualities, and administrative skills This department has teachin responsibilities for graduate student in anatomy, medical, dental an other students.

Please submit applications and curriculum vitae to: Antonietta I Hyche, Administrative Officer University of Alabama School o Medicine, Dean's Office – ME 306, Birmingham, Alabama 35294 An Equal Opportunity/Affirmativ Action Employer. (NW263)A

An den
MAX-PLANCK-INSTITUTEN
in Martinsried/München

werden im Rahmen einer Kooperation der Max-Planck-Institute für Biochemie und Psychiatrie mit dem Genzentrum an der Universität München und der Industrie zunächst auf folgenden Gebieten **gentechnologische Arbeitsgruppen** eingerichtet:

1. **Mikrosequenzierung** von Proteinen und biologisch aktiven Peptiden mit Schwerpunkt Gasphasensequenzierung.
2. **Molekulare Embryologie,** bevorzugt Frühentwicklung von Säugern.
3. **Transkription (Genexpression bei Eukaryonten):** Mechanismen und Kontrollen mit Betonung der Differenzierung.

Pro Arbeitsgruppe stehen ca. 150m^2 Nutzfläche, angemessene Mittel für wissenschaftliche und technische Assistenten, Geräte und laufenden Bedarf zur Verfügung. Die Anstellung der Gruppenleiter(innen) wird für die Dauer von 5 Jahren vereinbart; Bewerbungen mit einem tabellarischen Lebenslauf, einem Schriftenverzeichnis und einer Kurzdarstellung der bisherigen und der geplanten Arbeiten sind bis zum **15. März 1984** zu richten an die Geschäftsführende Leitung des Max-Planck-Instituts für Biochemie in 8033 Martinsried. (W740)A

UNIVERSITY OF STRATHCLYDE
Department of Pharmacy

LECTURESHIP

Applications are invited from graduates in Pharmacy for a Lectureship tenable in the Department of Pharmacy from 1 August 1984. The successful applicant will be expected to participate in a wide range of teaching in pharmaceutics with a preferred emphasis on pharmaceutical microbiology. Appropriate research experience would be an advantage.

Appointment on the national salary scale for Lecturers £7,190 — £14,125 per annum. USS benefit.

Application forms and further particulars (quote ref: 7/84) are available from the Academic Staff Office, University of Strathclyde, McCance Building, 16 Richmond Street, Glasgow G1 1XQ. Closing date for applications: 23 March 1984. (1191)A

WELSH NATIONAL
SCHOOL OF MEDICINE
(University of Wales)

DEPARTMENT OF CARDIOLOGY

POSTDOCTORAL
RESEARCH OFFICER

pplicants with experience in hysiology/pharmacology are ivited to apply for a 3-year post ubject to a probationary period) to in a small research team working on ontrol mechanisms in vascular nooth muscle, with particular ference to the role of endothelium. ndividual initiative will be couraged within this area.

Starting salary for suitably ualified candidates could be up to 3,530 on the Scale for University esearch and Analogous Staff Grade A.

Applications in the form of a rriculum vitae with names and ldresses of two referees should be nt to the Registrar and Secretary, elsh National School of Medicine, leath Park, Cardiff CF4 4XN uoting ref No C15/2/31) one onth from the date of appearance f this advertisement. Further formation from Dr Malcolm Lewis 222/755944 ext 2066 or 2058). (1182)A

CHAIRPERSON — Biochemistry. The Albert Einstein College of Medicine of Yeshiva University seeks a Chairperson for the Department of Biochemistry. Individuals with a commitment to research and a strong research record are invited to apply. The selected Chairperson will become a tenured professor and space and resources will be available to maintain and expand a high quality department. Interested candidates are requested to send cv's by May 1, 1984 to: E R Stanley, PhD, Associate Professor of Microbiology and Immunology, Chairman of Search Committee for Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461. An equal opportunity employer. (NW269)A

POSTDOCTORAL POSITION
IN PHYSICS

A postdoctoral position is available immediately at the Physics Department of Emory University. The research involves applications of biochemical and spectroscopic techniques to the study of metalloproteins. Recent doctorates in physical chemistry or biochemistry are invited to apply. Experience in protein purification is desirable.

Send résumé and two letters of recommendation to Dr B H Huynh, Physics Department, Emory University, Atlanta, GA 30322. Emory University is an Equal Opportunity/Affirmative-Action employer. (NW278)A

PAISLEY COLLEGE

A Scottish Central Institution

DEPARTMENT OF BIOLOGY

LECTURSHIP IN CELL BIOLOGY/GENETICS

The successful candidate will teach aspects of Cell Biology and Microbial Genetics on the BSc (Hons) Degree course in Biology. He/she will contribute to first and second year teaching and also to the Molecular Genetics and recombinant DNA Technology Units of the Honours year of the Biology Degree course and the Grad. I Biol. courses in Biochemistry and Microbiology.

Relevant qualifications as well as a special interest in Microbial Genetics are essential and recent research experience of recombinant DNA techniques would be preferred. Applicants will be expected to hold a research degree.

LECTURESHIP IN PHYSIOLOGY

This is a fixed term appointment running to 30th June, 1985.

The successful candidate will teach aspects of human and comparative animal physiology in the BSc Honours Degree course as well as contributing to Higher Certificate (day release) and HND (full-time) teaching.

Applicants should have a research degree and a qualifiction and special interest in Physiology. Recent research experience of modern immunological techniques would be an advantage.

SALARY SCALE LECTURER 'A' £8,313 — £13,125

Application forms and further particulars for the above two posts are available from the Personnel Officer, Paisley College of Technology, High Street, Paisley PA1 2BE (Tel: 041-887 1241 ext. 230). Closing date for applications is 27th February, 1984. (1175)A

UNIVERSITY OF STRATHCLYDE
Department of Applied Physics

TEMPORARY LECTURESHIP

Applications are invited for a Temporary Lectureship (tenable until 30 September 1986) from applied physicists, biophysicists and engineers. Applicants with industrial experience, eg on secondment, will be especially welcome.

The successful candidate will be expected to undertake varied teaching duties, and to join in one of the multi-disciplinary research activities of the Department: Photophysical Instrumentation, Environmental Physics, Renewable-Energy Technology, Superconducting Devices, and Population Ecology. Candidates should have experience in one of these areas.

Further details and application forms (quote 6/84) are available from Academic Staff Office, University of Strathclyde, McCance Building, 16 Richmond Street, Glasgow G1 1XQ. Closing date for applications: 28 February 1984. (1190)A

BIOTECHNOLOGISTS!

Are you reading Bio/Technology, the International Journal for Industrial Biology?

For a free sample copy of this new monthly Journal from the Publishers of *Nature*, please write or phone:

Mirta Soto
Bio/Technology
15 East 26th Street
New York 10010
USA
Tel: (212) 689 5900

Felicity Parker
Bio/Technology
Houndmills Industrial Estate
Brunel Road, Basingstoke
Hampshire, UK
Tel.: (0256) 29242

NEW SOUTH WALES GOVERNMENT
Growth and Development
Livestock Research Position
Agricultural Research Centre, Trangie

Position No Ag 84/ 13

A researcher is required to work in a multidisciplinary research team investigating the effect of selection for growth rate on total herd productivity in cattle. This is a major project of wide significance to the beef cattle industry and is well supported by field and laboratory facilities. Opportunities will also exist to undertake growth and development studies in sheep.

Salary: A$17,110 to A$30,119 p.a. depending on experience and qualifications.

Qualifications: Essential: A degree in Agricultural Science, Veterinary Science or equivalent.
Desirable: Post-graduate qualifications or considerable research experience related to the study of growth and development in animals.

Duties: The successful applicant will be required to initiate and undertake pen and field studies with the effect of selection for growth rate on growth and body composition in cattle. These studies will utilise cattle from high and low weight selection lines and a randomly bred control line established at Trangie. Experience in the areas of growth modelling, the problems in comparing genotypes of different mature size and techniques of carcase evaluation and good statistical background is desirable.

Inquiries: (02) 217 5264 (Dr. G. Robards) or (068) 8882 Manual 87 (Dr. D. Saville)

General: Some consideration will be given to payment of removal expenses, should an interstate or overseas applicant be successful, however expenses incurred in attending interview must be met by the applicant.

Applications: **Are to be submitted to the Personnel Officer (Recruitment), P.O. Box K220, Haymarket 2000, Australia.**

Equality of Employment Opportunity is Public Service Policy

Applications close 16th March 1984. (W741)A

UNIVERSITY
OF CAMBRIDGE

DEPARTMENT OF ANATOMY

POST-DOCTORAL
RESEARCH ASSISTANT

Applications are invited for a 3 year post-doctoral research appointment to investigate the neural and neuro-endocrine basis of maternal behaviour. The post is supported by ARC funds with salary starting in the range £7,630 to £9,875 depending on age and experience.

Applications in writing including full CV and names and addresses of two academic referees to Dr E B Keverne, Department of Anatomy, University of Cambridge, Cambridge CB2 3DY, as soon as possible. (1172)A

UNIVERSITY OF MANITOBA
DIVISION OF HUMAN GENETICS

MOLECULAR GENETICIST

The Division of Human Genetics of the Faculty of Medicine invites applications for a full time Assistant Professor to commence by July 1, 1984. Preference will be given to individuals with demonstrated research experience in molecular genetics using recombinant DNA technology on mammalian systems, particularly in the area of genome organization, control of gene expression or gene mapping. A PhD, postdoctoral training and potential for an independent career as an experimental scientist are prerequisites.

An interest in developing expertise and a desire to be an effective educator are desirable qualities. The position is open to both women and men. Both women and men are encourged to apply. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

For full consideration send a concise description of projected research goals and interests, names of at least 3 individuals for letters of reference, and a curriculum vitae to: Dr. P. J. McAlpine, Division of Human Genetics, Faculty of Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3. Deadline for receipt of applications: March 1, 1983. (NW254)A

Queen's University, Department of Radiation Oncology

CAREER SCIENTIST POSITION

A position is available as a "Career Scientist" of the Ontario Cancer Treatment and Research Foundation at the Kingston Regional Centre, Department of Radiation Oncology, Queen's University.

The applicant must have an MD or PhD or equivalent, must have had post-doctoral training relevant to cancer research, and must be capable of independent and innovative investigative work in the field of cancer. The ability to collaborate with existing scientists involved in tumour biology, tumour immunology and experimental cancer therapy is essential. The position is associated with adjunct academic rank and is renewable depending on satisfactory progress.

In conformity with Canadian immigration policy, preference will be given to Canadian citizens or landed immigrants. Address inquiries, with curriculum vitae, and the names of three referees to:

Dr J.S. Lott, Director
Ontario Cancer Foundation
Kingston Clinic
King Street West
Kingston, Ontario
CANADA K7L 2V7

(NW272)A

THE KENNEDY INSTITUTE

A recently qualified

POST-DOCTORAL
SCIENTIST

is required (supported by a 3-year grant to Dr I Olsen and Dr Helen Muir FRS) to work in the Cell Enzymology Unit. A newly discovered process of lysosomal enzyme transfer which is of potential clinical importance is being investigated. Experience of cell fractionation and protein/membrane purification procedures would be an advantage.

Salary on the Universities Research and Analogous Staff scales depending on age. London Weighting paid in addition.

Applications with curriculum vitae and names of two referees to The General Secretary, The Mathilda and Terence Kennedy Institute of Rheumatology, Bute Gardens, London W6 7DW. (1192)A

GUY'S HOSPITAL

CYTOGENETICIST
— (BASIC GRADE
SCIENTIFIC OFFICER)

Applications are invited for the po of Basic Grade Cytogeneticist. T Centre provides a diagnost cytogenetics service, includi prenatal diagnosis to the SE Tham Region. Practical experience in cyt genetics would be an advantage, b graduates in Biology or Genet could be given training in this post

Salary: £6,723 — £9,391 pa (Depending on experience).

For further information and application form please contact t Personnel Officer, Guy's Hospit St Thomas Street, London SE1 9R Tel: 01-407 7600 ext 3471 quoting r P/3.

Closing date for completed app cations: 29th February 1984. (1193)A

UNIVERSITY OF ARIZONA

ASSISTANT/ASSOCIATE PROFESSOR

Microbiologist/Immunologist

Applications are being invited for several tenure track positions in the recently reorganized Department of Microbiology and Immunology at the Assistant or Associate Professor level, to begin summer-fall semester, 1984. Duties include teaching undergraduate students, medical students, and graduate students, and conducting an independent research program. Research interests should be within the broad area of microbiology/immunology. Individuals with a strong background in immunology, pathogenics, bacterial physiology, and virology are particularly being sought. The successful candidate must have a doctoral degree and a record of excellence and productivity in scholarly work. Postdoctoral experience is preferred.

Qualified applicants should send a résumé and arrange to have three letters of recommendation sent to Professor William Meinke, Search Committee Chairman, Department of Microbiology and Immunology, College of Medicine, University of Arizona, Health Sciences Center, Tucson, Arizona 85724. Complete applications should be received by April 1, 1984. The University of Arizona is an Equal Opportunity/Affirmative Action Employer. (NW279)A

PATHOLOGIST

he Institute for Cancer Research is eking a Pathologist to head a athology laboratory that will be a source for a variety of programs in velopmental biology and onco-gy. Applicants should be board rtified in anatomic pathology, nowledgable in morphologic athology, particularly of laboratory imals, with interest in colla-orative research. Independent search will be encouraged. Staff pointment.

Direct inquiries and submit idence of research achievements id curriculum vitae to: Chairman of ie Search Committee for athologist, c/o Personnel Dept, stitute for Cancer Research of the ox Chase Cancer Centre. 7701 rholme Avenue, Phila, PA 19111. n Affirmative Action Equal pportunity Employer.

(NW282)A

UNIVERSITY OF MAINE
DIATOMIST/LIMNOLOGIST

esearch Associate position starting March for research on effects of idic deposition on lakes in northern ew England. Work includes the entification and enumeration of atoms in sediment cores, computer alyses, and rigorous field work. pplicants should be skilled atomists, have a working owledge of limnology, and hold a D or a master's degree with levant post-degree experience. lary $15,000/annum; one year sition.

Applications must be received by February 1984.

Apply by letter and résumé; list ree references; send to Ronald B avis, Dept Botany & Plant Patho-gy, University of Maine*, Orono, aine, 04469, USA *An Affirmative ction/Equal Opportunity Employer.

(NW274)A

KING'S COLLEGE SCHOOL OF MEDICINE AND DENTISTRY
King's College London
Denmark Hill,
London SE5 8RX
RESEARCH ASSISTANT (MLSO)

pplications are invited for the post Research Assistant to work on the velopment of coagulation in-bitors in the Thrombosis Research nit. Candidates should be aduates in biochemistry or armacology, and preferably with me experience in protein purifica-n and/or enzymology.

Salary £6,533 — £7,168 per num.

Applications, naming two referees id quoting Ref: CT/R2, should be nt to the Secretary of the School at e above address by 23 February 84. (1163)A

BIOCHEMISTRY

Biotechnology firm is seeking a few outstanding research scientists.

B.S./M.S./Ph.D.

IMMUNOCHEMISTS. Successful candidates will have a proven track record of industrial development of homogenous enzyme immunoassay test kits. Ph.D. Salary range of $35-50K.

MOLECULAR BIOLOGISTS. Successful candidates will have proven expertise in nucleic acid replication, enzymology, and recombinant DNA technology. Ph.D. Salary range of $32-50K.

Microgenics offers a scientific challenge and equity participation with competitive salaries and fringe benefits. For immediate consideration, forward your resume in strict confidence to:

MICROGENICS CORPORATION
2341 Stanwell Dr. • Concord, CA 94520

MICROGENICS

(NW275)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF ASTRONOMY

POST-DOCTORAL RESEARCH APPOINTMENT

An SERC-supported Post-Doctoral Research post is available for work on the determination of the geoid from satellite altimetry data. The project is linked with the UK interest in satellite laser ranging, and is intended to assist UK participation in future satellite altimetry programmes. The person appointed will be based in Leicester, but will collaborate with workers in Oxford and at the Institute of Oceanographic Sciences.

Applicants should have an appropriate background in physics or mathematics and an interest in geophysics and geodesy. The post is tenable for three years at a starting salary up to £8,080. The appointment is to be taken up, if possible, on 1 April, 1984.

Applicants should send a curriculum vitae, together with any other relevant information, and should arrange for two referees to write in their support. Applications and references should be received by 2 March 1984 and should be addressed to Professor A J Meadows, Astronomy Department, The University, Leicester LE1 7RH, to whom any enquiries should also be directed.

(1181)A

MOLECULAR BIOLOGY

PROJECT SUPERVISOR (C-DNA)

The research manager of a pharmaceutical company, a world leader in its market, is looking for a project supervisor for its ***GENE - SPLICING UNIT.***

His primary task, working with a team of biologists, will be to set up a bank of ***EUKARYOTIC C-DNA.***

He will be a man or woman at least 28 years old, a D.Sc., agricultural expert, pharmacist, Ph.D., etc., with a successful background in a molecular biology laboratory.

Potential applications are vast, and this biologist's work will have almost immediately practical consequences.

The location is a leading French university town.

Please send your application to Jacques TIXIER with the reference 333 N. (NW748)A

jacques tixier s.a.
7 rue de logelbach. 75017 paris

membre de syntec

AVAS CONTACT

UNIVERSITY OF DUBLIN
Trinity College

CHAIR OF PHYSICS

This Chair, carrying the title 'Erasmus Smith's Chair of Natural and Experimental Philosophy (1724)' will fall vacant in 1984 when the present occupant and head of department, Professor Brian Henderson, leaves to take up another post.

The department is at present organised into the following research groupings:

(a) SURFACE PHYSICS;
(b) NUCLEAR INSTRUMENTATION;
(c) SOLID STATE SPECTROSCOPY;
(d) NON-CRYSTALLINE MATERIALS;
(e) LASER AND OPTICAL ELECTRONICS.

Applications are invited from theoretical physicists with relevant interests and from experimentalists: in present circumstances, applicants whose interests relate to solid state physics may be at some advantage.

Further particulars may be obtained from: G. H. H. Giltrap, Secretary to the College, Trinity College, Dublin 2, to whom formal application may be made, preferably not later than 17 February, 1984. (1164)A

WASHINGTON UNIVERSITY
SCHOOL OF MEDICINE
POSTDOCTORAL
RESEARCH FELLOW

Available immediately. Applications are invited for a 2-3 year post-doctoral research appointment in molecular biology/protein chemistry to investigate structure/function of oncogenes, growth factors, and transforming proteins.

Apply with curriculum vitae and two references to Dr Thomas F Deuel, Division of Hematology, Washington University School of Medicine, 216 S Kingshighway, St Louis, MO 63110. (NW271)A

JAPANESE BIOTECHNOLOGY STUDY TOUR

An in-depth series of site visits, conferences and seminars with some of Japan's leading industrial scientists and executives in biotechnology.

This exclusive study tour is designed to give western scientists and executives an opportunity to become acquainted with the Japanese marketing and research capacity. The mission will be led by Christopher G. Edwards, Editor in Chief of BIO/TECHNOLOGY, and organized by the Technology Transfer Institute. Visits will be conducted throughout Japan from March 31-April 15.

For further information, contact: Technology Transfer Institute — New York, One Penn Plaza, Suite 1411, 250 W. 34th St., New York, N.Y. 10119. 212-947-2648 telex 420057TTIUI. Attention: Mr. Hashizume. (NW174)C

WORKSHOP

EMBO INTERNATIONAL WORKSHOP
THE APPLICATION OF MOLECULAR BIOLOGY TO THE NERVOUS SYSTEM
Lady Margaret Hall, Oxford
September 23-25, 1984

Organisers: E.A. Barnard, M.R. Hanley, J.F. Jackson, Department of Biochemistry, Imperial College, London SW7 2AZ, UK

Topics: Approaches using molecular genetics and complementary molecular and immunological techniques applied to: receptors, ion channels, neurotransmitters, brain structures and neural development.

Investigators experienced in these areas have been invited to review current approaches and lead discussion periods, which will be extensive. The meeting will be in residential workshop format and will be limited to 65 participants.

Applications to attend should be sent, with a short statement of recent research work, to Professor E.A. Barnard (marking the envelope "EMBO Workshop").

A number of places are being reserved for younger postdoctoral workers interested in this field, and some funds are available for their support. The registration fee of £100 (payable on acceptance) will include full board and accommodation for four nights, conference dinner and reception. (1189)V

ASSISTANTSHIPS

UNIVERSITY
OF LIVERPOOL
DEPARTMENT OF GEOGRAPHY
Applications are invited for
POSTDOCTORAL SENIOR
RESEARCH
ASSISTANTSHIP

in the Department of Geography to work on an investigation of global cloud data archiving and analysis.

Applicants should hold a PhD in atmospheric science or physics and have considerable experience in computing. He or she must be able to undertake independent research; be prepared to attend international meetings and to collaborate with research scientists at the US Air Force Research Geophysics Laboratory in Massachusetts. Considerable experience has been established by this group. It is hoped the successful candidate will be able to draw upon developed techniques as well as contributing new ideas to the understanding of cloud observations from surface bases and satellites.

The project is financed on a year-by-year basis for two years from 1st March, 1984. The initial salary will be on the scale £7,190 — £8,530 per annum.

Applications, together with the names of three referees, should be received not later than 28 February, 1984, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/222/N. (1179)P

THE MIDDLESEX
HOSPITAL MEDICAL
SCHOOL
(University of London)
RESEARCH
ASSISTANTSHIP IN
FREEZE-FRACTURE
STUDIES

Applications are invited for a post-doctoral research assistantship to investigate the fine structure of the myelinated nerve fibre using the freeze-fracture technique. It is desirable that applicants have some experience in the freeze-fracture technique but not necessarily in neurobiology. The post, tenable for two years, is funded by The Wellcome Trust. Starting salary according to age, qualifications and experience up to £9,267 per annum inclusive of London Allowance.

Applications together with curriculum vitae and the names of three referees should be sent to L M J G Harrison, Director, Reta Lila Weston Institute of Neurological Studies, The Middlesex Hospital Medical School, London W1N 8AA. (1186)P

Please mention
nature
when replying to
these advertisements

ANNOUNCEMENT OF OPPORTUNITIES FOR THE SUPPORT OF RESEARCH AND DEVELOPMENT IN THE EUROPEAN COMMUNITY

Experimental action, 1984 to Stimulate the efficacy of Europe's scientific and technical potential

1. OBJECTIVES

The Commission of the European Communities has undertaken an experimental Stimulation Action designed to improve the efficacy of European research and development. This experimental action is intended to test various methods of support appropriate to the Community level, methods which are innovative by comparison with the range of available approaches which have been used by the Commission for several years in implementing its scientific and technical programmes.

2. AREAS AND ACTIVITIES SUPPORTED

The following may be supported:

- projects put forward jointly by teams of researchers or engineers from the various countries of the European Economic Community, and requiring collaboration — on a mono or multidisciplinary basis — between these teams (either in the public or private sector) in order to be brought to a successful conclusion;
- projects involving the secondment for relatively long periods of time (more than six months) of a researcher from a laboratory in one country of the European Community to a research centre in another country of the European Community;
- activities which enable communication and dissemination of information (especially where this is multidisiciplinary in nature) to be strengthened within the scientific and technical system of the European Economic Community.

Seven areas of activity have been selected for the experimental phase.

- Pharmacobiology is a multidisciplinary approach to the numerous scientific problems posed by the application of modern biology in this field particularly as regards inter and intra cellular communication;
- Solid State Physics: for example structure phenomena and the processes of preparing composite materials;
- Optics: particularly optoelectronics and the applied or theoretical development of the mathematics involved in this sector;
- Combustion: a theoretical approach involving new basic knowledge of systems experiencing imbalance and ignition phenomena (changes in material undergoing combustion);
- Photometry — Photoacoustics: particularly their application to the field of non destructive analysis;
- Interface phenomena: particularly catalysis and emulsification;
- Climatology: the study of transitory phenomena.

3. SUPPORT METHODS TO BE TESTED IN 1984

- Research Grants: payment of a grant to cover the travel, accomodation and research expenses, involved in the secondment of scientists to a laboratory in a country other than that in which they are currently employed upon research (within the EEC);
- Assistance intended to increase the mobility of researchers: a contribution to the dissemination of the best available knowledge and the development of contracts between researchers (subsidies);
- The twinning of laboratories in different countries: to enable researchers working in parallel in an advanced field of study in various countries of the Community to work together in order to achieve a "critical" scale: the allocation of a grant making it possible for researchers to meet, to carry out joint experiments and exchange results.

4. THE ALLOCATION OF COMMUNITY FINANCIAL SUPPORT

The Commission hopes to receive proposals corresponding to the specifications set out at points 2 and 3; these proposals should show the amount of support required. They will be evaluated by means of a system of referees set up under the aegis of the Committee for the European Development of Science and Technology (CODEST) which is responsible for advising the Commission on this matter.

The level of support given by the Commission will vary according to the method of intervention.

5. PROCEDURE

Any person or body (either in the public or private sector) wishing to learn more about the experimental stimulation action, or who wishes to submit a proposal should contact the following address as soon as possible:

General Directorate for Science Research and Development,
Division XII-A-2
Stimulation Action
Commission of the European Communities
200, Rue de La Loi
B — 1049 BRUSSELS
BELGIUM

Detailed information on this second call for proposals, and the forms upon which submissions should be made will be available upon request. Formal proposals which reach the Commission before 1 April 1984 will, if accepted, result in a contract for support during the same year. (W747)H

THIS LIST IS NEITHER EXCLUSIVE NOR EXHAUSTIVE,
AND ANY FIELD OF ACTIVITY OR ORIGINAL,
PROMISING DEVELOPMENT WHERE A TEST OF
STIMULATION SUPPORT METHODS CAN BE MADE,
MAY BE TAKEN INTO CONSIDERATION.

FELLOWSHIPS

Atlas Research Fellowship

St. Catherine's College, Oxford University

The Rutherford Appleton Laboratory of the Science and Engineering Research Council and St. Catherine's College invite applications from engineers, mathematicians or scientists for a joint Research Fellowship. The aim of this "Atlas Fellowship" is to encourage theoretical studies, particularly those involving computation, in an area of research supported by the Rutherford Appleton Laboratory and of interest to some Fellows of the College. These areas include the following broad fields: study of condensed matter by scattering of neutron beams; computer simulation of condensed phases, especially liquids; finite element methods applied to transport processes. Candidates will be asked to present the programme of research they would propose to follow.

The holder of the Fellowship will be expected to undertake independent research work and to spend the greater part of his/her time at the Rutherford Appleton Laboratory, where exceptionally powerful computer facilities are available. He/she will also be a Research Fellow of St. Catherine's College with the full rights and privileges of a Fellow and will be associated with a relevant University Department. The Rutherford Appleton Laboratory's involvement with many University research groups provides an excellent opportunity for contacts with other research workers engaged on a wide range of research programmes. The Fellowship provides an opportunity for some teaching experience, and for close personal contact with the College in other ways.

The Fellowship will be tenable for a period of three years and may be extended for a further two years for the completion of a total period of five years. The stipend will depend upon the age and experience of the candidate selected within the broad range £9,500 to £14,800.

For details of research fellows' rights at St. Catherine's College and application forms, write to Recruitment Office Personnel Group, Rutherford Appleton Laboratory, Chilton, Didcot, Oxon OX11 0QX, or telephone Abingdon 0235 445435 quoting reference No: VN 202.

The closing date for applications is 2nd March 1984

(1154)E

serc Rutherford Appleton Laboratory

THE ANIMAL HEALTH TRUST WOOLDRIDGE FARM LIVESTOCK FELLOWSHIP

Applications are invited from veterinarians and scientists with post-graduate research experience, who will design and undertake studies of major factors influencing the health and productivity of cattle, sheep or pigs.

The Fellowship is tenable for up to three years at an agreed place of work in the UK, commencing on 1st October, 1984.

The value of the Fellowship is in the region of £5,000/£8,500 plus a reasonable contribution towards essential expenses.

Further details and application forms are obtainable from the General Secretary, The Animal Health Trust, Lanwades Hall, Kennett, Newmarket, Suffolk CB8 7PN.

Closing date 31st March 1984. (1166)E

UNIVERSITE CATHOLIQUE DE LOUVAIN POST-DOCTORAL FELLOWSHIPS

— Spectroscopic study (E.P.R.-N.M.R.) of the molecular interactions at the solid-water interface: adsorption of ions by model colloids and cell walls. Preference given to a candidate experienced in physical chemistry of interfaces and/or magnetic spectroscopies.

— Multidisciplinary research dedicated to the implications of the physical chemistry of interfaces in biotechnology (cell and enzyme immobilization). Preference given to a candidate experienced in physical chemistry of interfaces, and/or microbiology, and/or biotechnology.

Positions available immediately; one year renewable. Salary of 39.000 B.Francs per month after tax deduction, or above depending on age, experience and family.

Applications accompanied by curriculum vitae, detailed information on the scientific background and the names of two references should be sent to

Prof. P. ROUXHET
Laboratoire de Physico-Chimie des Interfaces
Faculté des Sciences Agronomiques
Place Croix du Sud 1
B-1348 LOUVAIN-LA-NEUVE, Belgium (W733)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF MICROBIOLOGY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for this ARC-funded post, to work on the replication strategy of potexviruses and aspects of resistance. Experience in plant virology or nucleic acid biochemistry would be an advantage.

Post tenable for up to seventeen months from 1 May 1984.

Salary on the Research Fellow 1A scale £7,190 — £11,615 plus superannuation.

For further particulars phone 021-472 1301 ext 2559, quoting reference M2.

No formal application form. Three copies of application, including full Curriculum Vitae and naming three referees to Assistant Registrar, (Science and Engineering), PO Box 363 Birmingham B15 2TT by 24 February 1984. (1178)E

APPOINTMENTS WANTED

APPOINTMENTS WANTED

advertise your qualities and qualifications through the most influential science weekly in the world at a special reduced cost of *25p a word.* (Personal Box Numbers £1). Advertisements must be pre-paid and sent to: Nature Classified (Appts Wtd), 4 Little Essex Street, London WC2R 3LF. (890)B

STUDENTSHIPS

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

RESEARCH STUDENTSHI
October 1984

Applications are invited for t above positions from graduates a undergraduates who hold or expe to obtain at least an upper seco class honour degree by this summe

The major research activities of t department are:-
(a) Membrane proteins: structur mobility and function
(b) Molecular mechanisms of enzyn regulation in mammalian cells hormones and drugs; genetic diseas associated with these enzymes.
(c) Use of recombinant DNA tec niques to study DNA structure a function; regulation of ge expression in procaryotes a eucaryotes.

Applications including a f curriculum vitae and names a addresses of three referees Professor P Cohen, Department Biochemistry, The Universit Dundee DD1 4HN, Scotland.

(1151)F

MRC REPRODUCTIVE BIOLOGY UNIT Edinburgh

RESEARCH STUDENTSHI

Applications are invited for t above PhD studentship tenable f three years from October 1984 work on the molecular basis human sperm function.

Applicants should have a goo honours degree or MSc in bi chemistry or a related subject.

Additional information can obtained from Dr R J Aitke Telephone 031-229 2575. App cations, together with the names two referees, should be sent to Administrative Officer, MR Reproductive Biology Unit, Cen for Reproductive Biology, Chalmers Street, Edinburgh EH 9EW, by February 29th 1984.

(1155)F

nature classified

LONDON OFFICE
Keith Walesby
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

NEW YORK OFFICE
Miss Gene Fein
15 East 26 Street
New York, NY 100
Tel: (212) 689 59

TORONTO OFFICE
Peter Drake Associates
32 Front Street West
Suite 200, Toronto,
Ontario M5J 1C5
Tel: (416) 364 1623

INTERNATIONAL SYMPOSIUM ON MONOCLONAL ANTIBODIES: BIOLOGICAL AND CLINICAL APPLICATIONS
Florence, Palazzo dei Congressi, October 16-19, 1984

Scientific Secretariat: A. Pinchera — G. Doria — F. Dammacco — A. Bargellesi
The meeting is intended to cover three broad areas of biological and clinical applications of monoclonal antibodies: Markers of the Immune System; Hormonal Receptors and Related Autoimmune Disorders; Experimental and Clinical Oncology.
The Scientific Program will consist of Invited Lecturers and Poster Sessions. A limited number of Posters will be selected for oral presentation.
Invited Speakers: L. Adorini (Italy), R. C. Bast (USA), C. A. Bona (USA), M. I. Colnaghi (Italy), P. Comoglio (Italy), G. Corte (Italy), S. Dower (USA), P. A. W. Edwards (UK), A. Falaschi (Italy), I. D. Goldfine (USA), M. F. Greaves (UK), G. L. Greene (USA), C. Grossi (Italy), S. Hakomori (USA), L. D. Kohn (USA), J. Lindstrom (USA), R. G. Lynch (USA), J. P. Mach (Switzerland), S. L. Morrison (USA), K. Rajewski (FRG), M. D. Scharff (USA), R. H. Schwartz (USA), J. Strominger (USA), J. Schlessinger (Israel), T. Tada (Japan), I. S. Trowbridge (USA), P. E. Thorpe (UK), J. C. Venter (USA), T. A. Waldmann (USA), L. N. Warner (USA).
The deadline for submission of Abstracts is June 15, 1984. For further information write to the *Organizing Secretariat:* Organizzazione Internazionale Congressi (O.I.C.), Via Gustavo Modena 19, 50121 Florence (Italy). Tel: (055) 53962-571082 — Telex 570243 OICCONGRE.

Preliminary Program — International Symposium on MONOCLONAL ANTIBODIES '84: BIOLOGICAL AND CLINICAL APPLICATIONS
Florence, October 16-19, 1984

Tuesday, October 16 — 17:00 — 19:00 OPENING CEREMONY
R. Masi (Italy): Presidential Address
A. Falaschi (Italy): New Frontiers of Biotechnology

Wednesday, October 17 MARKERS OF THE IMMUNE SYSTEM
Morning 8:30 — 12:20
Chairmen: G. DORIA and A. BARGELLESI
M. D. SCHARFF (USA): Advances in Monoclonal Antibody Technology
S. L. MORRISON (USA): Ig Gene Expression in Transformed Lymphoid Cells
C. A. BONA (USA): Regulatory Idiotopes
K. RAJEWSKI (FRG): Monoclonal Antibodies in the Analysis of the Idiotypic Network and the Selection of the Antibody Repertoire
J. STROMINGER (USA): Human Histocompability Antigens: Genes and Proteins
12:20 — 14:00 — LUNCH AND POSTERS
Afternoon 14:00 — 18:20
Chairmen: M. RICCI and F. DAMMACCO
L. ADORINI (Italy): Antigen-Specific Monoclonal T Cell Products
R. H. SCHWARTZ (USA): Cellular Co-Recognition of MHC and Antigen
T. TADA (Japan): The Immunoregulatory Molecules Studied with Monoclonal Antibodies
C. GROSSI (Italy): Multiple Marker Analysis of Human Lymphocyte Subpopulations
N. L. WARNER (USA): New Developments in the Definition of Human Leucocyte Subsets by Monoclonal Antibodies
M. F. GREAVES (UK): Human Lymphoid Malignancies
18:20-19:20
Chairmen: G. TRIDENTE and S. ROMAGNANI
POSTER DISCUSSION

Thursday, October 18 HORMONAL RECEPTORS AND RELATED AUTOIMMUNE DISORDERS
Morning 8:30 — 12:20 Chairmen: A. PINCHERA and A. ALBERTINI
L. D. KOHN (USA): Thyrotropin Receptor
I. D. GOLDFINE (USA): Insulin Receptor
J. LINDSTROM (USA): Acetylcholine Receptor
G. L. GREENE (USA): Immunochemical Studies of Estrogen Receptor in Normal and Neoplastic Tissues
B. MONCHARMONT (Italy): Application of Monoclonal Antibodies to the Study of the Estrogen Receptor Structure
12:20 — 14:00 — LUNCH AND POSTERS
Afternoon 14:00 — 17:00
Chairmen: M. SERIO and L. MARTINI
J. C. VENTER (USA): Monoclonal and Autoantibodies to Beta-Adrenergic Receptors
I. S. TROWBRIDGE (USA): The Biology of the Transferrin Receptor
J. SCHLESSINGER (Israel): Monoclonal Antibodies Against the Epidermal Growth Factor Receptor: A Versatile Tool for Structural and Mechanistic Studies.
T. A. WALDMANN (USA): Interleukin-2 Receptors on T Cells
17:00 — 18:00
Chairmen: M. MUGGEO and V. MACCHIA
POSTER DISCUSSION

Friday, October 19 EXPERIMENTAL AND CLINICAL ONCOLOGY
Morning — 8:30 — 12:20 Chairmen: G. SALVATORE and G. L. BURAGGI
P. COMOGLIO (Italy): Oncogene Products as Targets for Monoclonal Antibodies
S. HAKOMORI (USA): Glycolipid Tumor Cell Markers
R. G. LYNCH (USA): Myeloma Proteins as Targets and Inducers of Immunoregulatory Signals
M. I. COLNAGHI (Italy): Tumor Detection by Immunocytochemical and Radioimmunometric Methods
J. P. MACH (Switzerland): Detection of Tumors by Immunoscintigraphy Using Monoclonal Antibodies against Tumor Markers
12:20 — 14:00 — LUNCH AND POSTERS
Afternoon 14:00 — 17:40 Chairmen: U. MELDOLESI and G. DELLA PORTA
P. A. W. EDWARDS (UK): Human Monoclonal Antibodies and the Prospects for Therapy
R. C. BAST (USA): Treatment of Bone Marrow with Monoclonal Antibodies *in Vitro*
G. CORTE (Italy: Monoclonal Antibodies for the Control of GVH Reaction after Bone Marrow Transplantation
S. DOWER (USA): A Rational Approach to the Development of Monoclonal Antibody Reagents for *in Vivo* Tumor Diagnosis and Treatment
P. E. THORPE (UK): Monoclonal Antibody-Toxin Conjugates
17:40 — 18:40 Chairmen: A. NICOLIN and L. FRATI
POSTER DISCUSSION

(W726)M

SEMINARS and SYMPOSIA continued

ROYAL POSTGRADUATE MEDICAL SCHOOL
in collaboration with
GLAXO GROUP RESEARCH LIMITED
BASIC SCIENCE IN GASTROENTEROLOGY SYMPOSIA
Seventh Symposium of the Series

GASTROINTESTINAL CARCINOGENESIS

London — 24 April 1984

Organisers: J. M. Polak, S. R. Bloom, N. A. Wright, A. G. Butler

Invited speakers include

D. Britton, UK
W.W.L. Chang, USA
P. Correa, USA
M. Hill, UK
J. Jass, UK
M. Lipkin, USA
B. Morson, UK
R.R. Peto, UK
C.D. Potten, UK
R. Riddell, USA
J.P. Sunter, UK
J. Weisburger, USA
R.C.N. Williamson, UK

Topics include
Epidemiology, Chemical Carcinogenesis, Cell Proliferation, Environmental Factors, Dysplasia, Pre-cancerous Lesions, Atrophic Gastritis

Registration and Lunch — £10.00

For further details and Application Form please contact:
A.G. Butler, Medical Division, Glaxo Group Research Ltd., Ware, Herts. SG12 0DJ. Telephone No. 0920 3993.

Closing date for applications: 13 April 1984.

(951)M

FIFTH INTERNATIONAL OCEAN DISPOSAL SYMPOSIUM
At Oregon State University
Corvallis, Oregon, USA

Topics include:

* Biological and toxicological effects of wastes in the ocean;
* Biological accumulation and concentration of wastes;
* Dispersion, settling, and transport processes;
* Eutrophication in coastal ecosystems;
* Physical and biochemical modelling on the effects of wastes in the ocean;
* Physical, chemical, and biological interactions of wastes.

Send Abstract (200-300 words), by 1 May 1984, to: Prof. Iver W. Duedall, Executive Committee, Fifth International Ocean Disposal Symposium, Department of Oceanography and Ocean Engineering, Florida Institute of Technology, Melbourne, FL 32901 USA. Telephone: 305 723-3701.

(NW280)M

LEUKAEMIA RESEARCH FUND
International Research Symposium on **DIAGNOSIS AND CLASSIFICATION OF LEUKAEMIA** will be held at the Royal College of Physicians, London on Thursday 5th April 1984. Registration fee (including lunch) £12. For programme details and registration form apply to: **Leukaemia Research Fund, 43 Great Ormond Street, London WC1N 3JJ. (Tel: 01-405 0101)**

(1158)M

FOR SALE and WANTED

ADDA-Interface for Apple. Use your Apple II for data acquisition, storage and signal averaging. Converts your analog oscilloscope or monitor to a digital storage oscilloscope with storage on discs. Dual trace option and other features. Package contains a plug-in card, friendly program on disc and manual. Requires any Apple II with language card (64K) and 2 disc drives. Price $725. 10 day trial. For information: OSI, 430 S 46th St Phila. PA 19143. (NW270)L

CONFERENCES and COURSES

Royal Postgraduate Medical School
(University of London)

TOXICITY TESTING — THE HUMAN DIMENSION

A Workshop on the Toxicity Testing of Drugs and Chemicals
April 2-6 1984
Department of Clinical Pharmacology
Royal Postgraduate Medical School, London

The Workshop will consist of a series of invited lectures and discussion sessions and will cover the following topics: current procedures — are they adequate; new developments in toxicity testing; in-depth discussions of carcinogenicity, teratotoxicity and immunotoxicity; reviews of recent problems in drug toxicity including benoxaprofen and perhexiline; the detection of drug toxicity in man.

Speakers will include: Dr. H. Amos, Professor R. Batchelor, Professor F. Beck, Professor A. M. Breckenridge, Dr. R. Brimblecombe, Dr. D. M. Davis, Professor G. Dworking, Professor Sir Abraham Goldberg, Professor D. G. Grahame-Smith, Professor D. F. Hawkins, Dr. R. Hess, Dr. W. Inman, Dr. G. Jones, Dr. Barbara MacGibbon, Dr. D. W. Nebert, Dr. R. Peto, Professor D. E. Poswillo, Dr. I. F. H. Purchase, Dr. F. Sullivan, Professor P. Turner, Professor K. Weinbren.

For further information and registration forms contact: School Office (SSC), Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12 0HS. (Tel: 01-743 2030 ext 351).

Course Organisers: Dr. A. R. Boobis, Professor D. S. Davies and Professor C. T. Dollery.

WE APOLOGISE TO THOSE WHO MAY HAVE EXPERIENCED SOME DELAY IN RECEIVING FURTHER PARTICULARS OF THE COURSE. THIS WAS DUE TO A POSTAL STRIKE IN OUR LOCAL SORTING OFFICE DURING DECEMBER. YOUR ENQUIRIES HAVE NOW BEEN RECEIVED AND ARE BEING DEALT WITH.

Closing date for applications 24 February 1984. (1170)C

BIOCHEMICAL SOCIETY
HARDEN CONFERENCES

22nd: "PLANT GENES: STRUCTURE, EXPRESSION, MOBILITY"
Wye College, Ashford, Kent, 9—14 September 1984
Chairman and Organizer: C.J. Leaver (Edinburgh
Principal Topics: Nuclear Gene Structure and Organization; Organization and Information Content of Plastid and Mitochondrial Genomes; Developmental, Environmental and Hormonal Regulation of Gene Expression; Gene Mobility; Expression of Plant Genes in Micro-organisms; Plant Genes and Plant Breeding.
Conference fee and full board **£160** including VAT.

23rd: "MOLECULAR AND CELLULAR ASPECTS OF REPRODUCTION"
Wye College, Ashford, Kent, 16—21 September 1984
Chairman and Organizer: R.B. Heap (Babraham)
Principal Topics: Regulation of Gametogenesis; Developmental Control; Genome Manipulation; Sex Determination; Molecular Action of Hormones; Cellular Control of Hormone Secretion.
Conference and full board **£160** including VAT.

Information: Meetings Officer, Biochemical Society, 7 Warwick Court, High Holborn, London WC1R 5DP, U.K.

(1174)C

ANNOUNCEMENTS

THE INTERNATIONAL SPINAL RESEARCH TRUST

invites applications for funding of research projects concerned with CNS regeneration, bypass or repair, by pharmaceutical, surgical, electrical or other means.

For research application form please contact: THE INTERNATIONAL SPINAL RESEARCH TRUST, Strand House, 10 New Fetter Lane, London EC4A 1AD.

(1185)G

Typesetting: J.W. Arrowsmith Ltd, Bristol and Outline Graphics Ltd, London EC4Y 0HP. Printing: Kingsdale Press, Reading, Berks, member of the St. Ives Group, in association with Godfrey Lang Ltd, London WC1R 4EB and The William Byrd Press Inc., Richmond, Virginia, USA. Published by MACMILLAN JOURNALS LTD, 4 Little Essex Street, London WC2R 3LF. — 9 February 1984.



nature is keeping ahead. Are you being left behind?

New research results, the very latest international science news, key papers by the world's leading scientists and speed of publication ensure *Nature* remains one of the foremost weekly scientific journals in the world.

But if you're tied in to a circulation list at work, all this vital weekly information will undoubtedly reach you late, often incomplete and sometimes not at all.

With your own personal copy, you solve the problem. Every week you'll have all the latest research results and science news in your hands within days of publication:

News reports from throughout the world of science and technology.

News and Views section, where new ideas are analysed and discussed by leading researchers.

Articles and Letters in which key investigators disclose their work for the first time.

Book Reviews – Eminent figures offer expert judgments on a wide variety of recent publications.

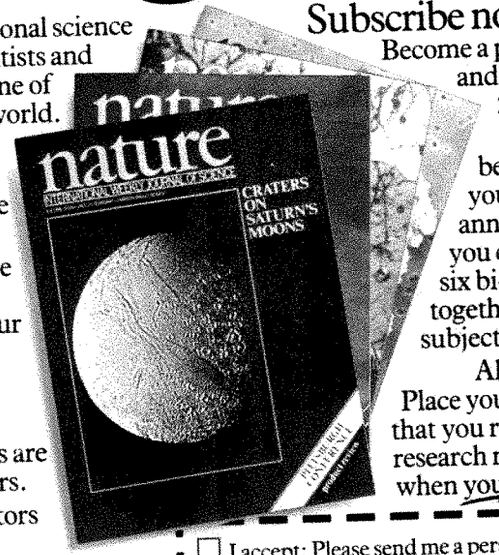
Special Issues providing an in-depth treatment of topics of importance.

Product Reviews – Detailed descriptions of laboratory equipment newly available.

Classified Advertisements for a wide variety of positions all over the world.

You will no longer be dependent upon other people passing down the 'list' copy, and because you will automatically receive every issue, you won't risk missing a single copy through absence from work. Moreover, you keep all this key information for permanent reference.

Through your door every week



Subscribe now – at Half Price

Become a personal subscriber now and you will receive a discount of 50% off the full rate.

All 51 weekly issues will be delivered to the address of your choice. You can start your annual subscription with any issue you choose. And you will receive six bi-monthly author indexes, together with the annual index of subject and author.

All this for just 94p a week.

Place your order now and make certain that you receive the scientific news and research results crucial to your work when you want them.

☐ I accept: Please send me a personal subscription to *Nature* for one year (51 issues) at the special half-price rate of £49.00.

☐ I enclose my personal cheque for £49.00 made payable to *Nature*. (Note: Institutional cheques cannot be accepted at this special rate).

☐ Please charge to my credit card account.
Access/American Express/Visa/Diners Club.

Account No. _____

Card Expiry Date _____

Signature _____

Name _____

Address _____

_____

_____

_____

Date _____ 7B18

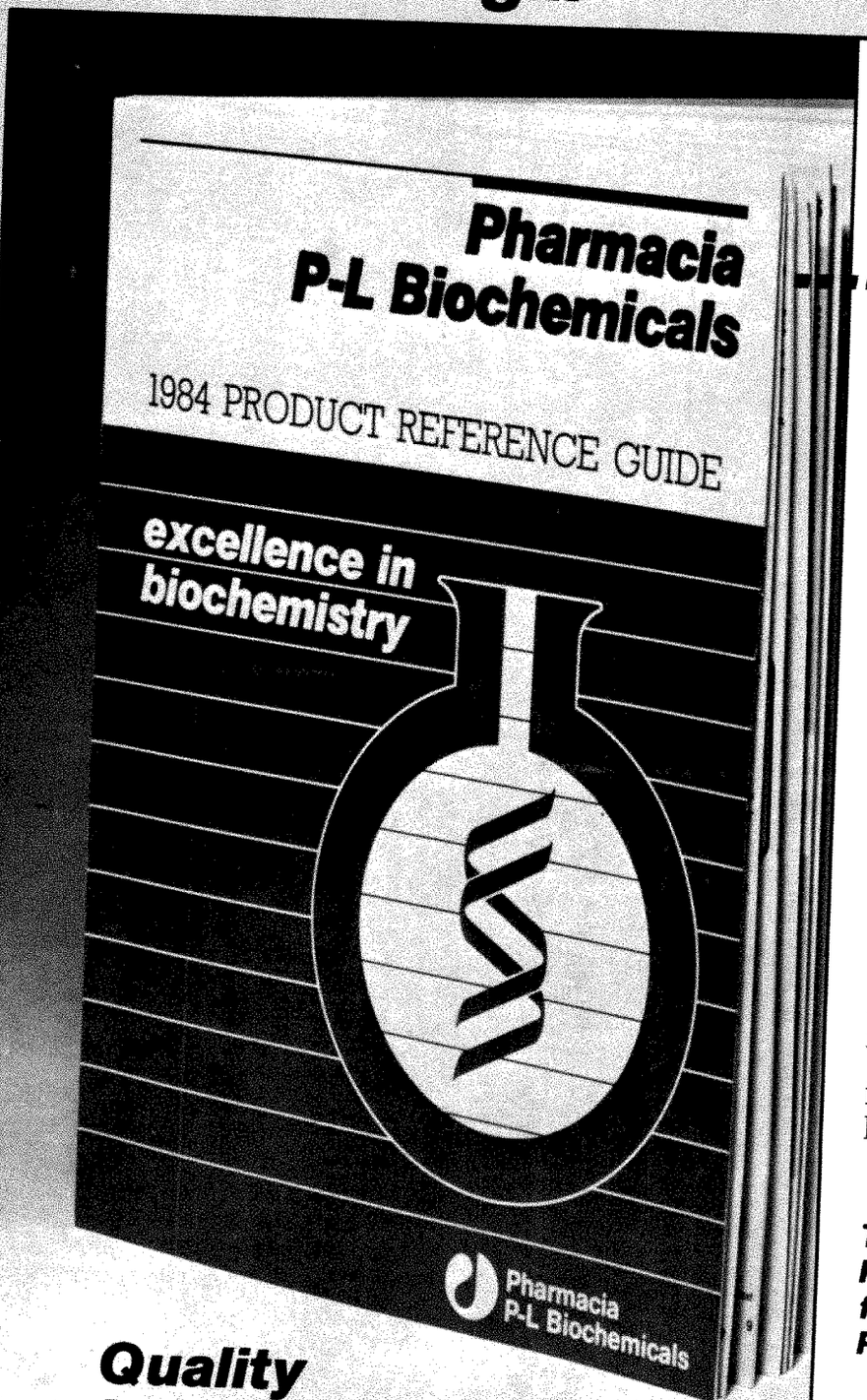
Please return this form to:
Circulation Manager
Nature
Brunel Road, Houndmills
Basingstoke
Hants RG21 2XS

This offer applies to UK and North America only. Please allow approximately 4 weeks for delivery of the first issue in your subscription year.

nature

Circle No.90 on Reader Service Card.

There is only one product in this catalog...



...Quality

Actually, the Pharmacia P-L Biochemicals Product Reference Guide lists over 2000 products for biochemistry and molecular biology. The products range from restriction nucleases to coenzymes, from lipids to polynucleotides – but our most important product is the quality that goes into each biochemical that we make.

At Pharmacia P-L Biochemicals, we take our commitment to "excellence in biochemistry" seriously. We make our biochemicals with quality – we know no other way.

The 1984 Product Reference Guide from Pharmacia P-L Biochemicals

Quality biochemicals for quality research.

For your copy, contact your local Pharmacia Fine Chemicals/Pharmacia P-L Biochemicals representative or write to one of the addresses below.

Pharmacia P-L Biochemicals Inc.
Subsidiary of Pharmacia Inc.
800 Centennial Avenue
Piscataway, NJ 08854

Pharmacia P-L Biochemicals Inc.
Subsidiary of Pharmacia Inc.
Box 175
S-751 04 Uppsala 1, Sweden

Pharmacia P-L Biochemicals

Circle No.09 on Reader Service Card.

nature

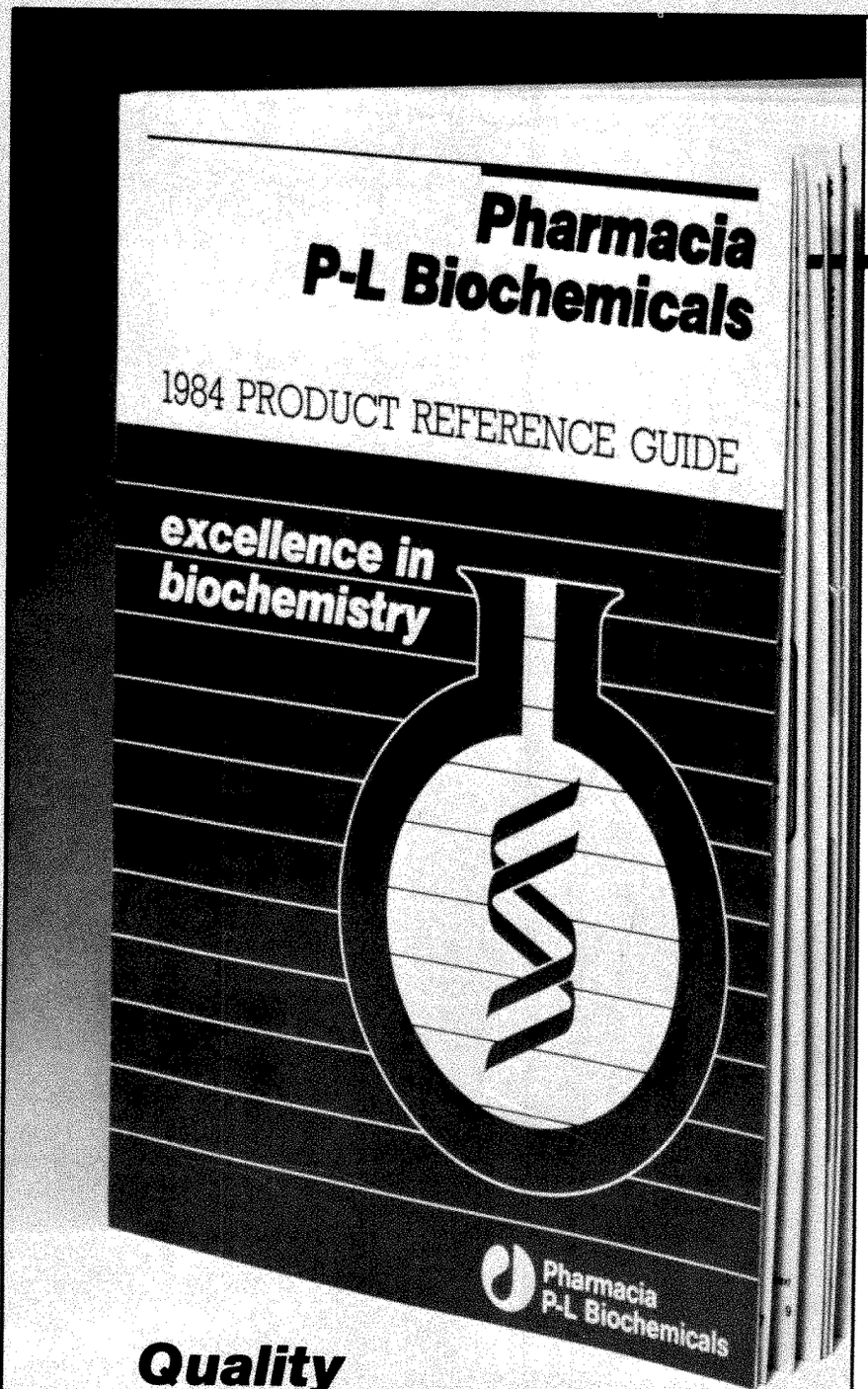
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

Vol 307 No 5952 16-22 February 1984 £1.80 $4.50



SHEEP-GOAT CHIMAERAS

There is only one product in this catalog...



Quality biochemicals for quality research.

...Quality

Actually, the Pharmacia P-L Biochemicals Product Reference Guide lists over 2000 products for biochemistry and molecular biology. The products range from restriction nucleases to coenzymes, from lipids to polynucleotides – but our most important product is the quality that goes into each biochemical that we make.

At Pharmacia P-L Biochemicals, we take our commitment to "excellence in biochemistry" seriously. We make our biochemicals with quality – we know no other way.

The 1984 Product Reference Guide from Pharmacia P-L Biochemicals

For your copy, contact your local Pharmacia Fine Chemicals/Pharmacia P-L Biochemicals representative or write to one of the addresses below.

Pharmacia P-L Biochemicals Inc.
Subsidiary of Pharmacia Inc.
800 Centennial Avenue
Piscataway, NJ 08854

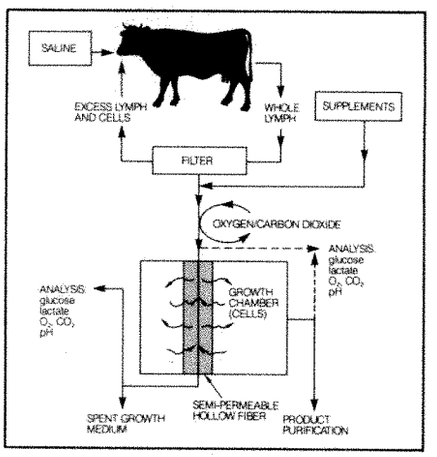
Pharmacia P-L Biochemicals Inc.
Subsidiary of Pharmacia Inc.
Box 175
S-751 04 Uppsala 1, Sweden

Pharmacia P-L Biochemicals

Circle No. 19 on Reader Service Card.

NATURE'S BIOREACTOR

The New Bio-Response MCT* (Mass Culturing Technique) System Delivers Monoclonal Antibodies And Other Mammalian Cell Products When You Need Them—From Grams To Kilograms.



Insufficient quantities of desired peptides are a key cause of expensive research and production delays. The MCT system is a *new* and *unique* mammalian cell culturing process designed to overcome the obstacle of insufficient quantities of peptides by delivering the quantity you need...*when you need it!* Additionally, every step in MCT production has been developed to contain costs now and in the future while maintaining exceptional purity and quality in mammalian cell proteins.

Quantity

Unlike conventional cell-culture methods, the MCT system uses "fresh," free-flowing lymph directly from a cow. Following treatment, the lymph is continuously diffused into and out of a growth chamber. This nearly *in vivo* growth environment stimulates continuous and optimal protein secretion. The result? Large quantities of desired proteins enabling you to move to the clinic or market...*faster.*

Cost

Unlike conventional cell-culture methods that require extensive labor forces and expensive growth mediums, Bio-Response's MCT is a closed, steady-state system. In essence, the MCT system starts with a low-cost lifeline—a feeding cow—and ends with a pure, quality product. MCT ...*an optimal system at low cost.*

Purity

Unlike conventional cell-culture methods, the MCT system is designed to insure purity without tedious processing. By directing the lymph across semi-permeable membranes, MCT provides both separation of media protein from desired cell products, and a continuous nutrient flow. The ability to add or withdraw from the chamber to maintain optimal cell density permits continuous harvest of a highly pure product.

Quality

Unlike conventional methods of production that attempt to create biologically active mammalian cell products, the MCT system provides a pure-mammalian product—an exact copy of the original protein and, in the case of non-antibody polypeptides, uncompromised by inappropriate glycosylation and disulfide bond formation. There is a growing consensus favoring products produced in a mammalian cell-culture system, such as the Bio-Response MCT system.

Circle No.07 on Reader Service Card.

For further information, call Bio-Response, Inc. at (415) 786-9744.

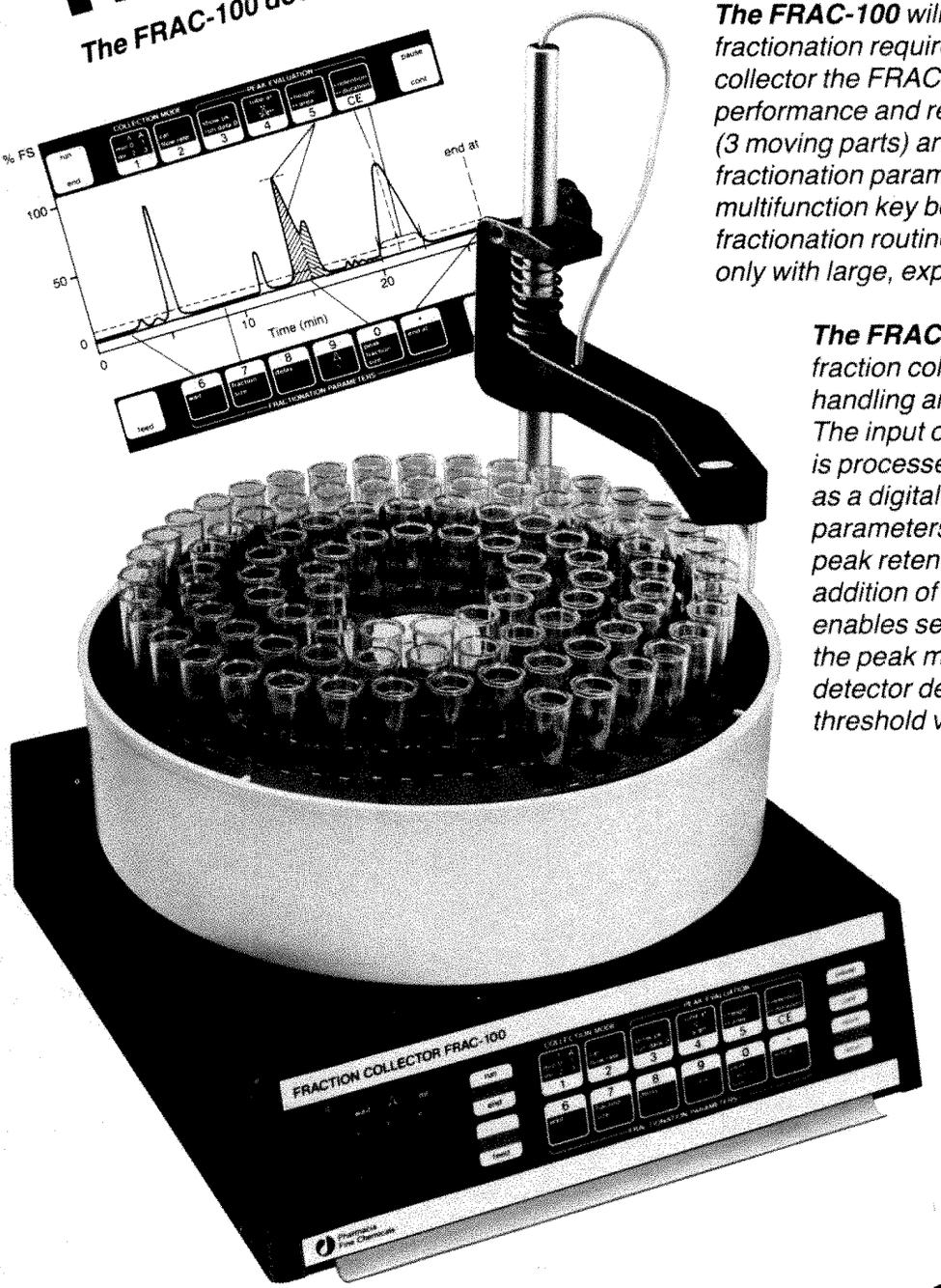
BIO-RESPONSE, INC.

*MCT (Mass Culturing Technique) is a trademark of Bio-Response, Inc.

© 1984 Bio-Response, Inc. All Rights Reserved.

The FRAC-100 Fraction Collector

The FRAC-100 does what any other small fraction collector does – collects fractions, PLUS...



The FRAC-100 *will fulfil all your needs for basic fractionation requirements. As a conventional collector the FRAC-100 is unequaled in performance and reliability. Simplicity in design (3 moving parts) and control (flexible fractionation parameters accessed by a multifunction key board) accomplish fractionation routines previously attainable only with large, expensive instruments.*

The FRAC-100, *as a sophisticated fraction collector presents unique data handling and integration capabilities. The input of a 0–10 mV detector signal is processed by the FRAC-100 to report as a digital display such separation parameters as peak height, peak width, peak retention and peak area. The addition of a PSV-100 solenoid valve enables selective fractionation of only the peak material as judged by a slope detector defined by a programmable threshold value.*

The FRAC-100 – as a small, simple inexpensive fraction collector – your best choice, – as a small sophisticated inexpensive collector, far beyond anything presently available – your only choice.

Pharmacia Fine Chemicals

Pharmacia Fine Chemicals AB,
Box 175, S-751 04 Uppsala 1, Sweden

Circle No. 14 on Reader Service Card.

The way ahead...

British Journal of Pharmacology

Published monthly on behalf of the British Pharmacological Society, the British Journal of Pharmacology contains original papers submitted from all parts of the world making it one of the most respected journals for all those professionally involved in the pharmacological sciences. It contains up-to-date papers of the highest quality on all aspects of drug action and is thus a vital source of information for physiologists, clinicians, immunologists, molecular biologists and biochemists as well as pharmacologists.

Subscription Prices
UK £145.00, USA/Canada US $360.00 surface, US $440 airmail,
Rest of World £160.00 surface, £210.00 airmail.

HUMAN TOXICOLOGY
An International Journal

Published bi-monthly this new journal features the following material:

* original papers of a high scientific standard on all aspects of human toxicology, embracing both animal research and studies with humans
* short communications
* invited comment on current matters of professional interest
* editorials on topical issues
* review papers
* book reviews

Subscription Prices
UK £65.00,
USA Canada
US $150.00 surface,
US $185.00 airmail,
Rest of World
£75.00 surface,
£95.00 airmail.

Pharmaceutical Medicine

New For 1984...

A new, independent, quarterly journal for the critical assessment of new and old methods employed to test drugs in man. To promote the discipline of pharmaceutical medicine and cover those activities germane to human testing of drugs, papers will appear on human pharmacology, clinical investigation, comparative clinical trials, and epidemiological studies. The latter will inevitably be associated with drug safety and post-marketing surveillance. The problems of quality control and analysis of laboratory tests will be addressed, and the handling of clinical trial data. The design and statistical analysis of clinical studies will also feature.

Pharmaceutical Medicine will provide invited contributions, reviews, news and opinion. A major feature will be abstracts of papers published elsewhere giving new means of assessing drugs.

The Journal will not take an overtly political or commercial position, but any matters affecting ways and means of assessing drug efficacy and safety, including governmental regulations, will be discussed.

Subscription Rates
UK and Eire: £45.00, USA Canada: Surface US $95.00, Rest of World: Surface £63.00

British Journal of Anaesthesia

The British Journal of Anaesthesia has consistently been the leading independent journal in anaesthesia enjoying international contributions and readership.

Published monthly the journal offers:

* Sections on laboratory investigation, clinical research, case reports and information on apparatus.
* A correspondence section in which views are exchanged through short communications and comments on matters of professional interest.
* Book Reviews.
* Postgraduate Educational Issues, on both clinical and basic themes, produced twice a year – indispensable to established clinician and trainee alike.

Subscription Prices UK £38.50, USA/Canada US $90.00,
Rest of World £53.00 surface, £95.00 airmail,
Trainee rates UK £24.00, US $50.00.

The British Journal of Cancer

Published monthly on behalf of the Cancer Research Campaign, The British Journal of Cancer is one of the leading cancer journals in the world, publishing articles in all the major scientific disciplines relevant to the subject. The journal provides papers and short communications submitted by scientists from countries throughout the world. In addition, the Proceedings of the meetings of the British Association for Cancer Research are regularly published in the journal. From time to time other selected national and international meetings are also covered. Up-to-date book reviews appear in each issue.

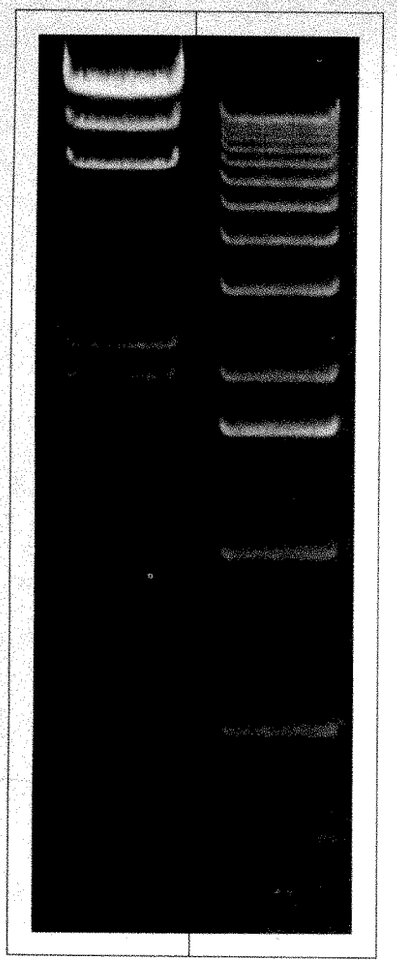
Subscription Prices
UK £99.00, USA/Canada US $240.00 surface,
US $285.00 airmail,
Rest of World £124.00 surface,
£160.00 airmail.

Sample copies available
All rates include postage
Back issues are available on request
Payment in any currency will be accepted at the current rate of exchange
Cheques should be made payable to Macmillan Journals Ltd.
Subscribers may make payments to UK Post Office Giro Account No. 5192455

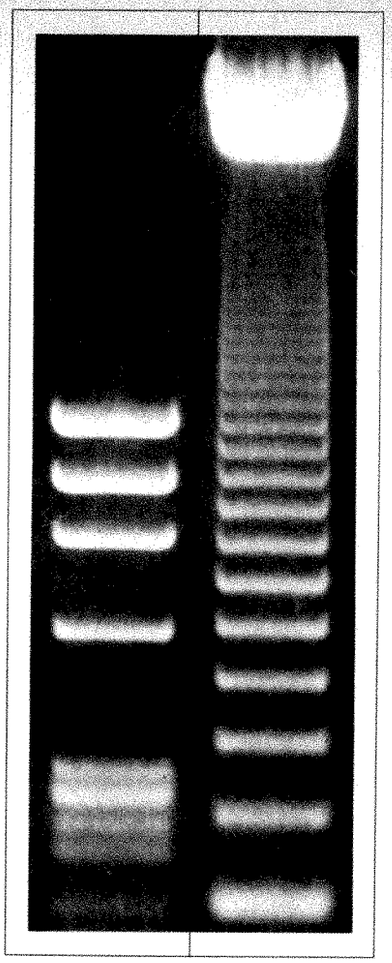
Scientific & Medical Division, The Macmillan Press Ltd,
Basingstoke, Hampshire, England, RG21 2XS

SCIENTIFIC & MEDICAL | M | MACMILLAN PRESS LTD

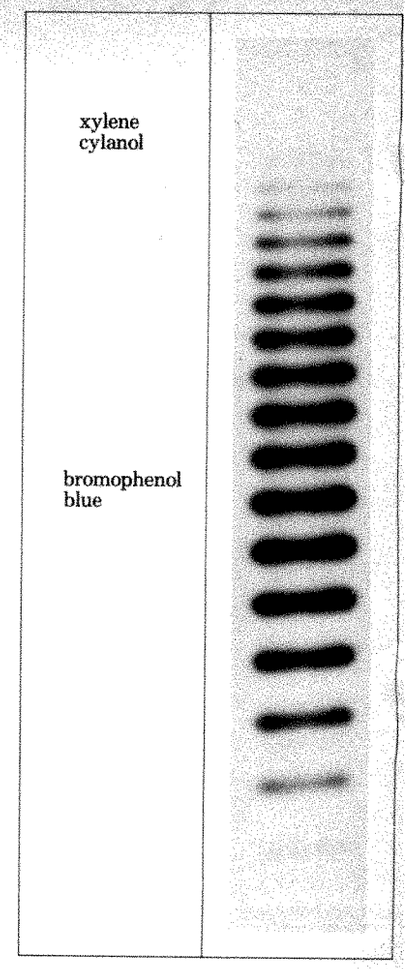
Reader Service Card



λ-*Hind* III 1 Kb Ladder



φX *Hae*III 123 bp Ladder



(Tracking dye) Oligo (dT) Ladder

Do your DNA markers measure up to the BRL "LADDERS"?

Do your markers provide enough reference points for accurate DNA sizing? Are the fragments spaced for confident calibration, or are there gaps that leave you guessing? BRL's exclusive Ladders measure up on both counts: these new markers contain *more fragments* than ordinary DNA standards, and the fragments are more evenly distributed. For calibration of DNAs over a wide range of sizes, BRL Ladders are the most accurate and convenient markers available.

For mapping and cloning applications, choose the 1 Kilobase (1 Kb) Ladder or the 123 Base Pair (123 bp) Ladder. The 1 Kb Ladder is composed of 12 fragments, ranging in size from 1,018 bp to 12,216 bp, in increments of 1,018 bp, and four additional fragments that act as reference markers. The 123 Base Pair Ladder consists of 34 multiples of a 123 bp fragment, covering the range from 123 bp to 4,182 bp. The smallest bands of both the 1 Kb Ladder and the 123 bp Ladder can be detected easily without overloading the gel, because smaller fragments are present in greater concentration than larger ones.

The Oligo $(dT)_{4\text{-}22}$ Ladder is the first marker available for nucleic acid sequencing and oligonucleotide synthesis. Consisting of 19 fragments, each increasing by 1 base from 4 to 22 bases, the Oligo $(dT)_{4;22}$ Ladder provides an accurate reference for determining the location and size of the smallest fragments on the gel.

BRL Ladders. The markers that measure up.

Bethesda Research Laboratories, Inc.
Gaithersburg, Maryland 20877
Telex: 64210 BRL GARG UW
To Order: (800) 638-8992;
(301) 840-8000
Technical Services: (800) 638-4045

Bethesda Research Laboratories (UK) Ltd.
Cambridge, CB4 4BE United Kingdom
Telex: 818710 BRLCAM G
To Order/Tech Services (0223) 315504

Bethesda Research Laboratories GmbH
6078 Neu Isenburg, West Germany
Telex: 417699 BRLNI D
To Order/Tech Services: 06102-3204

Bethesda Research Laboratories SARL
F-95220 Herblay, France
Telex: 695729 FBIMAGRE
To Order/Tech Services: (3) 978 8200

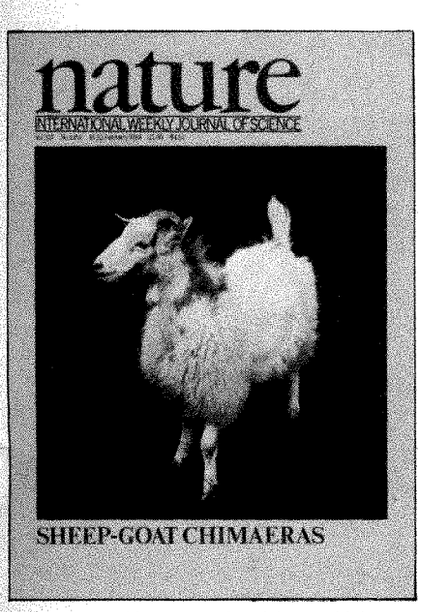
BRL Bethesda Research Laboratories

Circle No.05 on Reader Service Card.

nature

NATURE VOL. 307 16 FEBRUARY 1984

COVER



SHEEP-GOAT CHIMAERAS

A sheep-goat chimaera produced by combining one 8-cell sheep embryo with three 8-cell goat embryos. It is about 1-year-old and exhibits various characters confirming its chimaeric nature — a mixture of hairy and woolly areas in its coat, goat-like horns twisted like sheep horns and blood containing sheep and goat red blood cells. This animal and others are described on p.634 and p.637.

OPINION

No to nuclear decay? 581
How to help Orlov
What hope for Esprit? 582

NEWS

European space station 583
UN biotechnology
Recombinant DNA 584
Australian technology
Soviet Union 585
West German universities
La Palma's Newton
Remote sensing/mapping 586
Czechoslovak science
Nuclear waste 587
New man at zoo
Seabed geology
Unesco 588
US medical schools
Romanian casualties
Palaeontology 589
Israeli Arabs

CORRESPONDENCE

Human experiments/Anti-placebo/ Homer's drinking habits/etc. 590

NEWS AND VIEWS

What happens in the mantle?
John Maddox 591
An old illusion and a new theory of stereoscopic depth perception
John P Frisby 592
Membrane traffic and the problem of protein secretion
Miranda Robertson 594
The virus verified
Jon Darius 595
A quantum step towards artificial photosynthesis
J Barber 596
Onset of the ice age in the North Atlantic
Brian Funnell 597
Building acetylcholine receptors
Charles F Stevens
Evolution of the oaks
Peter D Moore 598

BOOK REVIEWS

Alan Turing: The Enigma *by A Hodges*
I J Good 663
Child's Talk: Learning to Use Language *by J Bruner*
John McShane 664
Intracellular Calcium *by A K Campbell*
P F Baker
Oncogenic Viruses, 3rd Edn *by L Gross*
Robin Weiss 665
Neogene Plankotonic Foraminifera: A Phylogenetic Atlas
by J P Kennett and M S Srinivasan
Gerta Keller 666
Earth's Earliest Biosphere
J W Schopf ed. A G Cairns-Smith
The Story of Astronomy in Edinburgh
by H A Brück David W Hughes 667
Koobi Fora: Researches into Geology, Palaeontology, and Human Origins, Vol.2 *J M Harris ed.*
Andrew Hill 668

ARTICLES

Acidic deposition and internal proton sources in acidification of soils and waters
N van Breemen, C T Driscoll & J Mulder 599

Expression of functional acetylcholine receptor from cloned cDNAs
M Mishina, T Kurosaki, T Tobimatsu, Y Morimoto, M Noda, T Yamamoto, M Terao, J Lindstrom, T Takahashi, M Kuno & S Numa 604

Two configurations of a channel-forming membrane protein
P N T Unwin & P D Ennis 609

LETTERS TO NATURE

Implications of 10^{16} eV γ rays from Cyg X-3
D Eichler & W T Vestrand 613

A simple non-markovian equation for a magnetoplasma
L C Woods 614

A possible mechanism for epeirogenic uplift
D McKenzie 616

Isotopic and geochemical evidence for the evolution of a cyclic unit in the Rhum intrusion
Z A Palacz 618

Oxygen isotope calibration of the onset of ice-rafting and history of glaciation in the North Atlantic region
N J Shackleton, J Backman, H Zimmerman, D V Kent, M A Hall, D G Roberts, D Schnitker, J G Baldauf, A Desprairies, R Homrighausen, P Huddlestun, J B Keene, A J Kaltenback, K A O Krumsiek, A C Morton, J W Murray & J Westberg-Smith 620

Seasonal fluctuations in deep-sea sediment community oxygen consumption: central and eastern North Pacific
K L Smith Jr & R J Baldwin 624

Phosphorus limitation of net production in a confined aquatic ecosystem
S V Smith & M J Atkinson 626

Mallomonadacean microfossils provide evidence of recent lake acidification
J P Smol, D F Charles & D R Whitehead 628

Photodriven charge separation in a carotenoporphyrin–quinone triad
T A Moore, D Gust, P Mathis, J-C Mialocq, C Chachaty, R V Bensasson, E J Land, D Doizi, P A Liddell, W R Lehman, G A Nemeth & A L Moore 630

Sensitivity for vertical retinal image differences
G Westheimer 632

Contents continued overleaf

Nature (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Annual Index (mailed in February). Annual subscription for USA and Canada US $230 (for subscription prices elsewhere, see next page). USA and Canadian orders only to: *Nature*, Subscription Dept PO Box 1018, Manasquan, New Jersey 08736, USA. All other orders to *Nature*, Brunel Road Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY10010 and additional mailing offices. US Postmaster send form for 3579 to: *Nature*, 15 East 26 Street, New York, NY 10010. Published in Japan by Macmillan Shuppan K.K., 5th Floor Eikow Building, 1-10-9 Hongo, Bunkyo-ku, Tokyo, Japan. © 1984 Macmillan Journals Ltd.

New ligands

- Affinity labelling reagent for oestrogen receptors
  [*ring*-^{3}H]Tamoxifen aziridine — TRK.788
- Calcium channel blocker
  (+)-[*methyl*-^{3}H]PN 200–110 — TRK.787
- Selective ligand for opiate receptor
  [*tyrosyl*-3,5(n)-^{3}H]Dynorphin (1-9) — TRK.795
- Muscarinic agonist
  [*N-methyl*-^{3}H]Oxotremorine M acetate — TRK.794
- Dopamine agonist
  [^{3}H]5,6-DPAT — TRK.792
- Selective α_2 antagonist
  [^{3}H]RX 781094* — TRK.799
- Exclusive adenosine agonist
  (–)-[^{125}I]HPIA — IM.153
- Receptor grade [^{125}I]insulin, human
  [^{125}I]-(A14)-monoiodinated human insulin — IM.166
  [^{125}I]-(B26)-monoiodinated human insulin — IM.167

*prepared by agreement with Reckitt and Colman plc.

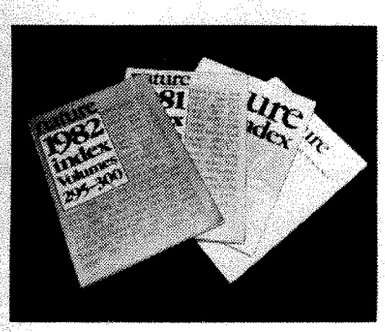
Amersham International plc
Amersham England HP7 9LL
telephone Little Chalfont (024 04) 4444
telex 83141 ACTIVA G

Amersham

Amersham Australia PTY Limited Sydney **Amersham Belgium SA/NV** Brussels **Amersham Buchler GmbH & Co. KG** Braunschweig W. Germany
Amersham Corporation Arlington Heights USA **Amersham Denmark ApS** Birkerød **Amersham France SA** Paris **Amersham Japan** Tokyo **Amersham Nederland BV** Utrecht

Circle No.50 on Reader Service Card.

Index



Comprehensive annual indexes of subject and author are available for all issues of **Nature** from 1971 to 1983.

Prices and address for orders are set out below.

Annual Subscription Prices

UK & Irish Republic		£98
USA & Canada		US$230
Australia & NZ	Airspeed	£150
Continental Europe	Airspeed	£120
India	Airspeed	£115
Japan	Airspeed	Y85000
Rest of World*	Surface	£120
Rest of World*	Airmail	£180

(not USA, Canada, Europe & Japan)

Orders (with remittance) to:

USA & Canada
Nature
Subscription Dept
PO Box 1018
Manasquan
NJ 08736
USA

UK & Rest of World
Nature
Circulation Dept
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to:
Japan Publications Trading Company Ltd
2-1 Sarugaku-cho 1-chome
Chiyoda-ku, Tokyo, Japan
Tel: 03-292-3755

Personal subscription rates
These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
15 East 26 Street
New York,
NY 10010, USA
Tel: (212) 689-5900

UK & Europe
Felicity Parker, Nature
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

Credit card orders only (in USA):
Call toll-free: (800) 824-7888 (Operator 246)
In California: (800) 852-7777 (Operator 246)

Back issues
UK, £2.50; USA & Canada, US$6.00 (surface), US$9.00 (air); Rest of World, £3.00 (surface), £4.00 (air)

Binders
Single Binders: UK, £4.50; Rest of World, $10.00
Set(s) of 3 Binders; UK, £12.00; Rest of World, $25.00

Annual indexes (1971–1982)
UK, £5.00 each; Rest of World, $10.00

Nature First Issue Facsimile
UK, 75p; Rest of World (surface), $1.50; (air) $2.00

Nature in microform
For Information:
University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

Interspecific chimaerism between sheep and goat
C B Fehilly, S M Willadsen & E M Tucker 634

Experimental chimaeras — removal of reproductive barrier between sheep and goat
S Meinecke-Tillmann & B Meinecke 637

An antigenic difference between cells forming early and late haematopoietic spleen colonies (CFU-S)
R A Harris, P M Hogarth, L J Wadeson, P Collins, I F C McKenzie & D G Penington 638

Glial heterogeneity may define the three-dimensional shape of mouse mesencephalic dopaminergic neurones
S Denis-Donini, J Glowinski & A Prochiantz 641

A dynorphinergic pathway of Leu-enkephalin production in rat substantia nigra
N Zamir, M Palkovits, E Weber, É Mezey & M J Brownstein 643

Dual role for *Dictyostelium* contact site B in phagocytosis and developmental size regulation
C M Chadwick, J E Ellison & D R Garrod 646

Induction of human vascular endothelial stress fibres by fluid shear stress
R-P Franke, M Gräfe, H Schnittler, D Seiffge, C Mittermayer & D Drenckhahn 648

Mapping by monoclonal antibody detection of glycosaminoglycans in connective tissues
J R Couchman, B Caterson, J E Christner & J R Baker 650

Is stepwise sarcomere shortening an artefact?
J D Altringham, R Bottinelli & J W Lacktis 653

Glycophorin is linked by band 4.1 protein to the human erythrocyte membrane skeleton
R A Anderson & R E Lovrien 655

Activation of the mouse cellular Harvey-*ras* gene in chemically induced benign skin papillomas
A Balmain, M Ramsden, G T Bowden & J Smith 658

MATTERS ARISING

Lunar magnetism
L L Hood, C P Sonett & L J Srnka
Reply: S K Runcorn 661

MISCELLANY

100 years ago 596
Erratum: A resetting of Phanerozoic community evolution L.M. Van Valen 660
Books received 669

NATURE CLASSIFIED

Professional appointments — Research posts — Studentships — Fellowships — Conferences — Courses — Seminars — Symposia: Back pages

Next week in *Nature*:

- What use are lie detectors?
- Rotation of Saturn's moon Hyperion
- New mine determination of Newton's gravitational constant
- New chemical oscillator
- Visualizing real and apparent movement
- Oestrogen receptors localized in target cell nucleus
- Evolutionary origin of eubacteria
- Calcium-activated potassium channels in secretion
- Simian virus genetic elements induce altered chromatin structures
- R.V. Jones reviews a new biography of Ernest Rutherford

GUIDE TO AUTHORS

Authors should be aware of the diversity of *Nature*'s readership and should strive to be as widely understood as possible.

Review articles should be accessible to the whole readership. Most are commissioned, but unsolicited reviews are welcome (in which case prior consultation with the office is desirable).

Scientific articles are research reports whose conclusions are of general interest or which represent substantial advances of understanding. The text should not exceed 3,000 words and six displayed items (figures plus tables). The article should include an abstract of about 50 words.

Letters to *Nature* are ordinarily 1,000 words long with no more than four displayed items. The first paragraph (not exceeding 150 words) should say what the letter is about, why the study it reports was undertaken and what the conclusions are.

Matters arising are brief comments (up to 500 words) on articles and letters recently published in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted.

Manuscripts may be submitted either to London or Washington (decisions being made only in London). Manuscripts should be typed (double spacing) on one side of the paper only. Three copies are required, each accompanied by copies of lettered artwork. No title should exceed 80 characters in length. Reference lists, figure legends, etc. should be on separate sheets, all of which should be numbered. Abbreviations, symbols, units, etc. should be identified on one copy of the manuscript at their first appearance.

References should appear sequentially indicated by superscripts in the text and should be abbreviated according to the *World List of Scientific Periodicals*, fourth edition (Butterworth 1963–65). The first and last page numbers of each reference should be cited. References to books should clearly indicate the publisher and the date and place of publication. Unpublished articles should not be formally referred to unless accepted or submitted for publication, but may be mentioned in the text.

Each piece of artwork should be clearly marked with the author's name and the figure number. Original artwork should be unlettered. Suggestions for cover illustrations are welcome. Original artwork (and one copy of the manuscript) will be returned when manuscripts cannot be published.

Requests for permission to reproduce material from Nature *should be accompanied by a self-addressed (and, in the case of the United Kingdom and United States, stamped) envelope.*

Announcing a new Elsevier journal...

Computers and Electronics in Agriculture

Editor-in-Chief: Professor Jerry Lambert
Dept of Agricultural Engineering
Clemson University
CLEMSON, S.C. 29631, U.S.A.

Aims: The aim of the journal is to provide an international forum for papers and notes on the application of computers and electronics to agriculture. Hardware and software aspects of computers are included. Electronics coverage includes instrumentation, data acquisition and control of any facet of agriculture. The term agriculture is interpreted broadly to include horticulture, forestry, veterinary medicine, crop and livestock production.

Subscription Information
1985: Volume 1 in 4 issues
Price: US$ 69.25 / Dfl. 180.00 excluding postage
Volume 1/1 is scheduled for late 1984

Submission of Articles
Manuscripts should be submitted, in triplicate, to the Editorial Secretariat, "Computers and Electronics in Agriculture", P.O. Box 330, 1000 AH Amsterdam, The Netherlands. Contributors from North America can submit papers directly to Professor Jerry Lambert.

COMPUTERS AND ELECTRONICS IN AGRICULTURE HAS NO PAGE CHARGES

For full details or a free sample copy please contact

ELSEVIER SCIENCE PUBLISHERS
Attn: Jef Lambach
P.O. Box 330
1000 AH Amsterdam
The Netherlands

Circle No.11 on Reader Service Card.

EDITORIAL OFFICES

London Office
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024

Washington Office
1134 National Press Building, DC 20045
Telephone: (202) 737-2355

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff: Alun Anderson, Tim Beardsley, Philip Campbell, Isobel Collins, Peter Gambles, Melanie Kee, Tim Lincoln, Naomi Molson, Sara Nash, Geoffrey North, Peta Pickering, Miranda Robertson, Robert Walgate, Charles Wenz, Nigel Williams, Guil Winchester

Washington Editor: Peter David
Washington Correspondent: Stephen Budiansky

Art and Design: Joan Godwin, Joel Chernin, Theresa George, Paul Norris, Marie Xeridat

Administration: Mary Sheehan, Jacqueline Cole, Ruth Cutts, Joanna Czechowska, Ruth Goldenberg, Sharon Isaacs and Penny Walker (Washington)

Publisher: Elizabeth Hughes

Marketing Director: Ray Barker

Promotion Manager: Felicity Parker

International Advertising Manager: Andy Sutherland
Features Advertising Manager: Marion Delaney

Group Classified Advertising Manager: John Barnes — Telephone: (01) 240 1101

Nature New York Office
15 East 26 Street, New York, NY 10010
Telephone: (212) 689-5900 Telex: 668497

General Manager: Gary Rekstad

American Advertising Manager: Henry Dale

Classified Advertising Manager: Miss Gene Fein

Circulation Manager: Mirta Soto

Canadian Display and Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1—(416) 690-2423

Display Advertising Representatives

New York: Nature Office
Chicago: Didier & Broderick, Inc.—(312) 498-4520
San Francisco: Jobson/Jordan/Harrison/Schulz, Inc.—(415) 392-6794
Los Angeles & Pasadena: Jobson/Jordan/Harrison/Schulz, Inc.—(213) 796-9200
Philadelphia: Daniel Adams Associates, Inc.—(215) 353-6191
Boston: CEL Associates—(617) 848-9306
Fort Lauderdale: Brinker & Brinker—(305) 771-0064
New Jersey: Mary Grunmeier—(201) 572 9018
Dalton, Pennsylvania: Donald E. Northup Associates—(717) 563 2572
West Germany and Austria: Franz Schrecklinger TMW Top Media Werbegesellschaft GmbH Frankfurt—(611) 726046
Holland and Belgium: Adele Struyck G. Arnold Teesing BV Amsterdam—(020) 2636 15
Switzerland: Werner Stähli Agentur Iff AG Schaffhausen—(053) 45821
Scandinavia: Andrew Karnig Andrew Karnig & Associates AB Stockholm—(08) 51 68 70
Japan: Mashy Yoshikawa Orient Echo Inc Tokyo—541-4923
Australia: Gus Bartel & Associates PO Box 1755, Southport, Queensland 4215 (075) 466 577

Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office

©1984 Macmillan Journals Ltd
Vol.307 No. 5952 16 February 1984

nature

No end to nuclear decay?

The stagnation of the nuclear power industry in the United States should be an incentive for radical thinking rather than an excuse for despair.

THE predicament of the nuclear power industry in the United States will get worse before it gets better. Last July, nuclear power provided the occasion for the biggest municipal default in history, when the Washington Public Power Supply System failed to meet its payments on bonds worth more than $2,000 million. More financial embarrassments are in store. It appears to be only a matter of time, for example, before the Long Island Lighting Company (LILCO) concedes that its Shoreham plant, which is already a decade behind schedule and seems likely to cost 15 times more than intended, will never be allowed to operate. LILCO's chief executive, Dr William Catacosinos, admitted for the first time last week that it might be simpler for the utility to abandon Shoreham than to continue its enervating struggle with the local municipality, Suffolk County. Suffolk, it will be remembered, has done its utmost to thwart LILCO's plans for Long Island by refusing to participate in the emergency evacuation planning mandated by the Nuclear Regulatory Commission (NRC).

Shoreham's plight is a depressingly familiar (but exceptionally acute) compound of engineering, economic and political failures, failures that have contributed to the fact that there has not been a single new order for a nuclear reactor in the United States since 1978. The nuclear industry, supported by the Reagan Administration, lays much of the blame at the door of NRC. Less cumbersome procedures, fewer opportunities for public intervention and a limit on the number of design "backfits" ordered by NRC could, it is suggested, transform the economics of reactor construction. But there is little point in looking for relief in that direction. Congress, backed by public opinion (half of the public is opposed to building nuclear plants, a third supports it), is in no mood to reduce the powers of NRC, a body which is criticized for too cosy a relationship with the industry at least as often as it is accused of over-zealous enforcement of its rules. There may indeed be a case for strengthening NRC procedures in order to revive public confidence in the safety of nuclear energy.

It is in any case far from clear that regulatory reform by itself would do much to alter the predicament of the industry. The two main causes of that predicament are the abrupt fall in the rate of growth in electricity demand since 1973 and the simultaneous increase in the costs of nuclear reactor construction. Between 1960 and 1972, electric utilities took for granted annual growth rates of 7 per cent, and it is on those assumptions that existing nuclear power construction was predicted. In practice, growth has averaged only 2.5 per cent a year since 1973. Moreover, the costs of building nuclear plants doubled in constant dollars during the 1970s and are expected to increase by another 80 per cent for plants now under construction. Some of this increase can be ascribed to new regulatory requirements but not much, according to a new study, *Nuclear Power in an Age of Uncertainty*, published by the Office of Technology Assessment (OTA) last week. OTA points out that of the group of plants now being built, the most expensive is expected to cost more than four times as much as the least expensive. The variation has something to do with regional labour costs and differences in weather but is primarily a result of differences in the competence and experience of management.

So it is to its own management, rather than to external agencies, that the nuclear industry should look for a way out of the impasse in which it finds itself. Unfortunately, industry managers show little enthusiasm for tackling any of the major structural problems that have to be solved. There is a temptation instead to ride out the hiatus in orders for new plants in the hope that demand for nuclear power will revive when the demand for electricity revives. Meanwhile there is a reasonable living to be made by servicing existing plants and, from time to time, selling reactors overseas.

That strategy might work, if the hoped-for recovery were to come within five years. But if the hiatus lasts a decade or more, the prospects for reviving the industry are much less promising. According to OTA, the lack of orders for new plants is already creating shortages of spare parts and personnel. Enrolment for nuclear engineering degrees has declined since the mid-1970s, and graduate numbers will barely be enough to fill the anticipated need for 6,000 engineers to operate plants by 1991. Instead of alternately sitting on its hands and wringing them, the industry should be acting now to ensure that the hiatus ends sooner rather than later.

What can be done? For a start, the industry could stop talking about standardized designs and actually begin to standardize them. Without some degree of standardization, as in France, construction of nuclear reactors is unlikely to be able to compete with coal-fired plants. Standardization would require ruthless surgery to reduce the proliferation of vendors and designers, but that would be better than seeing the whole industry gradually collapse under the weight of its own diversity. Second, the industry could begin to address some of the concerns of its critics, by taking more visible measures to ensure competent management and to enhance safety standards. Why not look seriously at alternatives to the much-criticized light-water reactors — at high-temperature gas-cooled reactors or the Swedish PIUS (process inherent ultimately safe) reactors, in which the core and other critical reactor components are submerged in a pool of water? □

How to help Orlov

Some of the schemes being considered in the West on behalf of Yuri Orlov will not work.

SEVEN years ago, the Soviet accelerator physicist, Yuri Orlov, was arrested in Moscow and charged with making anti-Soviet propaganda. At his trial, Orlov was sentenced to seven years in gaol followed by five years of exile. During the first part of his spell in prison, Orlov seems to have been a thorn in the flesh of the prison authorities, protesting at censorship of mail and the molestation of prisoners by their guards. During this period, he seems also to have kept his hand in with his physics. More recently Orlov seems to have been ill, perhaps gravely so. Orlov's case, like Sakharov's, has become to many in the West a test of Soviet attitudes towards professional people who happen to disagree with government policy. Excitement has come to a head (see page 585) now that the more rigorous part of Orlov's sentence is at an end. Will he be released into exile, perhaps abroad, or cynically be kept in gaol?

Orlov was the chief organizer of the group active in Moscow in the mid-1970s in the monitoring within the Soviet Union of the Helsinki agreements. This formless group of undertakings commits its signatories, among other things, to permit the free

0028-0836/84/070581-01$01.00

© 1984 Macmillan Journals Ltd

flow of ideas and even people between themselves. Orlov and company have plenty to complain about.

There have been several well-publicized international shouting matches, most recently at Madrid, but the agreements have also led to the Stockholm talks on European security, now under way, which may yet make ten years of diplomacy seem worthwhile. In any case, Orlov was not imprisoned for having discovered that the Helsinki agreements were being illiberally interpreted (more simply, ignored) by Soviet authorities, but for having said so, and for having distributed his complaints abroad.

By that test, Orlov is unquestionably a criminal. Did not a Soviet court, whose judicial independence is guaranteed by the Soviet constitution, come to that conclusion? Is it not, in any case, well known that the Soviet authorities, like their tsarist predecessors, have always taken a serious view of what seems to be disloyalty? With the obligations of individuals to the state counting for more than the state's obligation towards its people as individuals, Orlov must have known that he was asking for trouble. Orlov's activities had much in common with those of earlier trouble-makers. Medvedev, Solzhenitsyn and the like find some weakness in the system, such as the constitutional right that private correspondence should not be interfered with, exploit it to the full and, provided they keep within the law, sooner or later find themselves at an airport with an exit visa in their pocket. Orlov must have overstepped the line.

This is how Orlov's case seems to many honest Soviet citizens. People elsewhere distressed at Orlov's plight must take that disconcerting circumstance into their calculations. Two conclusions follow. First, on compassionate grounds, anything that will help to release Orlov from the plight in which he finds himself is to be welcomed. If the petition signed by 2,600 French physicists, or the proposal being canvassed among the accelerator people at Geneva that Orlov should be offered a job at the European Organization for Nuclear Research (CERN), will help towards that end, all well and good. But the chances are not high. That is why this sad occasion should be a reminder to complainants in the West that their quarrel with the Soviet authorities goes deeper than the circumstances of Orlov's imprisonment and is tantamount to a protest against Soviet law and the manner in which it is interpreted. But sovereign governments do not often change their legislation at the behest of people who live elsewhere. So, for the long haul, the complainants must find subtle ways of making their opinions felt. The proposal, put forward last week, that there should be an Orlov Prize to honour people like him, will not help and may even hinder. Breaking off such personal scientific collaborations with Soviet scientists as have survived the past few years would be similarly nugatory. It will be better to keep the links going and to use them as a means of convincing collaborators that there are good reasons why the law needs changing. Orlov's objective, after all, is that his own brave act should ensure that there is less need of an army of successors. □

What hope for Esprit?

The European plan for information technology research is likely to be launched too late.

THE European Communities are more conspicuously than usual travelling in cloud-cuckoo land. Last week, the Commission, the Communities' executive authority, put the finishing touches to its plan for supporting long-term research in information technology by means of an intricate system of research contracts to be let by Brussels to companies and other research organizations which are themselves willing to meet half the cost.

The European strategic programme of research and development in information technology (known as Esprit) has recently been nurtured by Etienne Davignon, the Commissioner for Industry in Brussels, who has been shuttling about Europe seeking promises of support. On Monday, Davignon spent 50 minutes at 10 Downing Street with the British Prime Minister; the hope now is that if British objections can be overcome, the project will be approved at a meeting of ministers on 28 February, whereupon the Commission will be free to spend roughly US$400 million sterling of its own money provided that its partners commit an equal sum over the succeeding five years. The drawback, for the time being set aside in Brussels, is that the Commission is certain on present form to run out of funds between June and September. In short, the Commission has no money for projects like Esprit, and will not know whether it will be in funds again until the meeting of the heads of European governments fixed for 20 and 21 March. On recent form, the chances that the Communities' deep-seated differences can be resolved at a single meeting, and before the last minute has arrived (which will not be until June), are negligible.

So will Esprit be another European dream impaled on the failure of European governments to agree what should be done with their common enterprise? If it is, it will not be the first casualty of this kind. In the 1950s, Euratom, then legally a community in its own right, embarked on an ambitious programme for the development of nuclear reactors whose hallmark was that their design had nothing in common with those of the reactors being developed in member states. Member governments would not countenance the threat of competition between their own national nuclear energy establishments and the common enterprise, but the result was what everybody would have been entitled to predict: Euratom's projects failed (as did many of those mounted nationally). The idea that there might be a European project to strengthen the technology of the computer and communications industry was the most prominent of the proposals canvassed in the late 1960s by M. Pierre Aigrain, then President Pompidou's chief scientist. That came to grief for two reasons — the resistance of European telecommunications monopolies to the suggestion that their own research was insufficient and the lack of a plan to suggest how a common research programme supported by established companies or nationalized industries could nevertheless yield benefits that would be widely shared. Aigrain's project came too soon.

The Commission's latest attempt to breathe life into European information technology has obviously been designed to avoid these pitfalls, but has weaknesses of its own. Participation in the programme will be voluntary, but collaborating companies or consortia will have to commit resources to projects that the Commission supports. This will ensure a degree of seriousness. There are also rules about the availability of the results of research and development projects although, inevitably and even equitably, the partners in the research projects will have an advantage over others in their exploitation deriving from familiarity with the work. Perhaps the most encouraging feature of the Esprit programme is that it seems already to have fostered a degree of collaboration between European companies hitherto not much used to talking to each other. But the question remains of whether Esprit is what Europe most needs.

That information technology offers great opportunities for European industry, and that technology of the kind now being applied is likely to seem, a few decades from now, a rudimentary tool, are nowhere denied. Most industrial organizations recognize which way the wind is blowing. But the impediments to faster development consist not so much of the lack of development projects as of the shortage of people able to turn bright ideas into working machines decisively and quickly, the lack of technical people able to service the machines actually sold to customers (which demands more skill than that required to order a package of software from a list) and too little support for really long-term projects, those that might lead to optical computers for example (see *Nature* 9 February, p.494). For all the delicacy with which Esprit has been conceived, the programme will help towards these ends only incidentally, by training people. Might not the same result have been obtained by other means? (The same criticism can be made of the British Government's Alvey programme.) The risk in what the Commission is proposing is that a great deal of money (which does not exist) may be spent ineffectually. The danger is that if the Commission fails with this huge project, it may not be given another chance. □

0028-0836/84/070582-01$01.00

© 1984 Macmillan Journals Ltd

European community

French visions of Europe and space

At last, a French phoenix has arisen from the ashes of the European summit at Athens last December. In Athens, President François Mitterrand of France apparently put paid to all hopes of negotiation with Britain over the future of the European Community. In The Hague last week, he explained his action: not directly, but by presenting a technological vision of a new Europe, in which the old conflicts would be something of a past age.

One element of this future would be a European space station, as a military observation post in the first place, but one that could also carry laser and other star wars weaponry for the defence of Europe.

Meanwhile, M. Mitterrand proposed only partial solutions to Europe's overriding political problem (the European budget), solutions which are unlikely to find favour with the crusading British Prime Minister, Mrs Margaret Thatcher.

Surprisingly, one of M. Mitterrand's most favoured ministers, M. Laurent Fabius (industry and research) seemed last week to have been caught a little un-



prepared by his president's commitment to a space station. Speaking at a meeting of European Parliament socialists in Toulouse, in south-west France, Fabius described the station as having commercial, scientific and strategic purposes — with, it was implied, the "strategic" element firmly relegated to third place. The French space agency CNES had been studying the possibility of such a station for a few months, said an aide to M. Fabius, although the CNES director-general had earlier denied its.

Certainly, President Mitterrand has been taking an increasingly independent approach to European policy lately, so much so that French officials have occasionally been seen to be announcing conflicting policies. This apparent chaos, however, is probably the consequence of Mitterrand's commitment to a vision which becomes more dazzling — and perhaps more blinding — the worse becomes France's international and internal position. (Internally, Mitterrand is facing rioting in factories against his restructuring policies, and a probable rout at the European Parliament polls in the summer.)

Putting it boldly, the Mitterrand vision is this: the European Community is out of date — and so are Europe's defence arrangements with the United States. So why not try for something completely new?

The European Community, for example, is based on post-war treaties which saw iron, steel, coal and nuclear as the key economic technologies. Now the key technologies are electronics, informatics, aerospace and biotechnology. The Community must be based on these, says M. Mitterrand, echoed by Fabius at Toulouse.

Equally, M. Mitterrand is disturbed by President Reagan's attitude to Europe, both technological and military, and — with France outside the North Atlantic Treaty Organization — he feels freer than most in Europe to propose something approaching an independent nuclear defence: hence the European space station.

Here, however, M. Mitterrand mixes military and industrial motives. While CNES has not admitted to a military space station study, it has since 1981 been studying a non-military one (dubbed Solaris), along with a recoverable low-orbit injector (Hermes) looking much like the space shuttle. Clearly, the object was to give Europe the possibility of recovery, repair, refuelling, construction and other options in space that the shuttle gives the United States but that Ariane has not given Europe (Ariane 5 — already on the drawing board — could launch a future Hermes).

In M. Mitterrand's mind at The Hague, these essentially commercial questions of Europe's industrial place in space in the 1990s were being mixed with other issues, but his overall dream was clearly of a brave new European Community based on brave new ideas.

Unfortunately, neither Mitterrand nor Fabius last week had much to say about mundane things such as the price of milk and the beef mountain, which may cause the French vision to become a mirage.

Robert Walgate

UN biotechnology

Let a hundred labs bloom

New Delhi

The contentious issue of the location of the International Centre for Genetic Engineering and Biotechnology for developing countries, sponsored by the United Nations Industrial Development Organization (UNIDO), seems to have been resolved. Although the original intention was to set up the institute in a developing country, the preparatory committee for the centre has now recommended that it is split into two components to be established in New Delhi, India, and Trieste, Italy.

The committee, meeting in Vienna at the end of January, suggested that the New Delhi component should concentrate on research in agriculture and human and animal health and that the Trieste laboratory should emphasize industrial microbiology and energy research. The offers from the two countries have been found to be most attractive.

A high-level meeting of the national representatives on 3 April will in all probability approve this decision, reached by the preparatory committee of 27 member countries. The committee was set up after the abortive Madrid meeting in September last year failed to decide on a location, following which more than one centre seemed a necessary compromise.

The decision reflects a major victory for India in the teeth of opposition from the UNIDO scientific committee which had favoured siting the centre in Belgium, Thailand or Italy. The committee said that these countries had sufficient merits to offer good prospects for the foundation of the international centre. India, like Cuba and Pakistan, had been ruled out on the grounds that its background in basic science is insufficient and that it would be difficult to attract good scientists.

This argument has now been rejected. What ultimately clinched the issue in favour of India was the specific outline of the offer involving some US $19 million. The offer includes land, buildings and laboratories; technical and auxiliary staff; permanent equipment and $5 million to cover recurring costs for running the centre for five years.

The government has promised that the New Delhi centre will have "high standards of scientific and laboratory facilities dedicated to solving problems relevant to developing countries in a highly focused and multidisciplinary approach". It will also provide training to scientific and technical personnel from developing countries.

The preparatory committee also tries not to displease the other nations that had also staked their claim to host the international centre. They can set up their own units affiliated with the New Delhi and Trieste centres and funds for them will also be provided through international sources. These affiliated units can be converted into centres by the board of governors of the new centre following a review after three years of operation by the New Delhi and Trieste Laboratories.

Sunil Saraf

© 1984 Macmillan Journals Ltd

Recombinant DNA

Watch on human experiments

Washington

THE Recombinant DNA Advisory Committee (RAC) in the United States last week reaffirmed its intention to review any experiments involving human subjects or the release of recombinant organisms into the environment. Under rule changes adopted overwhelmingly by the committee, any proposal to transfer recombinant DNA into human subjects would have to be cleared by the full RAC.

Although the rules are legally binding only upon recipients of federal research money, industry and all government agencies have agreed voluntarily to follow the RAC procedures.

RAC's decision formally to require a review of human genetic engineering experiments was prompted largely by the demise last year of the President's Commission on Bioethics and the resulting concern that no group was monitoring developments in this field. RAC also decided to establish a nine-member working group, made up of scientists, lawyers and ethicists, to look at any human genetic engineering proposals. This move parallels a legislative proposal by Representative Albert Gore (Democrat, Tennessee) that would create a human genetic engineering commission with similarly broad representation. That proposal passed the House last year, but was never enacted into law. RAC itself is dominated by scientists and physicians.

In a related discussion of the scope of RAC's oversight, RAC appeared committed to continuing to review deliberate environmental release experiments and industrial activities. The Food and Drug Administration and the Department of Agriculture offered strong support for RAC's role in these areas; the Environmental Protection Agency (EPA), which has announced its intention eventually to regulate deliberate release of recombinant organisms under the Toxic Substances Control Act, said at last week's RAC meeting that it supported RAC's handling these areas at least until EPA's regulations are issued. A report released last week by the staff of Representative Gore's subcommittee questioned whether RAC has the expertise or the administrative capacity to handle a large flow of proposals from industry.

Anti-genetic-engineering activist Jeremy Rifkin was much in evidence at last week's meeting, notably for obtaining a court order barring RAC from holding a closed session to discuss a deliberate-release experiment proposed by Advanced Genetic Systems, Inc (AGS). The company is the sponsor of the planned release of frost-resistant bacteria by Steven Lindow of the University of California, Berkeley, currently tied up in other litigation initiated by Rifkin. AGS decided to submit to RAC its own proposal to conduct a similar experiment. In accordance with standard procedures for dealing with proprietary information, RAC announced a closed session to review the proposal.

At the behest of several RAC members, a decision was later made to open as much of the discussion as possible. Rifkin argued in his lawsuit that RAC did not have sufficient grounds to close any of its discussions of the proposal and that the subsequent decision to open part of it was not announced 30 days in advance in the Federal Register, as required for public meetings. A US Court of Appeals panel, acting on the technicalities of Rifkin's complaint rather than its substance, ordered RAC not to hold its discussion until it had published proper advance notice and had it explained why any portion of the discussion should be closed to the public.

Rifkin also appeared at the meeting to protest against a proposal from researchers at Uniformed Services University of the Health Sciences to lower the containment required for a previously-approved experiment aimed at cloning the gene of a bacterial toxin responsible for dysentery. Rifkin, claiming the support of several prominent arms-control experts, said the experiments should not commence until an "Arms Control Impact Statement" was prepared on the research. The Arms Control and Disarmament Agency is charged by Congress with preparing such a statement on any government research with potential military applications.

Rifkin said these experiments could be used to create biological warfare agents, and that RAC members "would be personally liable under international law and the principles enunciated at Nuremberg in aiding the commission of crimes against humanity" should the work lead to biological weapon development.

Paul Warnke, former SALT negotiator and the first name on the list of supporters claimed by Rifkin, said in an interview that he had not endorsed Rifkin's remarks, but had only told Rifkin in a telephone conversation that he believed an impact statement would be important in judging the implications of the research. He said that he neither supported nor opposed the actual research.

Proponents of the experiment, which was approved by RAC last year at P4 containment, said the research was vital to the development of a vaccine against the leading cause of dysentery. RAC approved the request to lower the containment requirement to P2 by a 9 to 5 vote with 4 abstentions. The director of the National Institute of Allergy and Infectious Diseases, to whom RAC reports, has generally declined to accept recommendations made on split votes such as this one.

Stephen Budiansky

Australian technology

Tax perks the stimulus

Canberra

AUSTRALIAN investors and technologists are likely to benefit from the Management and Investment Companies Act that came into force at the beginning of this month. The act embodies a tax-deductible venture capital scheme designed to bring together investors, innovative small businesses and management and investment companies (MICs). A licensing board, appointed by Mr Barry Jones, Minister for Science and Technology, is to select about eight MICs from the large number of expected applicants and to approve the amount of tax-free capital each can raise.

The MICs will choose several small, local high-technology businesses which they believe can sustain an annual sales growth rate higher than 20 per cent over the next three years. They will supply the businesses with long-term equity, loan capital and entrepreneurial advice, especially in marketing and finance. The source of the venture capital supply to the MICs will be investors who may claim 100 per cent tax deduction on the capital subscribed in the year of the investment.

Like Britain, Australia has done poorly in bringing technological innovation to commercial success: the technologists blame the businessman's conservatism and financiers blame the inventor's lack of management skill. The MIC scheme is based on recommendations of the Espie committee, set up by the previous government to investigate ways of financing high-technology enterprise in Australia. The committee perceived a gap at the high-risk, high-return end of the Australian capital market. The scheme may go some way towards plugging it. As if to fend off the possible jibe that a business with sales expanding at more than 20 per cent a year needs no special assistance and that the gap is merely the embodiment of Australian lack of self-confidence, Mr Jones recently echoed the Espie committee's remark that it "knew of no country which had succeeded in establishing a climate for investment in high technology without the government taking positive action".

The first MIC licences are expected to be granted in April. Over the first five-year period of phased capital injections into the ventures, $A200 million will be licensed for subscription to the MICs, at an estimated tax revenue loss of $A100 million.

On 24 January, the Treasury altered the proposed tax-incentive schedule for investors to promote long-term "patient" equity investment in the MICs and to inhibit abuse of the scheme. Investment for less than two years now attracts no net tax deduction; for the full, 100 per cent deduction, a four-year investment is necessary.

Jeffrey Sellar

0028-0836/84/070584-01$01.00

© 1984 Macmillan Journals Ltd

Soviet Union

Orlov's fate in balance

Dr Yurii Orlov, the Moscow physicist and human rights campaigner, last week completed a seven-year prison sentence for slandering the Soviet system. Ironically, the expiry of his term (on 10 February), coincided with the announcement of the death of the Soviet leader, Mr Yurii Andropov, who at the time of Orlov's trial was head of the secret police (KGB). Dr Orlov is now due to serve a further five years in Siberian exile. Last week, however, his supporters in London and Geneva announced proposals aimed at ameliorating the remainder of his sentence.

At his trial, Dr Orlov openly admitted charges of circulating and distributing to the Western press documents alleging breaches of the Helsinki accords and international human rights conventions within the Soviet Union. The basis of the prosecution case was that these allegations were false. However, Mr John Macdonald, who failed in his attempts to travel to Moscow to serve as Orlov's defence lawyer, still maintains that they were true, and last week, at a press conference held at the Institute of Physics in London, announced his intention of petitioning the Soviet courts to reopen the case on the grounds that much pertinent defence evidence was never considered. Such a petition has little chance of succeeding because Mr Macdonald's proposed witnesses are either citizens of Western countries or else recent emigrants from the Soviet Union — people whose motives would be suspect from the Soviet point of view.

A more realistic proposal is a petition that Dr Orlov's "internal exile" be commuted into "external exile", that is, that he be sent abroad. One suggestion is that he could be offered a job at the European Organisation for Nuclear Research (CERN) with the LEP (large electron-positron) storage ring project. The governments of the thirteen member-states of CERN have been unofficially canvassed, and most of the replies so far received have been cautiously supportive. CERN has, incidentally, a long-term cooperation agreement with the Soviet Union which was extended six months ago, and which includes work on LEP.

As to Orlov's scientific fitness for such a job, this was beyond dispute before his imprisonment, and it is known that he has tried to keep up his scientific work while in the prison camp, although, after a few early letters (an extract from one was reproduced in facsimile in *Nature* **277**, 591; 1979) the camp censors clamped down on anything resembling a scientific equation for fear it could be a coded message. If he is, indeed, sent to Siberia, Mr Macdonald has suggested that scientists from CERN should apply for visas to visit him — and if refused should consider some form of "non-cooperation" with the Soviet Union. CERN has a vigorous "Orlov Committee" whose activities have included defence of scientists in Uruguay, Turkey, Morocco and Poland as well as the Soviet Union, mustering on occasion over 600 signatures out of a scientific workforce of 3,500. This committee supported Mr Macdonald's proposal but stressed that each individual must decide for himself the "appropriate" response to a refusal. (Ethics apart, it would make no sense for one solitary member of a large team to opt out of a joint project.)

Finally, Mr Macdonald suggested the establishment of a $50,000 annual "Orlov prize" for the defence of human rights, for which he would approach the Rockefeller Foundation in the United States for funds. The CERN Orlov Committee again approved the idea in principle but suggested that it should be funded either by a consortium of European universities or by neutral governments such as Sweden and Switzerland, since the backing of these bodies would disarm possible Soviet criticism that the prize was simply a capitalist propaganda ploy.

At the time of writing it is uncertain whether Dr Orlov has been released from prison. Soviet legislation provides for an extension of sentence if prison rules are contravened and Dr Orlov has already lost visiting and correspondence rights for alleged contraventions. **Vera Rich**

West German universities

The price of protest

Bublingen, West Germany

A protest in West Berlin last week by 90 university lecturers against the siting of Cruise and Pershing missiles in West Germany, in defiance of instructions from the presidents of the Free University and the Technical University, was echoed at a conference here organized by the anti-*Berufsverbot* campaign. *Berufsverbot* — the exclusion from employment in the government service of those whose political views are deemed to present a threat to the state — has been practised in West Germany since the early 1970s.

The procedure is deemed by many constitutional lawyers to be contrary to the guarantees in the West German constitution of civil liberties and freedom of conscience. The practice was authorized by a special decree of the federal government, and has never gone through the parliamentary law-making procedure. Its implementation varies in the different *Länder*, and in Hesse, where the Greens hold the deciding vote in a hung parliament, they are pressing for the practice to be dropped altogether.

The new development since the last anti-*Berufsverbot* conference in Hannover in 1982, the campaigners say, is that restrictive measures have been increasingly employed against peace campaigners. There were complaints that the practice of *Berufsverbot* has been supplemented by restrictions on demonstrations and penalties for those taking part on the grounds of public order.

Surprisingly, and in contrast with previous conferences, there was little to report this year from the university sector. University lecturers and schoolteachers, being paid by the state, are considered to be "public servants". One view is that all lecturers at risk have already been sacked, while new graduates with doubtful political views are unable to gain university employment.

The only striking new *Berufsverbot* case affecting the universities reported at Bublingen was that of Hans Joachim Wunderlich, a mathematician who was dismissed last year from the Karlsruhe University Computer Centre on the grounds that he constituted a security risk, since the computer was also leased for defence-related industrial research. So far, university staff taking part in peace activities have not been penalized. This contrasts sharply with school teachers, many of whom are now facing disciplinary proceedings for taking part in the "five-minute strike" for peace organized by the Trade Union Federation (DGB) on 5 October 1983, or for allegedly "over-emphasizing" the theme of peace in school projects and concerts.

The universities have not been directly affected by the "Strike for Peace" because it took place on a Saturday (in West Germany a working day for schools but not for universities). The Berlin protest may be different. **Vera Rich**

La Palma's Newton



The Whirlpool Nebula (M51) as seen by the United Kingdom's 2.5-m Isaac Newton Telescope, newly erected at the La Palma Observatory in the Canary Islands. The official "First Light" was received by the telescope on 13 February.

0028-0836/84/070585-01$01.00

© 1984 Macmillan Journals Ltd

Remote sensing/mapping

Call for UK investment

THE remote sensing market is an essential area for UK involvement, according to a report on *Remote Sensing and Digital Mapping* (HMSO, £7) produced last week by the House of Lords Select Committee on Science and Technology. And while the government is asked to increase investment in selected areas, most of the report's 52 recommendations concern changes in the infrastructure of the remote sensing and mapping communities. In particular, there is a call for greater effort in the handling and intepretation of digital data and the development of a network of linked computers in key centres.

The committee highlights the market potential of remote sensing, especially the current investment in the United States and France in satellite imaging systems. A major recommendation is that the National Remote Sensing Centre at the Royal Aircraft Establishment, Farnborough, should be given more staff and become the central "executive" node of a national computer network — a role similar to that of the Rutherford Appleton Laboratory in the astronomers' 'Starlink' network.

The aim would be to enhance the general accessibility of remote sensing data. The committee emphasizes that a significant challenge for the future will come not so much from advances in satellite instrumentation (although increased spectral resolution and radar sensivitity are two key areas of research) but through the sheer volume of data that such technical advances will produce. Two areas of strength, therefore, on which the United Kingdom should build are remote sensing radar and software development.

On satellite vehicles themselves, the committee advocates enthusiastic participation in European Space Agency projects such as ERS-1 — a remote sensing satellite due for launch in 1986 — but also bilateral collaborations, particularly with the United States and Canada.

Another important recommendation is that there should be a formal link between the research councils in the form of a joint committee for basic research. This is likely to be welcomed by those working on the proposed Satellite Radar Altimetry project (see *Nature* **306**, 108; 1983) now under consideration by the Natural Environment Research Council and the Science and Engineering Research Council. The project is not only costly (requiring about £18 million over ten years) but also falls within both councils' areas of responsibility, and, as a result, has encountered difficulties. Yet its supporters argue that it would incorporate the development of just the sort of expertise in instrumentation and data-handling that the House of Lords committee seeks.

Philip Campbell

● The report also concludes that the Ordnance Survey should increase its qualified research and development staff if it is to maintain its leading role among British mapping agencies and that the survey's digitization programme should be speeded up.

These conclusions will be as music to the ears of Ordinance Survey staff, who have been facing an uncertain future. Formally, the survey is a government department financed on a vote from central funds. A plan last year to re-establish it as a "trading fund" met with a chorus of protest from a variety of Ordnance Survey users, and was defeated in the House of Lords. Last month Mr Patrick Jenkin, Secretary of State for the Environment, announced that the survey will remain on voted funds but will be required to work to clearly defined financial targets.

The digital mapping programme is very much in its infancy but the committee believes — and some at the survey are ready to agree — that demand for digital maps is likely to increase because they can be displayed or printed very rapidly, to a variable scale and with user-defined edges. Moreover, particular features can be emphasized, or omitted for clarity, and digital maps are easily updated.

The committee wants a reversal of the recent "insensitively applied" manpower cuts of almost 25 per cent in five years. As a second best, even more of the digitizing work should be contracted out than at present, to make savings which would then pay for extra staff. In this way, the Ordnance Survey could become a national centre for digital mapping incorporating a development team headed by a chief scientist.

But the House of Lords committee does see some difficulties on the horizon. Perhaps the most important is that of copyright. For many years, some small societies, such as the Chiltern Society, have been selling their own annotated versions of Ordnance Survey maps. But, in keeping with its new business-like image, the survey will not allow the sale of such competing maps in areas where it has produced an adequately detailed series of its own. With digital maps the problems may be much more difficult: the Ordnance Survey supplies base data from which any number of maps may be produced and which may be continually updated. Their Lordships decline to offer a solution to the problem.

Tim Beardsley

Correction

SIR Peter Hirsch's share of the 1983–84 Wolf Foundation prize in physics was for his contributions to the utilization of the transmission electron microscope and not the scanning electron microscope as claimed in *Nature* 2 February, p.407. □

Czechoslovak science

Large gap to bridge

PLANNERS in Czechoslovakia must reject slogans about the "omnipotence" of science and technology, since these can foster "dangerous illusions", deputy federal premier Jaromir Obzina warned last month. He was addressing the first meeting of the new State Commission for Scientific, Technical and Industrial Development, set up to close the gap between research and development on the one hand and the practical implementation of results on the other.

Before the establishment of the Czechoslovak commission, there had been considerable discussion in the press about the reasons for the gap. The general opinion was that there is no serious lack of funds for research — indeed, some allocations are not taken up or spent in full. Instead, the main cause appears to lie in the personalities of those responsible for implementing the results, a view reiterated by Mr Obzina in his reference before the comission to "insufficient coordination" between research and production and to "serious shortcomings" in management.

The purpose of the commission, Mr Obzina said, is to reveal the real causes of the shortcomings, and to deal with real problems. Energy should not be wasted on theoretical "pseudo-problems" and discussions, "however brilliant and witty they might be". But the task, he implied, is likely to be a long haul. The process of "socialist industrialization" may be compared with collectivization in agriculture — "a programme of persistent work for a number of decades".

He warned the commission against associating itself with "naive people" who "today enthusiastically applaud scientific and technical progress", but whose personalities lack "persistence, diligence, perseverance and selflessness". Such people, he said, will be all too likely to turn against science when they find that "no cheap results have fallen into their laps".

Such remarks must, of course, be politically irreproachable, coming as they do from a deputy federal premier with the endorsement of party presidium member Milos Jakes, who pledged to the commission the "effective support" of the party "at all levels". In another context, and from a less eminent speaker, they might be construed as containing a certain veiled criticism not only of Marxism (which bases its validity on its "scientific" nature) but also of the Soviet Union where, during the later years of the Brezhnev era, there was undoubtedly a tendency to expect, in Obzina's words, "some kind of miracle" as a result of that continuously repeated slogan "accelerated application of scientific and technical research findings in practice".

Vera Rich

0028-0836/84/070586-01$01.00

© 1984 Macmillan Journals Ltd

Nuclear waste

UK procedures condemned

THE Nuclear Installations Inspectorate (NII) and the Department of the Environment (DoE) this week publish separate, highly critical reports of safety procedures at the British Nuclear Fuels Limited (BNFL) reprocessing plant at Sellafield (formerly Windscale). The reports investigate incidents at the plant last November that led to widespread radioactive contamination and a public warning from the Department of the Environment against the use of beaches in the area.

BNFL's latest problems began on 11 November, when operators pumped wastes into a sea discharge tank, apparently unaware that radioactive solvent and "crud" had not first been separated off. Standing instructions for the procedure, which was being undertaken as part of the annual shut-down, were "ambiguous", says DoE's radiochemical inspectorate, and the procedure for communications between managers was "inadequate, prone to error and not sufficiently formalized". Records were kept only in manual log books and instructions were confused by contradictory suggestions made in pencil.

As soon as the mistake was detected the flow was stopped. Attempts were then made to return the solvent and crud to the treatment plant, but because the available pipeline had only a 2-inch diameter, the procedure was partly unsuccesssful and the level of gamma radioactivity near the sea tank outlets necessitated evacuation of workers from the immediate vicinity of the pipes. By this time, senior management had been alerted and after further unsuccessful attempts to rescue the position they decided to flush the offending material down the sea pipeline to increase flow rates and reduce radiation dose to workers. DoE says this decision was taken "without adequate assessment of the available options": the presence of the solvent and highly active crud "does not appear to have been considered".

The company did not at this stage inform the relevant government departments because the total amount of radioactivity in the sea tank was less than the total authorized discharge; DoE says the company "would have been well advised to do so". BNFL maintains that a high proportion of the 4,500 curies of beta activity in the sea tank was returned to the plant, but NII is sceptical and thinks "a significant proportion" went to sea.

The first indication of problems outside the site was on 14 November, when divers from a ship belonging to the environmental group Greenpeace were contaminated with ruthenium 106 in solvent. Two days later, a DoE inspector was informed that ruthenium had been released but was told neither of the complex circumstances nor that more than 500 curies was likely to have been released. On 18 November, BNFL noticed a slick of solvent at sea and attempted to disperse it, but subsequently lost the slick. The slick and associated debris were later washed up on the beach, but, according to DoE, BNFL had no effective procedure for recording and assessing results. On 20 November, BNFL advised the public that all was back to normal and that beaches could be used again. This was later contradicted by DoE, and BNFL's decision is now criticized.

DoE says that current authorizations are not adequate to deal with sudden releases of solvent and crud. Although the total amount of radioactivity released was well within authorized limits, the sticky nature of the solvent, together with the highly active crud, led to the formation of local "hot spots" of activity, mainly on flotsam, and these were the main potential hazard.

Although little damage to public health is likely to have resulted from the November incidents, there is more concern over plutonium that is routinely discharged and which, contrary to expectations when the discharge pipeline was built, appears to bind to sediments on the sea bed. DoE's radiochemical inspectorate considers that solvent discharges in the past may be responsible for the enrichment of plutonium in the sea surface and its subsequent transfer to land. The National Radiological Protection Board is investigating whether plutonium in household dust in the Sellafield area could account for a local excess of childhood leukaemias claimed in some recent studies.

NII has required BNFL to make a number of immediate changes to operating procedures, and further long-term improvements are planned. Automatic cut-offs have already been installed to prevent solvent or crud finding its way to the sea discharge tanks.

Both reports are now with the Director of Public Prosecutions. The DoE report finds possible breaches of statutory operating requirements relating to record-keeping and maintaining doses to the public that are as low as reasonably achievable.

Tim Beardsley

Seabed geology

Gloria goes west

IN a few weeks' time, the MV *Farnella* will sail for the west coast of the United States carrying the unique long-range sidescan sonar "Gloria". The voyage is the result of a new cooperative agreement between the Institute of Oceanographic Sciences (IOS), a component body of the UK Natural Environment Research Council, and the US Geological Survey. It will cost the latter over £1 million.

Gloria (Geological Long Range Inclined Asdic) will be used to survey the ocean floor in all waters more than 500 m deep within the Pacific margin of the recently created US Exclusive Economic Zone. The objective is to produce maps of the shape and texture of the seabed, the interpretation of which may reveal features of economic as well as academic interest.

Gloria was developed by IOS and is at present the only sonar of its type in the world. Using acoustic back-scattering in a manner analogous to the use of oblique illumination during aerial photography, the system produces images of the seafloor up to 30 km either side of the track of the 8-m long towfish. In its six years of service, Gloria II has mapped approximately one-fiftieth of the world ocean floor.

Geological interpretation of the Gloria data may provide clues to the petroleum potential of the Exclusive Economic Zone. Of equal interest is the possibility of discovering significant quantities of heavy metal sulphides. Hundreds of kilometres of the Gorda and Juan de Fuca ridges lie within the zone and the heat generated at these spreading centres causes large-scale circulation of seawater. This extracts elements such as manganese, zinc, copper, cadmium and silver from the fresh basalt and emerges on the seafloor as spectacular "black smokers". Contact with the cold ocean water causes precipitation of sulphide minerals which may build up economically attractive deposits.

The investigation will yield immediate returns for the academic community but in commercial terms it must be seen as a speculative investment. **Peter Gambles**

New man at zoo

BARRING the unlikely emergence and the even less likely victory of an alternative candidate, Sir William Henderson FRS will succeed Lord Zuckerman as president of the Zoological Society of London. Sir William, a former secretary of the Agricultural Research Council, sees his most important task as reducing the society's budget deficit, now running at £2 million a year. The society has long been plagued with financial problems and many zoo buildings need renovating. Last year, the Department of the Environment turned down an ambitious expansion plan but agreed to write off the society's losses to the tune of £2 million a year until 1986.

Sir William is keen to preserve and enhance the society's scientific prestige: its expertise in reproductive physiology, for example, has allowed it to maintain in captivity species such as the oryx that are almost extinct in the wild. The only way to continue to finance such work will be to increase the number of visitors to the zoos.

Tim Beardsley

0028-0836/84/070587-01$01.00

© 1984 Macmillan Journals Ltd

Unesco

Turmoil and strife abound

Paris

THE science and technology programmes of the United Nations Educational, Scientific and Cultural Organization (Unesco) are putting a brave face on the threatened withdrawal of the United States at the end of the year. Indeed, there is even a possibility that for the next few months, science and technology, which have often been pleaded in aid in the past year, will be more generously dealt with.

One sign of this is that the annual grant to the International Council of Scientific Unions has been increased by a quarter to $500,000 for the year ahead, exclusive of contracts yet to be agreed. But Unesco's generosity will also take in for the first time the World Federation of Scientific Workers, which is to receive $5,000 or more to support its newsletter.

How the total funds for science and technology will be affected will be known only when the urgent review of the budget now under way is decided, in early April. The management has asked for a 10 per cent reduction, but this will not be equally spread. Much the same will happen to vacancies as they arise. Posts will be left vacant if possible but otherwise filled by transfers within Unesco.

Much change is in prospect. The much respected head of the Man and Biosphere Programme left earlier this month for the University of Montpellier. The Belgian head of the Science Policy Division, the work of which was criticized at the biennial General Assembly last year (particularly by the British), will retire later this year. It will be a test of Unesco's managers' mood to see whether that vacant post is filled. One other senior departure seems in the offing at least, but there is talk of a senior Soviet scientist joining.

The mood among the staff ranges from optimism to despair. Some see the director-general's attitude as intransigent, fear their life's work is being jeopardized — "It's just as much my Unesco as his" — and fear that prospects of promotion are blocked. Others consider that less money may encourage efficiency and point out that their financial contribution to outside activities such as training is often only token and could be further reduced without catastrophe.

Some administrative problems are as yet unsolved. For many collaborative projects, the United States provides not only its general contribution but also assistance in kind — research vessels, communications and administration. So will US agencies be allowed (or wish) to continue if withdrawal goes ahead?

The charge of bureaucracy levelled at Unesco is impossible to verify or deny. Some say it is a mess, others point out that repeated enquiries from member governments make endless work, as do the endless committee meetings at which a Unesco presence is thought necessary. More worrying are the sudden if small shifts of policy at the behest of top management or if an itinerant official should rashly promise to help a host government.

One impression is that Unesco's most serious problem in science and technology is that it expects its staff to behave as scientists when they are administrators, that the organization is as a result too ready to make judgements off its own bat without the benefit of external advice, and that the endless search for programmes that sound well often blinds it to projects it could usefully tackle. **John Maddox**

US medical schools

Admissions coaching challenged

Washington

THE administrators of the standard test for admission to US medical schools are taking new legal action to prevent a Pennsylvanian company from using bootlegged copies of past tests. The Association of American Medical Colleges (AAMC), which owns the standard Medical College Admission Test, or MCAT, charged in federal court last week that Multiprep, a company that offers to prepare students for MCAT, had reproduced questions from the tests verbatim in its course materials in violation of copyright law.

Multiprep is one of many small organizations offering coaching courses for students planning to take college admissions tests, such as MCAT, that tend to weigh heavily in admissions decisions. The test administrators' standard line has been to discount the effectiveness of such courses, which typically cost several hundred dollars, but otherwise to view them as a tolerable nuisance.

Last June, however, AAMC noticed that 250 students from the Philadelphia area who took MCAT in April 1983 did particularly well on questions that had been recycled from the test given in autumn 1980. Further investigation revealed that all had taken the Multiprep course. AAMC obtained a court order to have Multiprep's offices raided; on the basis of materials seized by federal marshals in the raid, including exact copies of MCAT question papers, AAMC was then granted a temporary injunction against Multiprep pending a trial on the merits of the copyright violation charge. The injunction specifically barred Multiprep from using MCAT materials or from advertising, as it had, its possession of "facsimile" MCAT tests.

In last week's action, AAMC claimed that Multiprep had violated the court order on both counts. AAMC said that 77 of the 87 chemistry and biology questions appearing in Multiprep's current course materials were word-for-word copies of MCAT questions.

According to AAMC, questions are "often" repeated from year to year in the test both for statistical reasons and because of the limited number of "quality" questions covering first-year college material in biology, chemistry and physics. The test administrators take pains to prevent the circulation of test questions; question papers are numbered serially and are collected and checked after each administration of the test. AAMC officials believe that Multiprep obtained their material by having an examinee remove the inner pages of a test booklet, replacing them with dummy pages.

No date has been set for the trial on AAMC's original accusation of copyright infringement, in which AAMC is seeking $1.5 million in damages against Multiprep. **Stephen Budiansky**

Accident casualties

ROMANIA's Minister of the Chemical Industry, Gheorghe Caranfil, and his deputy, Ion Bivoralu, were sacked last week as a result of a major accident last December in the pyrolysis installation at the Teleajen petrochemical combine. A joint party and government commission found that the accident was due to "serious deficiencies" in the organization and management of the pyrolysis installation during its commissioning, violations of safety regulations and "serious violations" of order and discipline. The Communist Party Secretariat of Prahova County (in which the Teleajen plant is situated) was rebuked for failing to take "appropriate political educational measures" to ensure the smooth operation of the plant, and the first secretary of the county party committee, Virgil Cazacu, and also a deputy prime minister, Ion Nicolae, received votes of censure.

No details of the accident have been published, but the tone of the official announcements, with their repeated use of the words "grave" and "serious", as well as the penalties imposed, point to major human and financial loss.

The petrochemical industry is a particularly sensitive issue in Romania. Mrs Elena Ceaucescu, the wife of the party leader, is herself a petrochemical engineer, and, as head of the National Council for Science and Technology, was responsible for the expansion of Romania's petrochemical capacity in the early 1970s — a programme which caused Romania, an oil-producer, to become a net oil importer, and which has been a major cause of the country's present energy problems. Vera Rich

0028-0836/84/070588-01$01.00

© 1984 Macmillan Journals Ltd

Palaeontology

New bones for old

AFTER 12 years of planning and legal battles, one of the world's top ten palaeontological sites — at Messel, near Darmstadt in West Germany — has been designated a rubbish dump. The decision, which will not be immediately disastrous, is a compromise between the obligation of the local bureau of mines to deal with dangerous old mine workings and the scientific community represented principally by the Senckenberg Museum in Frankfurt.

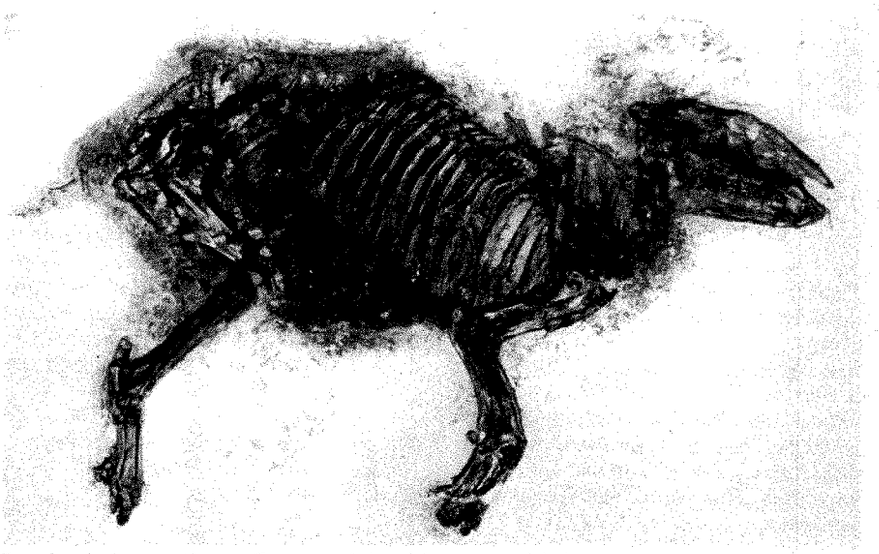
The main site covers about 10 per cent of a 90-year-old opencast mine working in soft oil shale that forms a pit 1 km long and 60m deep. An entire Eocène ecosystem is preserved with a huge variety of fossils of outstanding quality.

The first fossil, a crocodile, was found at Messel in 1875. Between 1886 and 1971, some 20 million tonnes of oil shale were mined. As the mining decreased in the 1960s, the pit became the focus of a rush of amateur fossil hunters. It was eventually closed to the public because of its dangerous state but, with a five-year grant from the Volkswagen Foundation, scientific excavation began in 1976.

To preserve the complete site permanently for palaeontologists would cost DM30 million (£7.5 million) on one estimate, a sum that could not be found by the mining authority of the *Land* of Hesse which has legal responsibility for the site. It has therefore decided that the pit will be filled with waste, although about 40 per cent of the area will be preserved for 20 years for scientific work, and an extra 9 hectares will be made accessible for a period of a year or two while drainage is carried out.

The site is the product of two rivers which bore detritus into a warm (20-30°C) still lake over a period of 1-2 million years. The oil shales preserve not only the contents of the lake but also the flora and fauna of the teeming palm forests that lined its margin. The fossils contain 40 per cent water and were difficult to preserve until the advent of replacement techniques using water-miscible plastics. These reveal skeletons and unique soft-part detail, fine enough to show replacement of many structural parts by bacteria and including feathers and hair. Stomach contents show, for instance, that the bat *Palaeochiropteryx tupaiodon* lived primarily on Lepidoptera and that the horse-like *Propalaeotherium* ate foliage including bay leaves and vines.

Every major group of the animal kingdom is represented at Messel and published papers already fill more than 130 monographs issued by the Senckenberg Museum. Finds have included many rarely preserved amphibians, skeletons of bats with hair and skin, a unique rodent genus and, last year, an anteater never before unearthed in Europe and indicative of later land links with America than has hitherto been supposed.



Propalaeotherium messelense, *a European relative of the ancestor of the horse.*

Given the legal position whereby responsibility is a matter solely for *Länder* government, even though most of the research on the site is supported by federal agencies, those involved have reluctantly accepted what appears to be the best compromise. The argument that Messel is so important that it should be designated by the United Nations Educational, Scientific and Cultural Organization as a site of special historical interest neglects the legal fact that an initiative in that direction would have to come from the *Land* of Hesse. □

Israeli Arabs

Finding technical jobs

Rehovot

FEW Israeli scientists can claim to have declined the offer of a job overseas in favour of one at one-quarter of the salary at home; even fewer are Arabs. One who fits both bills is Dr Nicola Yanaki, a nuclear physicist whose decision to work for one of Israel's most successful high-technology companies was made, in part, to help fellow Israeli Arabs.

Yanaki, who lives in the predominantly Jewish town of Upper Nazareth, is product manager for mammography at the Elscint plant in that town and is largely responsible for the company's advanced systems for the detection of breast cancer. Called Man 2 and Man 3, they provide extremely fine resolution and optimal contrast for accurate differential diagnosis.

Yanaki grew up in the town of Ramle, near Tel Aviv, and received his first scientific training at the Arab Orthodox College (a high school) in Haifa. He then went to Tel Aviv University, where, in August 1981, he became the first Israeli Arab to win a doctorate in nuclear physics. During his stay at Tel Aviv University, Yanaki developed and patented a special gamma-radiation detector. This device, while not yet produced commercially, aroused interest among oil companies because it has proved useful in oil exploration projects.

Indeed, it was because of this detector that a US oil company offered Yanaki a well-paid job, which he turned down when asked to join the staff of Elscint, which now sells $110 million worth of medical imaging equipment a year. Yanaki clearly feels that he should repay the nation for having provided him with a good education, as well as being motivated by a desire to help other Israeli Arabs. Those with advanced training in science and engineering have limited employment opportunities because many science and engineering jobs are in defence industries and hence, Yanaki says, "are naturally closed to Arabs in present circumstances".

Until these circumstances change, Israeli Arabs with Yanaki's background must look to civilian science-based industry. By taking a job with Elscint, Yanaki feels that he has "opened the way for others". At Elscint itself, this is clearly the case. There are two Arab engineers in Yanaki's own staff, and another Arab, Anvan Nujam, is carrying out research in Elscint's ultrasound division.

At a time when many others are pessimistic about the chance of peace in the Middle East, Yanaki is optimistic, pointing out that the people of the region have everything to gain by working together. He looks forward to the day when he and his colleagues at Elscint will be able to work on joint projects with researchers in neighbouring Arab countries. And, he adds, "I'm sure that day will come more quickly than anyone now believes possible".

Nechemia Meyers

0028-0836/84/070589-01$01.00

© 1984 Macmillan Journals Ltd

Human experiment

SIR — The relative rarity with which the results of experimental work in human beings are reported in *Nature* must be the excuse, if not a good one, for the publication of a study on pain in which (1) no indication of whether consent was sought is given (and the study design probably excludes the possibility); and (2) pain experienced by individuals was *increased* by the experimenters' hidden actions. ("Placebo and naloxone can alter post-surgical pain by separate mechanisms", by R.H. Gracely *et al.*, *Nature* **306**, 264-265; 1983). Although dental pain may be regarded as trivial, I, and I am sure many of my colleagues, would regard this study as unethical by any standard.

H.A.F. DUDLEY
Academic Surgical Unit,
St Mary's Hospital,
London W2 1NY, UK

● R.H. GRACELY REPLIES — Professor Dudley's letter challenges whether consent was obtained and the ethics of giving a drug that may increase pain. It is unfortunate that the consent procedure was not described in the text. Human research protocols at the National Institutes of Health (NIH) must be approved by a formal review process that includes written informed consent. In this case, the consent form indicated that "withdrawal from the study may be accomplished at any time without jeopardy or prejudice" and informed the subjects that they may receive "a maximum of 10 mg naloxone — a drug that may be less effective than placebo in pain relief and that theoretically may increase any pain". Professor Dudley is correct in citing this omission from the manuscript but he inappropriately suggests that this study could be performed without consent.

His description of this study as unethical is a more serious matter. He criticizes not only our ethics, but those of other investigators. A dose of 10 mg naloxone has been administered to dental patients after third molar extractions in several highly publicized studies, including one published in *Nature* (**272**, 826-827; 1978). The increase in pain after naloxone administration was slight and caused no excessive discomfort in these experiments. Our use of naloxone was accompanied by knowledge of its effects in previous studies and by an informed consent procedure that included termination at any time. Patients not only completed this study (involving extraction of third molars on one side) but returned for a similar extraction of their contralateral third molars. They described the naloxone experiment as uncomfortable but tolerable.

Professor Dudley is apparently unaware of the previous studies assessing the effects of naloxone on postoperative dental pain or of the magnitude of the naloxone effect. Naloxone causes a minimal and temporary increase in discomfort in this situation. However, a great majority of human medical research involves discomfort and risks. It is not the presence of these factors but their magnitude, along with important issues of consent and voluntary termination, that are of ethical concern. The discomfort and risk in this experiment were considerably less than those experienced in many routine medical studies. Unfortunately, Professor Dudley has not addressed previous studies (cited in our article) or the relative magnitude of the discomfort, and has also questioned the research policies at NIH.

RICHARD H. GRACELY
National Institute of Dental Research,
National Institutes of Health,
Bethesda, Maryland 20205, USA

Placebo defect

SIR — A recent light-hearted account of the banning of urea foam insulation in Canada (*Medical Post*, 15 November 1983), has drawn attention to the lack of a word in the English language to describe the opposite of a placebo. A placebo may be defined as something which, in itself, is inactive, such as a simple lactose pill. However, a placebo has been found to be effective therapeutically in about 30 per cent of patients suffering from a wide spectrum of diseases.

Little attention has been paid to the possibility that a harmless substance produces harmful effects when the patient believes it to be toxic. The author of the article in *Medical Post* suggests the word "garblow". The urea foam insulation was banned precipitately by the Government of Canada after a media crusade against the "dangerous" formaldehyde gas which the insulation may emit. Of about 80,000 households which had installed foam insulation, about 10 per cent developed symptoms which they were encouraged to attribute to the formaldehyde vapour. The English have learned to live with the particular peril of foam insulation but there must be many examples of garblow in our own press threatening our health and sanity.

M. J. BARRIE
Toronto East General
and Orthopaedic Hospital Inc.,
Toronto, Ontario, Canada M4C 3E7

Not by corn alone

SIR — With reference to J. R. S. Fincham's interesting article "Transposable elements and plant gene structure" (*Nature* **306**, 425, 1983), I write to point out that P.A. Peterson is a member of the faculty of Iowa State University and not, as the article states, of our sister institution, the University of Iowa.

Incidentally, D.S. Robertson, mentioned in the article as the discoverer of the *Mu-1* transposable element, is also a member of our faculty.

ETHAN HACK
Department of Botany,
Iowa State University,
Ames, Iowa 50011, USA

Homer's wine

SIR — Wright and Cattley's chemical hypothesis about Homer's "wine-dark sea" (*Nature* **303**, 568; 1983) is both interesting and evidence of a commendable piece of interdisciplinary cooperation.

But the topic of Homer's epithet is many-sided. Any "solution" of the "puzzle of the wine-dark sea" will be a function of one's particular couching of the question. The chemical hypothesis would certainly be appropriate to the question "Why did Homer call the (Mediterranean) sea wine-coloured (he mentions only red wine in his epics) when it is celebrated for its blueness?" The hypothesis that adding alkaline water to the wine of Homer's time and region turned it from red to blue complies with the scientific requirement of being empirically verifiable, in principle if no longer in practice. Yet a positive test result would not solve, or even invariably be appropriate to, other aspects of the puzzle unless one asserted reductionistically that a scientific answer is the "one proper" one.

Among the many other equally tantalizing aspects of the sea/wine topic are:

(1) Irrespective of the actual colour of the wine, should not the phrase "wine-dark sea" be seen purely or primarily as a formulaic device of the oral Homeric tradition, a recurrent epithet or a fixed metaphor? Homer uses it many times in diverse contexts in both the *Iliad* and the *Odyssey*.

(2) Should we doubt that the Mediterranean sea can look wine-red? The classicist Stanford and others have noted that it is at times a "deep purple crimson" or "mulberry" in places. So "wine-dark" could in fact mean "wine-red".

(3) How adequate, in aesthetic and/or other respects, are the various translations of Homer's original Greek phrase, namely οινοπα ποντον, to quote one inflexion? Down the ages it has been translated into several languages and with divergent meanings. Examples are "wine-dark sea" (Rieux, Shewring), *les vagues vineuses* (Bérard), *weinrote Meer* (Schadewalt), and *wijnkleurige zee* (Boutens). Among English translations, we have variations such as "wine-coloured sea" (Simcox), "wine-faced sea" (Cook) and even "the gloomy flood" (Cowper). The translator Cook, in fact, sees no colour connotation whatever in the original Greek phrase.

(4) If we focus on just one English translation, say "wine-*dark* sea", and consider it simply as an aesthetically piquant English phrase, then what is its poetic merit as such? This question entails the usual multiplicity of considerations of imagery, associations, allusions, euphonics, and phonetic symbolism, and decisions made in terms of facets of these criteria may scarcely be a function of what colour the wine was.

MICHAEL MACNAMARA
Philosophy Department,
University of South Africa,
Pretoria 0001, South Africa

0028-0836/84/070590-01$01.00

What happens in the mantle?

While it is generally agreed that continental drift occurs by the mechanisms of plate tectonics, the forces that drive these motions remain largely undescribed. But change may be on the way.

AFTER more than 20 years of contentment bordering on complacency, the theory of plate tectonics seems in for a bout of disputation that may be as fierce (and as entertaining) as that of the late 1950s, when geophysics was divided into the two camps of neo-Wegenerians and the conservative rest. That the continents move relative to each other is not now substantially in dispute, of course: the reality of that phenomenon, originally inferred from observations of the deep oceanic crust, is now within the range of direct measurement using radio interferometry. The contentious question now is what drives continental drift? And what, in any case, goes on in the mantle on which the tectonic plates appear to float?

One small sign of which way the wind is blowing can be seen in the note by D.P. McKenzie on page 616. Without saying as much, McKenzie deals with a point often raised by his Cambridge colleague, R.A. Lyttleton, in the past few years — how can the new orthodoxy account for the several recognized episodes of mountain-building in regions far distant from the places at which continents are colliding with each other, or where one plate is riding over another, as in the formation of the Himalayas and the Sierras?

On the standard view, the continents, with an average density of 2.8 g cm^{-3}, float on the more dense mantle beneath in the particular sense to which geophysicists have made us familiar — adjustments take place on a timescale comparable with that of the plastic deformation of the solid material of the mantle. What McKenzie now says is that the mantle material beneath continents may differentiate into dense and less-dense components just as it does beneath the Earth's great spreading oceanic crust. But beneath the continents, the lighter material is either added to the basement or intruded at high levels as horizontal basalt slabs. In either case, the result is to make blocks of continental material more buoyant than they would otherwise have been. The result, the numbers suggest, may be some kilometres of uplift.

Orthodox geologists should be content. Mountain-building other than that provoked by the more spectacular tectonic events is made explicable. So too is the occurrence near the surface of metamorphic rocks, some even derived from sedimentary material, which have obviously in their time been deeply buried. This was precisely the phenomenon that C.J. Allègre and his colleagues from the Institut de Physique du Globe in Paris were describing last month (*Nature* **307**, 17; 1984). Whether this simple notion will settle the long-standing controversy about the origin of the Colorado plateau is less certain, given the great pleasure it gives to orthodox geologists.

Meanwhile, it may be thought puzzling that such a simple idea as McKenzie's has not been put forward long before this. The explanation seems to be that geophysicists have been unduly impressed with the apparent constancy of the thickness of the continental crust, almost everywhere within 5 km or so of 35 km, and at the same time attracted by the simplicity of the notion that the continental crust is essentially primitive material which, once formed, remains on or near the surface. That some of it must do so is shown by the ages of the rocks of the ancient shield areas, exceeding 3,000 million years. But not everything is quite that simple.

Dr D.L. Anderson from the California Institute of Technology seems ready to make a more striking break with the new orthodoxy of plate tectonics (*Science* **223**, 347; 1984). Starting from his own recent analysis of the speeds of seismic waves in the upper reaches of the Earth's mantle, Anderson outlines a framework within which the entire standard model of what happens in the mantle might be recast. The observations show that seismic speeds vary from one place to another, as gravity anomalies are known to do, while there is the now long-standing expectation that the regions of continental crust will act as an insulating blanket for the mantle beneath, where the temperature should as a consequence be greater than that elsewhere. Briefly, low seismic velocities signal relatively high temperatures.

Part of Anderson's argument is that these lateral temperature gradients must have an important influence on what happens within the mantle. Few will dissent from that. So what happens to the standard view of what happens in the mantle and of what drives continental drift — the notion that the mantle is one huge pattern of thermal convection cells in which hot material rises (along the lines marked out by the spreading centre) and sinks as cold material where the edges of oceanic plates re-enter the Earth by the process called subduction at the continental margins (south-east Pacific) and at island arcs (western Pacific)?

The remarkable feature of this convection process is that it has been so vague. If the present disposition of the continents and of the plates on which they are mounted is simply to be explained by a pattern of convection cells, at least some features of that pattern must have persisted for some 180 million years, the age of the oldest rocks so far found in the oceanic crust (in the south-east Pacific). So one would expect that the outlines of these convection cells would by now have been fully described, and the outlines of their ascending and descending walls drawn out on maps as befits important influences on the surface structure of the Earth. Then, by comparison with some theory of convection, it should be possible to tell what fraction of the driving force is represented by the thermal energy of the Earth's interior and how much by other influences. But in reality, even the depth of the supposed convection cells has been uncertain, although the discontinuity of seismic velocities at 670 km has been most people's favourite candidate.

Anderson's view of the upper reaches of the mantle is radically different. First, he says, it is not even vertically homogeneous but, rather, layered, as would be expected for a large body of material that has undergone continual zone-refining over aeons. The zone-refining consists of the liberation from the deeper rocks of those components which form liquids, or which can melt, and, on the same view, the buoyancy of the molten material is what drives upward convection. The apparent discontinuity of 670 km is not a phase transition within chemically homogeneous material but a region where both chemical composition and physical properties are changing.

If this way of describing what happens in the mantle proves correct, the consequences will be interesting. First, mantle convection need not be a continuous process but may be episodic — indeed, Anderson argues that the fragmentation of Gondwanaland may be most simply understood as the consequence of the accumulation of heat beneath it, setting off the instability that has continued ever since. And — this is the real radicalism — the occurrence of positive gravity anomalies may not be accidental but, instead, a sign that one consequence of mantle activity is that the Earth's moments of inertia can be changed so as to reorient the mantle as a whole with respect to the spin axis of the Earth. **John Maddox**

0028-0836/84/070591-01$01.00

© 1984 Macmillan Journals Ltd

Vision

An old illusion and a new theory of stereoscopic depth perception

from John P. Frisby

OGLE'S induced effect, a visual illusion first studied in the 1930s[1], has over the past year been the subject of an intriguing series of papers[2-5] (the latest of them in this issue of *Nature*, p.632). The reasons for the renewal of interest in the induced effect can be found in the severe challenge it poses for conventional theories of binocular depth perception and the development of a new computional theory of stereoscopic vision within which it can be encompassed.

Stereoscopic depth perception is generally accepted to derive from the slightly different horizontal positions from which the two eyes view the world — small differences arise in the horizontal positions of corresponding points in the left and right retinal images of objects lying at different distances. But Ogles's induced effect is a change in depth perception caused by introducing vertical, not horizontal, disparities between corresponding points in the two images. The effect is usually produced by placing a meridional size lens, which has the property of magnifying an image in one axis only, in front of one eye such that it enlarges the image in the vertical dimension. As this magnification does not affect the horizontal axis, the lens should have no effect on the perception of depth, given conventional theories. In fact, however, depth perception is markedly altered. For example, a fronto-parallel surface appears rotated about the vertical axis through the fixation point, seeming to protrude towards the observer on the right-hand side if the right eye's image is vertically magnified.

Ogle called the illusion the induced effect because it seemed in many ways as if the vertical magnification 'induced' a whole-field magnification of the other image (or its neural representation)[1]. This compensated for the vertical size difference introduced by the lens but at the price of creating a horizontal size difference between images which then produced the illusory rotation via the normal mechanisms of stereoscopic vision. Gillam and Lawergren[2] provide a good review of the attempts of Ogle and others to explain the induced effect.

Recent interest has centred on the relevance of the induced effect to a new computational theory of binocular depth perception advanced by Mayhew and Longuet-Higgins[3-5]. The theory was not specifically designed to account for the effect but it predicts its occurrence as an inevitable side effect for any visual system that implements the theory. The fact that the human visual system is susceptible to the induced effect can thus be regarded as evidence that it contains mechanisms which implement the theory, a claim which has caused some controversy.

The new theory is based on a geometrical analysis of the spatial distribution of horizontal and vertical disparities. Figure *A* shows a symmetrically converged pair of eyes observing a square lying in the fronto-parallel plane containing the fixation point. Because the sides of the square are at different distances from each eye, each image of the square is a trapezium. Figure *B* shows how the two images possess horizontal (H) and vertical (V) disparities, defined respectively as the difference in x and y coordinates of matching points. It can readily be appreciated from the figure that the larger the observed square, the larger will be H and V: in other words, H and V will be scaled by retinal eccentricity. It is also true that H and V will vary with the fixation distance (d) and the angle of gaze (g; see figures *C* and *D*) with which the fixation point is viewed because both d and g will affect the relative sizes of images in the two eyes. The equations derived by Mayhew[3] that determine the relative contributions of these factors are:

$$V = \frac{xyI}{d} + \frac{ygI}{d}$$

$$H = \frac{x^2I}{d} + \frac{xgI}{d} + \frac{zI}{d^2}$$

where I is the interocular separation and z is the depth relative to the fixation point (both in centimetres; all other quantities in radians). Note that the z term in the equation for H reflects the fact that H values are sensitive to depth differences in the scene, which is why they provide the fundamental information for stereoscopic vision. In sharp contrast, V values have no z term: as a result they can be used to compute the viewing parameters d and g. With these parameters known it is then possible to interpret the ambiguous information in the equation for H and find the value of z: that is, it is possible to extract absolute values for depth differences in the scene being observed. Of course, all these computations depend upon the difficult problem of stereo correspondence having already been solved, that is, the problem of which points in the left image correspond to which points in the right image (for an introduction to this problem see ref.6, Ch.7).

The equations for V and H are remarkably simple and constitute a theory of binocular depth perception that shows that the absolute depths of all points in a scene can be recovered from visual information alone. Indeed the equations are so simple that, at least in principle, V values for only two matched points (of differing x eccentricity and not falling too close to the horizontal meridian) are required for the solution of two simultaneous equations in V to recover the parameters d and g. This is remarkable, because it has been a commonplace belief that d and g, so essential for interpreting the horizontal disparities, have to be obtained from extra-retinal information about eye positions. Curiously, although Helmholtz[7] knew that information about absolute distance was contained in vertical disparities, he gave no details and his comments have been neglected, perhaps because it has been assumed, incorrectly for near distances, that vertical disparities are too small to be detectable by the human visual system.

Why is it that Ogle's induced effect is produced as a side effect of implementation of the theory? It can be seen from figure *C* that V varies with retinal eccentricity in x, that is, the lines defining the top and bottom of the square become more and more separated as they extend into peripheral vision. Another way of saying this is that the vertical magnification difference between images is scaled by x, and a useful way of visualizing the equation for V is as a plot of the vertical magnification factor (given by V/y) against x. This graph is illustrated in figure *E*. It is a straight line whose slope is I/d so that, as I is known, the slope directly yields $1/d$. The reason why the slope is inversely proportional to d is that the further away the fixation point, the smaller will be the vertical size difference between images.

The intercept with the ordinate is given by gI/d, which means that it is scaled both by d and by g. For any given d, if convergence is symmetric ($g = 0$), then the plot goes through the origin; but if it is asymmetric ($g \neq 0$), then the line is shifted up or down by an amount proportional to g (fig. *E*). Changes in g do not alter the slope of the line and hence do not have to be taken into account in the computation of d from the gradient of change in vertical magnification with x eccentricity.

Consider now what happens if one image is vertically magnified. This will not alter the slope of the line but it will add a constant to the quantity V/y present at each x eccentricity, thereby reproducing the state of affairs for a non-zero gaze angle. Hence, a vertical magnification of one image will be interpreted as indicating asymmetric convergence. And by some straightforward algebra it can be shown that, if the pattern of H values created by a frontoparallel surface viewed with $g = 0$ are found to be present when $g \neq 0$, then they must have arisen from a surface rotated about a vertical axis through the fixation point. Hence any visual system using vertical disparity information for computing the viewing parameters d and g will be susceptible to an induced effect due to a spurious g being computed when vertical magnification is introduced. Interestingly, observers do not report feeling that they are 'not looking straight ahead'

0028-0836/84/070592-02$01.00

© 1984 Macmillan Journals Ltd

when experiencing the induced effect, so if it is due to a visual computation of g it must be one that is either solely used for interpreting H values or one that is over-ruled by extra-retinal information about the positions of the eyes as far as 'felt position' is concerned.

The logic of Mayhew and Longuet-Higgin's theory of binocular vision is quite independent of the question of whether any particular visual system uses it. That is, the theory provides a method for recovering d and g from visual information alone (for near distances) which may or may not have been discovered during the evolution of biological visual systems. Nevertheless, it is of considerable interest to ask whether the human visual system uses the method and the induced effect is *prima facie* evidence that it does indeed do so. However, the 'd/g hypothesis' of the induced effect, as it has been called[8], is by no means the only explanation currently being debated (see ref. 2) and it therefore becomes necessary to enquire whether psychophysical details of the induced effect fit its predictions.

To date, psychophysical predictions drawn from the d/g hypothesis have survived attempts to falsify them as long as the requirement is met that experimental conditions present vertical size differences between images which could arise in natural viewing. Not surprisingly, this is a requirement that adherents of the hypothesis regard as reasonable. They point out, for example, that the fact that the induced effect reaches a maximum at magnifications of about 4–6 per cent is as expected because larger magnifications could only be produced by unrealistically large gaze angles.

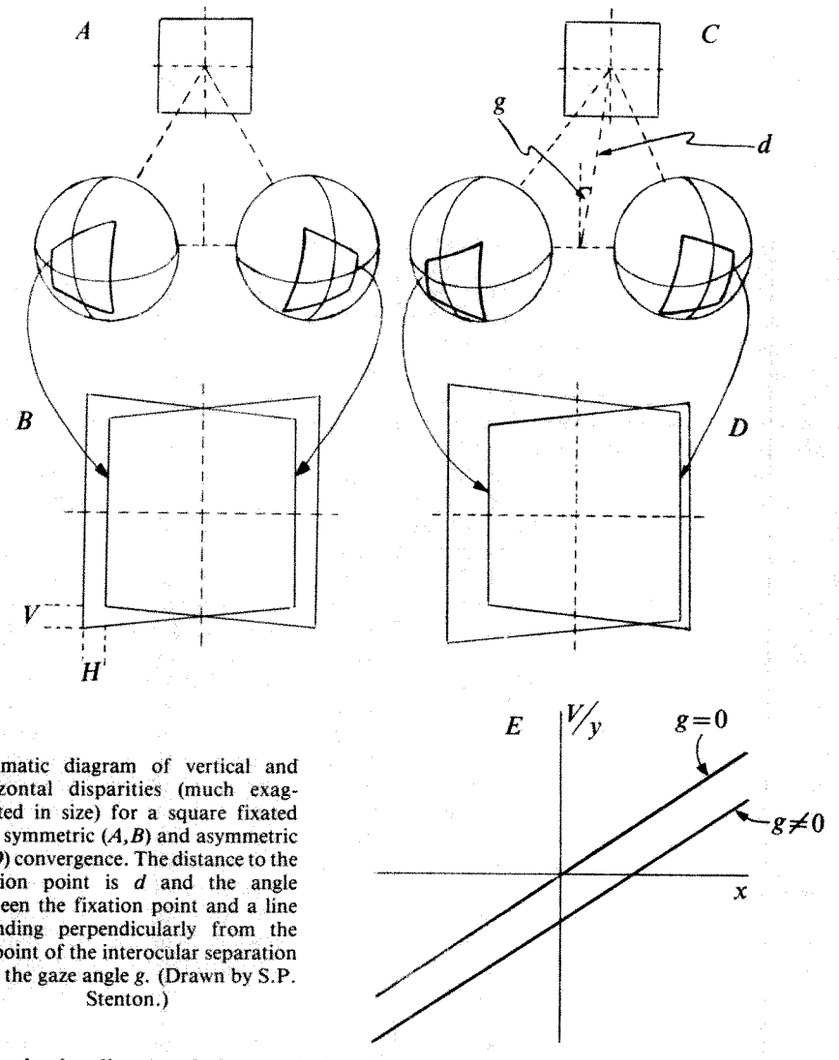
An objection to the hypothesis can be drawn from Westheimer's report[9] that sensitivity to vertical magnifications falls well below that for horizontal ones. This appears quite at odds with a hypothesis which demands V values be measured with great precision. However, when Westheimer's experimental conditions are examined with the d/g hypothesis in mind, it becomes clear that the large d he used (3 m) means that he was presenting magnifications that would need impossibly large g values to obtain them. In such circumstances, it can be argued that an induced effect would be difficult to observe.

A crucial prediction of the hypothesis is that the size of the induced effect should be scaled by d. Gillam[10], who has independently arrived at a theory of the induced effect which is much the same as that of Mayhew and Longuet-Higgins, recently reported data that she initially though falsified this prediction. However, when she re-analysed her results excluding those conditions with d/g combinations that could not occur in natural viewing, she found that the remaining data fitted the prediction remarkably well.

One characteristic of the induced effect which Ogle[1] emphasized is that it is very much a 'whole field' phenomenon. For example, he discovered that an induced rotation can be seen for a field of vertical rods even when only one rod carries vertical disparity information (by fixing some beads to it). That is, V values in one part of the field can create a rotation of the entire field of view. This result is consistent with the d/g hypothesis because d and g are intrinsically whole-field viewing parameters: only one d and g combination can exist at any one time and so it makes obvious sense to compute a single d/g pairing and to use it for interpreting H values over the whole field of view. The same kind of interpretation can be given of results, soon to be reported in *Nature*, showing that it is the average vertical magnification that seems to determine the size of the induced effect[8]. This might reflect a sensible pooling strategy for computing whole-field values of d and g given the small size of vertical disparities.

It may be that the new theory will cause a flurry of new papers on vertical disparity processing as the d/g hypothesis and its competitors are exposed to critical psychophysical examination. If so, this will be a case of experimental interest being led by a clear theoretical exposition of the nature of a given visual computation, and not by the discovery of a new illusion whose potential computational significance is quite unclear, as has so often happened in the history of psychophysics (for example, selective adaptation effects such as the McCullough effect). Conducting psychophysics within the context of a computational theory is very much in keeping with the research framework proposed by Marr[11] and it will be interesting to see whether the outcome in this case will achieve that all too rare occurrence: a clear and definite conclusion about what some psychophysical effects really mean. □

S.P. Stenton is thanked for his help in preparing this article.



Schematic diagram of vertical and horizontal disparities (much exaggerated in size) for a square fixated with symmetric (A,B) and asymmetric (C,D) convergence. The distance to the fixation point is d and the angle between the fixation point and a line extending perpendicularly from the midpoint of the interocular separation (I) is the gaze angle g. (Drawn by S.P. Stenton.)

John P. Frisby is Professor in the Department of Psychology, University of Sheffield, Sheffield S10 2TN.

1. Ogle, K.N. *Researches in Binocular Vision* (Saunders, Philadelphia, 1950).
2. Gillam, B. & Lawergren, B. *Percept Psychophys.* **34**, 121 (1983).
3. Mayhew, J.E.W. *Perception* **11**, 387 (1982).
4. Mayhew, J.E.W. & Longuet-Higgins, H.C. *Nature* **297**, 376 (1982).
5. Longuet-Higgins, H.C. *Perception* **11**, 377 (1982).
6. Frisby, J.P. *Seeing* (Oxford University Press, 1979).
7. Helmholtz, H. von *Physiological Optics* Vol. 3 (Dover, New York, 1962).
8. Stenton, S.P., Frisby, J.P. & Mayhew J.E.W. *Nature* (in the press).
9. Westheimer, G. *Nature* **307**, 632 (1984).
10. Gillam, B. Paper read to Rank Prize Funds Meeting on *Hypercuity and Depth Perception* (January 1984).
11. Marr, D. *Vision* (Freeman, San Francisco, 1982).

© 1984 Macmillan Journals Ltd

Cell biology

Membrane traffic and the problem of protein secretion

from Miranda Robertson

THE problem of how to get hydrophilic proteins across hydrophobic membranes is fundamental to both prokaryotic and eukaryotic life; so it would not be unreasonable to expect to find the solution conserved across the evolutionary spectrum from bacterial to animal cells. In fact, however, the conservation of secretory mechanisms is a controversial issue — a state of affairs which may turn out to reflect the existence of more than one secretory mechanism. The present state of the evidence, now beginning to show signs of convergence, was one of the topics of a recent conference* which also dealt with the exclusively eukaryotic problem of what controls the continuous and complex traffic of membrane and secreted proteins through the membrane-bounded compartments of the cell.

The main point of contention is whether secretion in bacteria depends on signalling, guiding, regulating and possibly pore-forming molecules analogous to those that comprise the machinery of transit across the endoplasmic reticulum of eukaryotes[1]. It is already well established that most secreted proteins of both bacterial and animal cells are synthesized with amino-terminal leader — or 'signal' — peptides that are enzymatically cleaved from the completed molecule after transport; and that eukaryotic signals are intelligible to bacterial enzymes and vice versa: *Escherichia coli* will, for example, process and secrete insulin[2] — albeit not very efficiently. Yet according to the work of W. Wickner (UCLA) and L. Randall[3], bacteria and even liposomes can transport proteins across their membranes post-translationally and without assistance from proteins other than the leader peptidase[4,5]; while it is clear that in eukaryotes things are much more complicated.

Two components of the eukaryotic secretory mechanism have already been identified and purified. The first, the signal recognition particle (SRP), binds to the leader peptide of nascent proteins and halts translation until the ribosome has engaged with the endoplasmic reticulum (ER). The SRP is now known to consist of a 7S RNA bound to six proteins[6], the two smallest of which bind specifically to *Alu* repeats at the ends of the RNA (P. Walter *et al.*, Rockefeller University) — which incidentally suggests that, in general, the hitherto mysterious *Alu* transcripts found in the cytoplasm may all turn out to mediate important functions, and calls into question the selfishness of the DNA that encodes them.

The second identified secretory component is the docking protein on the cytoplasmic face of the ER. This protein engages with the SRP, releasing the translational block so that synthesis can proceed and the nascent polypeptide is transported co-translationally into the lumen. There is preliminary evidence that the translocation step involves at least one more protein (D. Meyer, EMBL): secreted molecules are not translocated through artificial lipid bilayers supplied only with purified SRP and docking protein. Furthermore, ER membranes inactivated by mild proteolysis can be reconstituted with a purified proteolytic fragment of the docking protein, though after more prolonged proteolysis of the membranes this is no longer possible, presumably because of the destruction of a further component of the translocation mechanism more closely associated with the membrane.

There is clear evidence that the SRP component has been highly conserved in eukaryotic evolution: it can be reconstituted from *Xenopus* or *Drosophila* 7S RNA and mammalian proteins. And now it seems possible that an analogous particle is involved in the secretion of at least some bacterial proteins. Through the analysis of temperature-sensitive mutants of *E. coli* J. Beckwith (Harvard University) and colleagues have been able to identify proteins essential for the secretion of some bacterial products. Antibodies to one of these, the Sec-A protein, precipitates a 6S RNA with some homology to the 7S eukaryotic RNA (H. Liebke, Yale University).

But if Beckwith's work on *E. coli* mutants is beginning to make bacterial secretory mechanisms look more like eukaryotic ones, G. Schatz's (Basel University) analysis of yeast mitochondrial proteins is making eurkaryotic mechanisms look more like the prokaryotic ones described by Wickner. According to Wickner, the efficient post-translational transport of the M13 coat protein by *E. coli* is independent of proteins but dependent on membrane potential[5]. The transport of proteins encoded in the eukaryotic nucleus into mitochondria, which can be regarded as an inside-out version of the same process, is also post-translational and dependent on membrane potential. Furthermore, a recent analysis of the effects of mutations introduced into the leader peptides of bacterial secreted proteins lends weight to the increasing evidence[3] that there may be not be any fundamental difference between post- and co-translational mechanisms of transport: M. Inouye (SUNY) reports that certain mutations will convert a co-translationally transported protein into a post-translationally transported one.

The mechanism of transport is not the only important issue that arises in connection with mitochondrial proteins. Unlike the membrane proteins made by a bacterial cell, those made in eukaryotes have a choice of membranes with which to become associated, and must be targeted to the right one. One obvious possibility is that the leader sequences that target proteins to the membrane are specific for different cellular membranes. Not all mitochondrial proteins however are synthesized with leader peptides; and Reizman and his colleagues in Schatz's laboratory have now been able to show that a 70,000-molecular weight protein specifically targeted to the mitochondrial outer membrane in yeast will get lost in the cytoplasm if part of its N terminus is deleted by manipulation of the cloned gene[7,8].

The great advantage of yeast for the study of eukaryotic mechanisms is that mutants are relatively easily come by; and it is yeast mutants that may in the end provide the key to one of the most important and least understood of all problems in intracellular traffic: the differential routing of different membrane proteins through the specific formation and fusion of membrane vesicles. It is clear, for example, that once a secreted protein has been successfully extruded into the ER, there is more than one possible route by which it may reach the cell surface; and mutations selectively affecting the different paths would be invaluable. This is the importance of the approach of R. Schekman and his colleagues (University of California, Berkeley) who have isolated temperature-sensitive mutants of yeast blocked at four different stages in the progress of secreted proteins from the ER to the cell surface. The general usefulness of these mutants will of course depend — once again — on how far the secretory mechanisms have been conserved. Schekman and his colleagues have already established, for example, that, as in animal cells, different secreted proteins reach the cell surface at different rates; and that glycosylation is necessary for the correct processing and secretion of some, though not all, of them[9].

The crucial question, however, is whether these differences are due to the differential interaction of the proteins with components of a common path, or whether they reflect different paths. In animal cells there is little doubt that distinct paths exist — most obviously in those cells in which the secretion of some specialized products is inducible by hormones. Distinctive properties of the inducible path are storage in granules in the absence of stimulation, and the processing of secreted prohormones to their mature form. Thus, for example, if fibroblasts, in which secretion is not normally inducible, are transfected with pro-

*The Ninth EMBO Symposium on Membrane Traffic in the Animal Cell was held in Heidelberg on 26–29 September 1983.

0028-0836/84/070594-02$01.00

© 1984 Macmillan

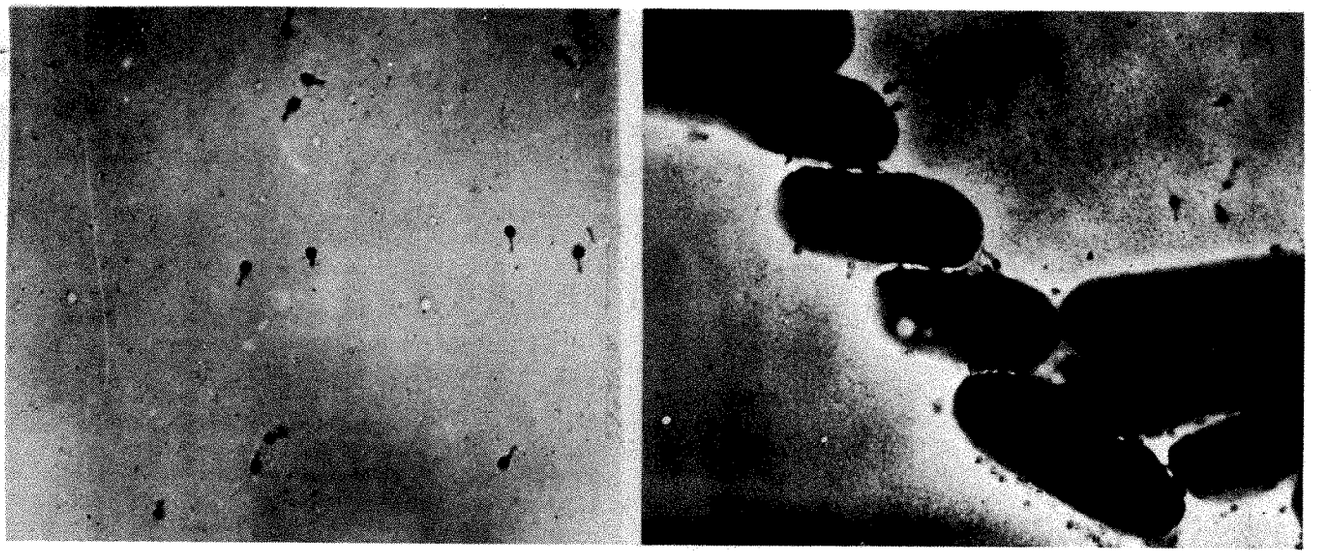
The virus verified

UNTIL the advent of the electron microscope, viruses were to the biologist what atoms had long been to the physicist: entities which could not be seen on account of their small size but which proved exceedingly fruitful in accounting for observed phenomena.

The earliest electron micrographs to reveal unmistakably the head-and-tail structure of viruses were of the viral strain originally called 'anti-coli PC', and now known as bacteriophage T2, taken by Thomas Anderson(RCA Research Laboratories) and Salvador Luria (Columbia University) in the week of 2 March 1942 and published the same year in *Proceedings of the National Academy of Sciences U.S.A.* (**28**, 127). Anderson recorded the phage particles at a magnification of x45,000 (below left) and their attachment to the gamma or PC strain of *Escherichia coli* bacilli at x50,000 (below right), both on Kodak Lantern slide glass plates using an RCA model B electron microscope and very highly concentrated stocks of bacteriophage.

A typical bacteriophage T2 particle possesses a head 60–80 μm in diameter and a tail 120–130 μm long and 20 μm thick. In the right-hand micrograph, the bacteriophage is seen attaching itself to the cell walls of a bacterium, living up to its name of 'bacterium eater'. With hindsight, Anderson describes the ensuing viral attack as follows: rather like a hypodermic syringe, the "attachment causes the tail sheath to contract which drives the needle [an extension of the neck below the head, invisible on the 1942 micrographs] into the bacterial wall, which somehow induces the DNA contained in the head to pass through the needle into the bacterium. There the DNA takes over the synthetic machinery of the bacterium to make some 100 daughter phage particles in 20 minutes. The bacterium then lyses to release the daughter particles into the medium where each of them can encounter a fresh bacterium to repeat the life cycle". The infecting phage particles adsorbed onto the bacterial wall remain stuck fast, their delivery complete and their role fulfilled. **Jon Darius**

Selected from Beyond Vision *by Jon Darius of the Science Museum (Natural History), London (to be published in April by Oxford University Press). Micrographs reproduced by courtesy of T.F. Anderson, now at the Fox Chase Institute for Cancer Research.*



insulin they will secrete the unprocessed molecule (R. Kelly, University of California, San Francisco). Conversely, AtT-20 cells, which inducibly process and secrete adrenocorticotropic hormone, can also be induced to process and secrete insulin after transfection with the proinsulin gene. Unstimulated AtT-20 cells will secrete the unprocessed hormones at low levels, presumably via the constitutive path. This is suggestive, though not strictly conclusive, evidence for two separate vesicular pathways.

And the most recent work of Schekman and his colleagues suggests that yeasts also have distinct secretory paths — although the evidence in this case concerns only constitutive secretion. Schekman and his co-workers find that isolated secretory vesicles contain all the secretory enzymes of yeast but only a subset of the plasma membrane proteins. If the membrane proteins are travelling by different routes, however, they converge at the final step: mutations affecting the last stage in secretion also prevent the appearance of any membrane protein at the cell surface.

For vesicle traffic originating at the cell surface — and particularly concerning the recycling of membrane receptors — there is some hope that human mutants may soon provide important insights. Receptor recycling has already been reviewed in *Nature*[10] and elsewhere[11,12] and it is now generally accepted that surface receptors enter the cell via clathrin-coated pits whence they may either return to the cell surface after releasing their ligand, or progress to the lysosomes for degradation.

With the discovery that some forms of familial hypercholesterolaemia are due to mutations that interfere with the biosynthesis or post-translational processing of low-density lipoprotein (LDL) receptors[11], it has become clear that defects in cholesterol uptake may make it possible to identify and understand branchpoints in intracellular vesicle pathways. J.L. Goldstein and his colleagues (University of Texas, Dallas) have, for example, identified a mutant receptor that binds cholesterol normally but fails to aggregate in coated pits; and they are now in the process of cloning LDL receptor cDNA, which will enable them to analyse such defects at the molecular level.

Mutant receptors, and mutant viruses borrowing cellular secretory paths, may help to reveal mechanisms that for the time being can only be inferred from morphological and kinetic studies. But the most powerful approach should lie with the temperature-sensitive mutants of yeast: first, because they may affect components of the selective mechanism rather than the selected molecule; and second, because their temperature sensitivity makes it possible to examine defects that would otherwise be lethal. □

Miranda Robertson is an associate editor of Nature.

1. see Warren, G. *Nature* **297**, 624 (1982).
2. Talmage, K., Brosins, J. & Gilbert, W. *Nature* **294**, 176 (1981).
3. Randall, L.L. *Cell* **33**, 231 (1983).
4. Wickner, W. *Trends biochem. Sci.* **8**, 90 (1983)
5. Ohno-Iwashita, Y. & Wickner, W. *J. biol. Chem.* **258** 1895 (1983).
6. Walter, P. & Blobel, G. *Cell* **34**, 525 (1983).
7. Reizman, H. *et al. EMBO J.* **2**, 216 (1983).
8. Hase, T. *et al. EMBO J.* **2**, 169 (1983).
9. Novick, P. & Schekman, R. *J. Cell Biol.* **96**, 541 (1983).
10. Hopkins, C.R. *Nature* **304**, 684 (1983).
11. Brown, M.S., Anderson, R.G.W. & Goldstein, J.L. *Cell* **32**, 663 (1983).
12. Helenius, A., Mellman, I., Wall, D. & Hubbard, A. *Trends biochem. Sci.* **8**, 245 (1983).

0028-0836/84/070595-01$01.00

© 1984 Macmillan Journals Ltd

Photochemistry

A quantum step towards artificial photosynthesis

from J. Barber

ACCORDING to an article by Moore and colleagues published in this issue of *Nature* (see p.630), one of the major obstacles to the development of an artificial photosynthetic system has been overcome. For many years the goal of photochemists has been to provide an artificial system that mimics the unique properties of natural photosynthetic reaction centres — particularly their striking ability to facilitate a very rapid and efficient light-induced separation of charge without a significant back-reaction. To date, it has proved difficult to make model systems which do not suffer from high back-reaction rates; now Moore and colleagues seem to have achieved success.

Several different types of reaction centre are found in photosynthetic organisms. Higher plants and algae, which use water as a source of electrons and protons to reduce carbon dioxide and which produce oxygen as a by-product, contain two different types of reaction centre, known as photosystem one and photosystem two. Photosynthetic bacteria, which do not oxidize water, but oxidize more reducing substances such as H_2S and organic acids and therefore do not produce oxygen, contain only one type of reaction centre. Despite these differences, all photosynthetic reaction centres consist of a chromophore (P), which absorbs light energy, and a closely associated electron donor (D) and acceptor (A). The organization of these three components allows the following sequence of events to occur when a photon is absorbed by the chromophore:

$$DPA \xrightarrow{h\nu} DP^*A \rightarrow DP^+A^- \rightarrow D^+PA^-$$

The D^+PA^- state is further stabilized by rapid secondary electron transfer processes. P is always a chlorophyll molecule but the chemical nature of D and A varies, with A most often being a quinone. For photosystem one and the bacterial system, D is a metalloprotein, either plastocyanin or cytochrome *c*; the nature of the primary electron donor in the photosystem two reaction centre is unknown but under certain circumstances can be the carotenoid β-carotene. Unlike photosystem one and two, functional reaction centres of photosynthetic bacteria have been isolated free of their light-harvesting proteins and subjected to detailed physical and chemical studies.

With these preparations it has been shown that P^+A^- formation occurs within 100 picoseconds of an excitation flash from an ultrafast laser. The back-reaction is 100 times slower than the forward reaction; normally the P^+A^- radical pair will be quickly converted to D^+PA^- which then has a much longer lifetime.

Moore and his co-workers have succeeded in synthesizing a very stable molecule which mimics the photochemical properties of natural reaction centres. Their molecule consists of three covalently linked entities: a chromophore (P), a donor (D) and an acceptor (A). The chromophore used is tetraarylporphyrin which has a structure and light-absorption properties comparable to those of chlorophyll. The acceptor is a quinone and the donor is a substituted β-carotene.

Because D and A are spatially separated by the porphyrin, the tripartite molecule has some remarkable properties. Flash spectroscopy shows that D^+PA^- is generated within 200 picoseconds and has a long lifetime — charge recombination occurs on a microsecond time scale. The quantum yield for its formation is also very high and, in certain conditions, can reach 25 per cent. The energy stored in the charge complex is at least 1 V; the conversion of energy is thus remarkably efficient given that only 1.8 eV is available from the absorbed photon. Surprisingly, the stabilized charge-transfer complex is generated from the singlet state of the excited porphyrin, with triplet states occurring only when the quinone is chemically reduced before illumination. In this situation, the porphyrin triplet is converted in 20 nanoseconds to a less reactive carotenoid triplet by a protective mechanism very reminiscent of a process seen in photosynthetic tissue. In nature, carotenoids not only function to quench chlorophyll triplets but also serve as light-harvesting pigments. Interestingly, Moore and colleagues have found that in their molecular triad, 10 per cent of light energy directly absorbed by the β-carotene moiety is transferred to the porphyrin.

Clearly Moore and colleagues have synthesized a very interesting molecule which, like photosynthetic reaction centres, can efficiently use the singlet energy of an excited porphyrin molecule to bring about stabilization of positive and negative charges to store chemical potential energy. Not only does the molecular triad give a convenient synthetic system for studying very fast photochemical reactions similar to those which occur in photosynthesis, but it could perhaps be exploited in a solar cell in which the molecule would catalyse further secondary reactions which generate a usable chemical or electrical fuel. □

J. Barber is Professor of Plant Physiology and Head of the AFRC Photosynthesis Research Group in the Department of Pure and Applied Biology, Imperial College, London SW7 2BB.



100 years ago

PROFESSOR Ruskin's utterances are perhaps to be taken least seriously when he is himself most serious, and probably he was never more in earnest than in his jeremiad on modern clouds, delivered at the London Institution on the 4th and 11th inst. Probably none of the readers of Nature have been terrified by the storm cloud of the nineteenth century, but should it be otherwise we hasten at once to their relief. Twenty years before the date fixed by Mr. Ruskin for the first appearance of his portentious "plague-cloud", the writer of the present article commenced a series of observations on the forms and structures of clouds, followed a few years later by such daily charts of wind and weather as could be constructed from the data, somewhat meagre, that were then accessible. As might be expected, cyclone and anticyclone were then as they are now. The dimensions and densities of the cloud layers have not altered, neither has our moral degeneracy nor the increased smoke of our manufacturing towns developed any new form of cloud. Neither (until the phenomenal sunrises and sunsets of the last three months) has Nature, in painting the clouds, employed upon her palette any fresh tints, whatever artists may have done. Further, we have not observed, nor met with any one, except Mr. Ruskin, who has observed, that the wind during the last thirteen years has adopted a "hissing" instead of a "wailing" tone.

Admiration ought ungrudgingly to be bestowed on one who has done good service as an art critic and as a contributor to English literature. The sympathy, moreover, which, denied to those who are in advance of their age, is naturally accorded to the archaic type of mind, is enhanced by the attractiveness of a personality whose idealism is as lofty as that of Mr. Ruskin. But we maintain that there is a further sentiment which contributed to the applause which Mr. Ruskin's audiences bestowed upon him. Speaking generally, "broadly and comfortably", as he would say, Mr. Ruskin is not a representative man, yet he represents a certain spirit of Philistinism (for it merits this name), which is far from being unpopular, and which shows itself in opposition to scientific culture. He is the spokesman of that mental attitude which misinterprets the province of science and affects to misunderstand the plainest utterance of the physicist. "The first business", he says, "of scientific men is to tell you things that happen, as, that if you warm water it will boil". "The second and far more important business is to tell you what you had best do under the circumstances — put the kettle on in time for tea." "But if beyond this safe and beneficial business they ever try and explain anything to you, you may be confident of one of two things — either that they know nothing (to speak of) about it, or that they have only seen one side of it, and not only have not seen, but usually have no mind to see, the other".

From *Nature* **29**, 353, 14 February 1884.

0028-0836/84/070596-01$01.00

© 1984 Macmillan Journals Ltd

Palaeoclimatology

Onset of the ice age in the North Atlantic

from Brian Funnell

IN a paper published in this issue of *Nature* (see page 620), Shackleton and colleagues[1] complete a careful interdisciplinary study of sediment cores obtained in the north-east Atlantic and establish with admirable precision that ice-rafted debris first appeared there about 2.5 Myr BP, with the first major input of debris slightly later, at 2.37 Myr BP. The results make it possible to assert with confidence that the North Atlantic and its bordering continents have been subject to periodic glaciation for no less than the past two and a half million years.

Although earlier investigations[2] of deep-sea sediments had indicated ice-rafting of debris into the North Atlantic from as long ago as approximately 3 Myr, it was not until the hydraulic piston corer was added to the Deep Sea Drilling Project's range of equipment that good-quality cores, suitable for detailed analysis, could be extracted from deep enough into ocean sediments to allow a full interpretation of the time period earlier than half a million years ago.

Shackleton and colleagues investigated one of the earlier cores to be obtained with the hydraulic piston corer (Leg 81, site 552A of the Deep Sea Drilling Project, from a depth of 2,300 metres) using a combination of micropalaeontological, palaeomagnetic and stable oxygen isotope methods.

The magnitude of the oxygen isotope signal (which mainly reflects global ice volumes) and the abundance of ice-rafted debris at 2.37 Myr leave no doubt that the extent of this episode of glaciation in and around the North Atlantic is fully comparable with the most recent glacial episode, which culminated only 18,000 years ago and melted back between 11,000 and 6,000 years ago.

This new date for the onset of general Northern Hemisphere glaciation accords well with a date of 2.4 Myr proposed by workers in the Netherlands[3] for the first indication of major cooling in and around the southern margins of the North Sea. Here there is unequivocal evidence of a massive conversion of a temperate forest cover into cold heath conditions, although there is so far no evidence of actual glacierization.

The carbonate content of the deep-sea sediments[1] (which varies in inverse proportion to the ice-rafted component) shows that before 2.5 Myr BP there was no significant sea ice in the North Atlantic. The stable oxygen isotope record shows that before 2.37 Myr ice volume maxima were absolutely less extreme, although subdued fluctuations, presumably mainly due to variations in the volume of the earlier and older Antarctic polar ice cap, still continue into the more remote past.

From the carbon isotope record of the core sediments it is clear that the formation of North Atlantic deep water by winter cooling at the surface of the Norwegian–Greenland Sea was already established by 3.5 Myr ago, before the onset of glaciation. The surface water, being cold and dense, sinks to the bottom of the North Atlantic. There it gives rise to a higher ^{13}C content in bottom-living calcareous microfossils than do the 'older' Antarctic bottom waters which have had more time to gain ^{12}C from the oxidation of ^{12}C-rich organic matter falling through the water column.

Since site 552A was drilled, Leg 94 of the Deep Sea Drilling Project has explored six more sites in the North Atlantic using the hydraulic piston corer[4], with the specific intention of elucidating the history of North Atlantic waters over the last few million years. What we can look forward to now is a detailed spatial and temporal record of past climatic changes in this critical North Atlantic area which will be invaluable for a better understanding of the mechanisms that control its physical oceanography and surrounding climate. □

Brian Funnell is Professor in the School of Environmental Sciences at the University of East Anglia, Norwich NR4 7JJ.

1. Shackleton, N.J. *et al. Nature* **307**, 620 (1984).
2. Berggnen, W.A. *Init. Rep. DSDP* **12**, 953 (1972).
3. Suc, J-P. & Zagwijn, W.H. *Boreas* **12**, (1983).
4. Kidd, R.B. *et al. Nature* **30**, 532 (1983).

Neurobiology

Building acetylcholine receptors

from Charles F. Stevens

THE Kyoto group has done it again. Just three months ago, *Nature*'s report on the C Cold Spring Harbor Symposium on molecular neurobiology noted that an important advance in channel research could be achieved by the expression in cultured cells of cDNA coding for the four kinds of acetylcholine receptor (AChR) subunits (see *News and Views* **306**, 14; 1983). Numa and his colleagues at Kyoto University have now taken this prize and at the same time made an unexpected observation on the relation between receptor structure and function (see this issue of *Nature*, p.604).

Recall that channels are integral membrane proteins responsible for the electrical activity of the nervous system and that the AChR is the best studied such molecule. The AChR is a pentameric membrane protein composed of four subunits assembled with the stoichiometry $\alpha_2\beta\delta\gamma$. Binding of acetylcholine to the receptor opens the door of a water-filled cation-selective pore for about a millisecond and lets half a million ions traverse the membrane. The channel protein has been purified, partially sequenced, functionally reconstituted, the cDNA for all four subunits has been cloned and sequenced, and genomic clones for at least one of the subunits have been identified and partly sequenced. The function of single copies of the AChR has been studied with the single-channel recording method.

As the report of the molecular neurobiology symposium pointed out, one of the next steps in clarifying the relationship between molecular structure and function would be to get the cDNA expressed in an appropriate system. Once expression has been achieved, protein structure can be altered rationally and systematically by modifying the DNA, providing a powerful tool for discovering what part subunits, domains and specific amino acids play in the operation of the AChR. The Kyoto group has made a significant start on this programme and has used an interesting approach.

There are three steps to success: constructing an expression vector, finding a method for making appropriate quantities of AChR mRNA and using a system that synthesizes and assembles receptors, and inserts them into surface membrane. Numa and co-workers combined three approaches that previously had been successful in different contexts.

O'Hare *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **78**, 1527; 1981) constructed an expression vector that can be used to transfect cultured cells so that they will express a foreign protein. The Kyoto group used a version of this vector to transfect a cell line, designated COS, prepared by Gluzman (*Cell* **23**, 175; 1981) that contains as part of its genome the simian virus 40 (SV40) DNA required for replication of that virus. The vector used by the Kyoto group contains the replication origin of the SV40 DNA and is thus easily replicated by COS cells: the result is that DNA coding for the AChR subunits is amplified and a relatively large amount of mRNA is made. Cultures were separately transfected with cDNA for each of the four types of AChR subunit, and mRNA coding for each subunit was isolated from the transfected cell lines. This message was then combined and injected into frog oocytes for transla-

© 1984 Macmillan Journals Ltd

tion, receptor assembly and insertion into surface membrane; Barnard *et al.* (*Proc. R. Soc.* **B215**, 241; 1982) had previously used this system to make functional AChRs from message isolated from *Torpedo* electric organs (a rich source of AChR message). The advantages of having separate systems for making message and for using it are that proportions of the message for various subunits can be controlled — for example, mRNA for a given subunit can be completely omitted — and quantity of message can be varied at will.

The Kyoto group find transfected cells make AChR message that is properly processed. Furthermore, when mRNA for all four subunits is injected into oocytes in normal proportions, functional AChRs appear in the surface membrane in numbers that reflect the quantity of message injected; these receptors have apparently ordinary electrophysiological properties and bind appropriately the snake toxin used to identify AChRs. An unexpected result is that functional receptors appeared in oocytes injected with only three of the four subunits. Apparently one of the larger subunits can substitute for another to form working receptors. Although the possibility of artefact is not entirely ruled out, substitution probably can occur because the gross structure of the various subunits is quite similar — there are strong homologies between all four subunits — and at a certain functional level they are interchangeable. That the two largest subunits are interchangeable supports the pseudosymmetric channel structures that have been proposed by several authors (for references see the paper by Numa *et al.* and the symposium report).

Before this new work from the Kyoto group, the extent to which expression of this rather complicated and delicate membrane protein could be achieved was uncertain. We now know it works; next will come an exploration of altered regulation and function due to systematic and rational changes in amino acid sequence. □

Charles F. Stevens is in the School of Medicine, Yale University, 333 Cedar Street, New Haven, Connecticut 06510.

Plant science

Evolution of the oaks

from Peter D. Moore

OUR knowledge of the fossil history of the oaks has recently been greatly enriched by a description of a range of fossils from an Oligocene locality near Huntsville, Texas, by C.P. Daghlian and W.L. Crepet[1]. They have found numerous leaves, fruit and a fragment of a catkin, all of which clearly belong to an oak, or possibly several oak species, and suggest that the oak underwent a rapid evolutionary radiation between the Eocene and the Oligocene.

The genus *Quercus* (the oaks) is a large one, containing somewhere in the region of 300–500 species depending upon whose system of taxonomy you follow. It is characterized within the family Fagaceae (which includes beech and sweet chestnut) by pendulous male catkins, separate female inflorescences and by a nut partially enclosed by a cupule of bracts — the characteristic acorn. Within the genus there is a high level of interspecific fertility, so hybrids are frequent, and there is a great deal of variation in such characters as leaf shape and evergreen or deciduous habit. A high level of variation is also found within many of the species, which further complicates their taxonomy.

A number of attempts have been made to subdivide the genus into subgenera, but authors are not agreed upon the way in which this should be effected. The result is that some species are placed in different subgenera by different authorities. Given such a confused and difficult taxonomic state of affairs among the modern oak, one can only express profound sympathy for palaeontologists attempting to unravel the fossil history of the genus.

The family Fagaceae was probably present in the late Cretaceous and was certainly well represented by the early Tertiary[2]. *Quercus* wood has been found in Eocene deposits in Britain[3] and leaves and fruits in the United States[4].

The new set of fossils found in Texas were not in intimate association, so it is not possible to be sure whether they do in fact belong to a single species — indeed, careful examination suggests that they possess characters indicative of not only separate species, but even separate sub-genera.

The flower structure within the catkin, with six stamens having extrorse anthers, leads the authors to propose that it belongs to a member of the sub-genus Erythrobalanus, currently found in Central and North America. The pollen grains are coarsely scabrate, which is also true of the Erythrobalanus oaks. Some leaves found near the flowers resemble those of this sub-genus too, having finely pointed vein endings.

The fossil acorn, however, has basally thickened cup scales which are more frequently associated with another sub-genus, Lepidobalanus. This is a rather heterogeneous sub-genus, containing our two British oaks *(Q. robur* and *Q. petraea)*, some other European species (such as *Q. ilex)* and some North American species (for example *Q. alba* and *Q. arizonica)*.

The outcome of these findings is that the authors propose three new oak species, assigning the catkin to *Q. oligocenensis*, the leaves to *Q. catahoulaensis* (both sub-genus Erythrobalanus) and the acorn to *Q. huntsvillensis* (sub-genus Lepidobalanus).

The fact that this mixture of characters should be present in the same sediments, whether or not they are derived from just a single species or several, suggests that *Quercus,* by the Oligocene, had undergone a considerable degree of evolutionary development and had acquired some of the features now characterizing its present-day sub-genera. Before one can accept this rapid evolutionary radiation in oak, however, one must examine the reliability of characters used in defining sub-genera. The subdivision of the genus *Quercus* adopted by *Flora Europaea*, for example, differs considerably from that accepted by Daghlian and Crepet. Lepidobalanus is not used there, but some of its members (for example, *Q.robur*) are taken into sub-genus *Quercus* and others (for example *Q. ilex*) to a further sub-genus Sclerophyllodrys. Evidently the sub-generic boundaries within the genus are far from clear.

Pollen morphology in *Quercus* has been studied fairly intensively, but the criteria upon which any subdivision of the genus can be firmly based are a matter of dispute. Spoel-Walvins[5], for example, proposed that some of the south European oak species can be separated by pollen form and sculpturing, but the use of such characteristics in the analysis of Quaternary sub-fossil pollen in Spain has met with very limited success[6]. Examination of type material from numerous sources reveals a very wide degree of intraspecific variations in pollen sculpturing.

One must be cautious, therefore, before fully accepting the rapid evolution of *Quercus* between the Eocene and the Oligocene. The proposal does, on the other hand, tie in well with what is known of environmental conditions in that period. It was a time of climatic cooling, though probably not such a rapid cooling as was once thought[7], and of falling sea levels that left new tracts of land exposed and created new ecological opportunities. Daghlian and Crepet feel that this environmental instability could underlie the diversification of the genus *Quercus* in the early Tertiary. The next step must be the extraction and analysis of more oak fossils from Eocene and Oligocene rocks in order to examine the extent of this diversification. □

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences, King's College, 68 Half Moon Lane, London SE24 9JF.

1. Daghlian, C.P. & Crepet, W.L. *Am. J. Bot.* **70**, 639 (1983).
2. Muller, C.J. *Bot. Rev.* **47**, 1 (1981).
3. Brett, D.W. *Palaeontology* **9**, 86 (1960).
4. Manchester, S.R. *Oregon Geol.* **43**, 75 (1981).
5. Spoel-Walvins, M.R. van der *Acta bot. neerl.* **12**, 525 (1963).
6. Stevenson, A.C. thesis, Univ. London (1981).
7. Collinson, M.E., Fowler, K. & Boulter, M.C. *Nature* **291**, 315 (1981).

0028-0836/84/070598-01$01.00

© 1984 Macmillan Journals Ltd

Acidic deposition and internal proton sources in acidification of soils and waters

N. van Breemen*, C. T. Driscoll† & J. Mulder*

* Department of Soil Science and Geology, Agricultural University, 6700 AA, Wageningen, The Netherlands
† Department of Civil Engineering, Syracuse University, Syracuse, New York 13210, USA

Enough is now known of the sources of hydrogen ions (protons) in natural ecosystems for the relative importance of external and internal sources to be compared. In acidic soils in the northeastern United States and western Europe, external often exceeds internal proton loading, resulting in soil acidification by aluminium dissolution and sulphate retention. One consequence is the export of ecologically significant quantities of hydrogen ion and aluminium in drainage water.

THERE is considerable controversy over the environmental impact of 'acid rain'. Some researchers claim that atmospheric inputs of anthropogenically-derived acidic substances cause acidification of soils and surface waters with serious effects on vegetation and fish[1,2]. Others contend that internal proton production in soils, sometimes intensified by changes in land use, overshadow the effects of acidic atmospheric deposition[3,4]. Thus an evaluation of the relative contributions of atmospheric inputs and internal proton sources to acidification of soil and water would be very useful. We have recently proposed an approach to identify and quantify proton fluxes and soil acidification[5,6]; we now use this approach to evaluate proton transformations for several ecosystems and discuss the implications for soil acidification and water quality.

Concepts

When evaluating acidification processes it is essential to distinguish between intensity factors (such as *p*H) and capacity factors (such as acid- and base-neutralizing capacity). Failure to make this distinction[4] lies at the root of much confusion about the nature of soil and water acidification. In aqueous systems the acid-neutralizing capacity (ANC (aq)) is determined by strong acid titration to a reference *p*H of about 4.5 and is defined as the aqueous base equivalence minus the strong acid equivalence[7].

$$\mathrm{ANC\ (aq;\ ref.\ 4.5)} = [\mathrm{HCO_3^-}] + 2[\mathrm{CO_3^{=}}] + [\mathrm{A^-}] + [\mathrm{OH^-}] - [\mathrm{H^+}]$$

where $[A^-]$ is the molar concentration of a potential proton accepting anion (such as a natural organic anion).

In an analogous manner we define the acid-neutralizing capacity of the inorganic fraction of a mineral soil as the sum of basic components minus the strongly acidic components [brackets denote molar concentrations].

$$\mathrm{ANC\ (s)} = 6[\mathrm{Al_2O_3}] + 6[\mathrm{Fe_2O_3}] + 2[\mathrm{FeO}] + 4[\mathrm{MnO_2}] + 2[\mathrm{MnO}] + 2[\mathrm{CaO}] + 2[\mathrm{MgO}] + 2[\mathrm{Na_2O}] + 2[\mathrm{K_2O}] - 2[\mathrm{SO_3}] - 2[\mathrm{P_2O_5}] - [\mathrm{HCl}]$$

Weak acid components (such as CO_2 (s), SiO_2 (s) and H_2S (s)) do not contribute to ANC (s) because of their inability to accept or donate protons at reference *p*H values of soils (generally *p*H 2–5)[5]. Also because the nitrogen content of mineral material is generally low, nitrogen components are not included in our definition of ANC (s).

The capacity factor ANC, rather than the intensity factor *p*H, is directly influenced by addition or depletion of protons, and hence is fundamental to acidification and alkalinization. *p*H generally, but not always, decreases during addition of protons and generally, but not always, increases during depletion of protons[5]. While *p*H is of more direct ecological importance than ANC, the relationship between *p*H and ANC is beyond the scope of this paper. Detailed discussions on the functional relationship between *p*H and ANC (aq)[7] or soil mineralogy[8] (which in turn determines ANC (s)) are available elsewhere.

Soil acidification is defined as a decrease in ANC (s) and is accomplished by removal of cationic components (such as CaO) from a mineral soil or, to a lesser extent, by addition of acidic components (such as SO_3). Conversely soil alkalinization is an

Table 1 Proton producing and consuming processes in terrestrial and aquatic ecosystems

Proton-producing processes		Proton-consuming processes
(1) Atmospheric input	H^+ (aq(rain/drain water)) $\rightleftharpoons$ H^+ (aq(soil solution))	(1) Drainage
(2) Assimilation of cations	M^{n+} (aq) + nR−OH (org) $\rightleftharpoons$ (R−O)$_n$M (org) + nH$^+$ (aq)	(2) Mineralization of cations
(3) Mineralization of anions	nH$_2$O + R$_n$−A (org) $\rightleftharpoons$ nR−OH + A^{n-} (aq) + nH$^+$ (aq)	(3) Assimilation of anions
(4) Dissociation of acids	H$_n$A (aq) $\rightleftharpoons$ A^{n-} (aq) + nH$^+$ (aq)	(4) Protonation of anions
(5) Oxidations	Red (aq, s, g) + mO$_2$ + nH$_2$O $\rightleftharpoons$ Ox^{r-} (aq, s, g) + rH$^+$ (aq)	(5) Reductions
(6) Reverse weathering of cations	M^{n+} (aq) + $\frac{1}{2}n$H$_2$O $\rightleftharpoons$ $\frac{1}{2}n$M$_{2/n}$O (s) + nH$^+$ (aq)	(6) Weathering of metal oxide components
(7) Weathering of anionic components	NO$_{(m+n)}$ (s) + mH$_2$O $\rightleftharpoons$ NO$_{(2m+n)}^{2m-}$ (aq) + 2mH$^+$ (aq)	(7) Reverse weathering of anions

Quantitatively important cations (M^{n+}) include Ca^{2+}, Mg^{2+}, Na^+, K^+ (equations (2), (6)), NH_4^+ (2) and Al^{3+} (6). Important anions (A^{n-}) are $H_2PO_4^-$ (equations (3, 7)), NO_3^- (3) and SO_4^{2-} (3, 7). Mineralization and assimilation of SO_4^{2-} and NO_3^- involves oxidation and reduction processes but the net reactions are equivalent to equations (3). Important reduction (Red)–oxidation (Ox) couples in gas/water/soil systems include NH_4^+/NO_3^-, N_2/NO_3^-, H_2S/SO_4^{2-}, SO_2/FO_4^{2-} and Fe^{2+}/Fe_2O_3. Reduction/oxidation reactions (5) have been coupled to the O_2—H_2O system. In actuality, organic matter (CH_2O) is usually the electron donor in reduction reactions (5); CH_2O oxidation is implicitly expressed in this reaction because organic matter is oxidized during aerobic respiration ($O_2 + CH_2O \rightleftharpoons H_2O + CO_2$). Cation and anion exchange reactions can be represented by reactions (6) and (7).

0028-0836/84/070599—06$01.00

© 1984 Macmillan Journals Ltd

Table 2 Proton fluxes, rates of soil acidification, and external/internal proton ratios of different ecosystems

Case	Ecosystem	Soil, vegetation	Measuring period	H^+-sources (kmol hectare^{-1} yr^{-1})					H^+-sinks (kmol hectare^{-1} yr^{-1})					External/internal proton ratio	Δ ANC (s)
				External		Internal									
				Input/N-transfer	CO_2	Biomass	Weathering	Σ+	Σ−	Weathering	Biomass	N-transfer	Stream		
1	World average	No net biomass assimilation	—	0.0/0.1	2.6	0.0	0.5	3.2	3.2	3.2	0.0	0.0	0.0	0.03	−2.7
Soil alkalinization															
2	Lake Chad, Chad	Bottom of alkaline lake	1967–72	0.0/0.0	−3.3*	0.0	3.4	3.4	3.3	0.0	0.0	0.0	0.0	0	+3.4
Low rates of soil acidification															
Bogs															
3	Thoreau's bog, Mass.	Sphagnum bog	1976–77	0.9/0.0	0.5†	0.1	0.0	1.6	1.3	0.4§	0.3	0.1	0.5	1.33	−0.1‖
4	Storgama, Norway	Oligotrophic peat, *p*H 4	1974–76	0.5/0.2	0.3‡	0.0	0.0	1.0	0.9	0.4	0.0	0.2	0.3	1.67	−0.4
Well drained soils															
5	Birkeness, Norway	80% Forested podzols, 20% peat	1974–76	1.2/0.7	0.5‡	0.0	0.0	2.4	2.3	1.3	0.0	0.7	0.3	2.40	−1.3
6	Lüneburger Heide, FRG	Haplorthod, Calluna	1978–79	0.6/0.5	0.0	0.6	0.7	2.4	2.4	1.6	0.3	0.2	0.2	0.69	−0.9
7	HBEF, New Hampshire	Haplorthod, *p*H 3.6–5, hardwoods	1963–74	1.3/0.1	0.1	1.0	0.1	2.6	2.5	2.2	0.2	0.0	0.1	1.17	−2.1
8	Gårdsjön, Sweden	Podzols, *p*H 4.5, *Picea, Pinus*	1979–81	1.0/0.7	0.7‡	1.0	0.3	3.7	3.5	2.4	0.0	0.7	0.5	0.50	−2.1
9	Mondo¶, Indonesia	Andosol, *p*H 5.5, *Agathis* plantation	1976–77	0.0/0.0	4.0	1.2	1.4	6.5	6.5	3.5	2.8#	0.2	0.0	0	−2.1
Intermediate rates of soil acidification															
10	Solling, FRG	Acid brown forest soil, *p*H 4, *Fagus*	1969–79	1.7/0.8	0.0	1.5	0.1	4.1	4.3	3.0	0.3	0.5	0.5	1.25	−2.9
11	Solling, FRG	*Picea*	1973–79	3.7/1.1	0.0	2.1	0.2	7.1	7.0	6.1	0.6	0.0	0.4	2.09	−5.9
12	Hackfort A, Neth.	Haplaquept, *p*H 4, *Quercus, Betula*	1981–82	0.4/4.2	0.0	0.8	1.1	6.4	6.2	6.1	0.1	0.0	0.1	2.42	−5.0
13	Hackfort B, Neth.	Dystrochrept, *p*H 4, *Quercus*	1981–82	0.4/3.9	0.0	1.1	0.8	6.2	5.8	5.6	0.1	0.0	0.1	2.26	−4.8
14	Hackfort C, Neth.	Udipsamment, *p*H 4, *Quercus, Betula*	1981–82	0.4/2.6	0.0	0.7	0.7	4.4	4.4	3.9	0.1	0.3	0.0	1.93	−3.2
15	Andrews**, Oregon	Dystrochrept, mature *Pseudotsuga*	1973–75	0.3††/0.0	0.9	0.0	3.2‡‡	4.5	4.4	4.4	0.0	0.0	0.0	0.07	−4.4§§
High rates of soil acidification															
16	North. Cascades, Wash.	Quartz-diorite + glacial till no vegetation	1970	0.4/0.0	7.2	0.0	2.2‡‡	9.8	9.9	9.9	0.0	0.0	0.0	0.04	−9.9§§
17	Hackfort D, Neth.	Haplaquoll, *p*H 7 *Quercus, Alnus, Populus*	1981 (1 event)	0.4/5.9	1.7	2.4	0.8	11.1	10.1	10.0	0.1	0.0	0.0	1.07	−9.2
18	Castricum I, Neth.	Undipsamment, 3.5% $CaCO_3$, no vegetation	1952 (5 events)	1.5‖‖/1.0	12.6	0.0	0.0	15.1	15.6	15.6	0.0	0.0	0.0	0.20	−15.6
19	Castricum III, Neth.	Udipsamment, *Quercus*	1952 (5 events)	1.6‖‖/0.6	12.4	0.0¶	0.2	15.0	15.1	14.5	0.0	0.6	0.0	0.13	−14.3
20	Castricum IV, Neth.	Udipsamment, *Pinus*	1952 (5 events)	1.7‖‖/0.6	7.8	0.1¶	0.2	10.3	10.3	9.7	0.0	0.6	0.0	0.21	−9.5
Deforested system															
21	HBEF, New Hampshire	Haplorthod, *p*H 3.6–5; deforested	1966 (2 events)	1.0/11.2##	0.0	0.8	2.1	15.1	13.9	4.3	5.9	3.1	0.6	0.07	−2.2

See Table 3 for references and hydrological characteristics of ecosystems.

* Proton neutralization due to protonation of HCO_3^-.

† Organic acid deprotonation rather than CO_2 deprotonation (measured).

‡ Organic acid deprotonation rather than CO_2 deprotonation (estimated by discrepancy in charge balance of drainage water).

§ Largely sulphate reduction.

‖ Proton neutralization due to sulphate reduction is not included in ΔANC (s).

¶ Anion input, output and assimilation data from L. A. Bruynzeel (personal communication). The assumptions are: sulphur input equals sulphur output; HCO_3^- in drainage water estimated from charge balance discrepancy.

High rates of net Na^+, K^+ and Mg^{2+} mineralization in a young (recently cut, <40 yr) forest plantation.

** Input of Cl^- and output of SO_4^{2-} are estimated from discrepancy in charge balance. Data from weir are used as output. Cl^- output is assumed to equal Cl^- input.

†† Dry deposition of S was assumed to be SO_4^{2-} in throughfall less SO_4^{2-} in precipitation.

‡‡ Assumed to be due to pyrite (FeS_2) oxidation.

§§ The transformation of H_2S_2 (s) to SO_3 (aq) that is removed by leaching does not change ANC (s), so ΔANC (s) is equivalent to H^+ consumed in weathering.

‖‖ Wet deposition was estimated from Cl^- output in the unvegetated lysimeter, assuming that Cl^- input equalled Cl^- output and the stoichiometry of ions in precipitation in 1952–55 were similar to values in 1980 [14]. Sea spray on vegetation surfaces was calculated by assuming that Cl^- output less Cl^- input equals Cl^- dry deposition. Any SO_4^{2-} output that exceeded SO_4^{2-} inputs from wet deposition and sea spray was attributed to dry deposition of SO_2.

¶¶ Biomass accretion was estimated by measured increases in tree height and literature values of nutrient content [15,16].

Mineralization of organic N.

increase in ANC (s). Changes in ANC (s) may involve both the weathering/formation of soil minerals, and adsorption/desorption reactions. Although Fe_2O_3 and Al_2O_3 are included in ANC (s), they are relatively insoluble at *p*H values above 3 and 5 respectively and therefore are important only in the acidification of moderately to strongly acidic soils. Oxidation of FeO to Fe_2O_3, common in weathering zones with FeO-containing minerals, increases ANC (s). Because this increase is difficult to quantify and is of no practical significance we will neglect the effect of Fe_2O_3 on ANC(s).

Soil acidification occurs by an irreversible flux of protons to the soil. Proton sources include: (1) atmospheric inputs of acidic or potentially acidic substances (such as SO_2); (2) net assimilation of cations by vegetation; (3) net mineralization of anions from organic matter; (4) deprotonation of weak acids; (5) oxidation reactions; (6) precipitation (reverse weathering) of cations; and (7) mineral weathering of anionic components (Table 1).

Protons entering or produced in the soil may be removed by (1) export in drainage water, (2) net mineralization of cations in organic matter, (3) net assimilation of anions by vegetation, (4) protonation of anions, (5) reduction reactions, (6) weathering of cationic components and (7) precipitation of anions (Table 1).

Free or bound protons (such as H_2CO_3) are transferred to the soil predominantly through the dissolution of cationic components.

If a soil completely neutralizes proton inputs, basic cations

(Ca^{2+}, Mg^{2+}, Na^+, K^+) are released and exported from the soil by vegetation uptake or by outflow of drainage water, resulting in soil acidification. Aqueous alkalinization (an increase in ANC (aq)) generally occurs as a counter-process to soil acidification. Alkalinization of the percolating soil solution is accomplished by neutralization of free protons in solution by soil, or by the donation of bound protons to the soil resulting in the formation of unprotonated anions (such as HCO_3^-).

If there are considerable proton inputs to a soil, and soil-bound basic cations are present in low concentrations or have characteristically slow dissolution kinetics, or the retention time of water within the mineral soil is short, the neutralization of proton inputs may be incomplete and result in the export of free protons in drainage water from the soil. A consequence of elevated concentrations of free protons (low *p*H) is the dissolution of aluminium from soil. Aluminium dissolution is realized in acidic conditions ($pH < 5.5$) and results in a solution that contains both hydrogen ion and aluminium base neutralizing capacity[9]:

$$\text{BNC (aq, ref. } p\text{H } 5.5) = H^+ + n[Al^{n+}].$$

Elevated levels of dissolved aluminium are ecologically significant due to toxicity to aquatic organisms[10,11] and vegetation[12].

There are two approaches to the quantification of soil acidification. A historic approach requires the determination of ANC (s) in a complete soil profile, including parent material, or a chronosequence of soils. An actualistic approach involves the measurement of all proton fluxes so that a proton budget can be calculated. The measurements required to compile a proton budget include: the flux of solutes entering and leaving the ecosystem; the net assimilation (or mineralization) of substances by living or dead biomass; the net quantities of potentially acidic gases and aerosols entering and leaving the ecosystem; and the net sorption (for example, by precipitation) or desorption of substances in the soil. The advantages of the proton budget approach over the historic approach are obvious; it reflects the current conditions of an ecosystem, identifies various processes involved in soil acidification, is sensitive compared with the analysis of soil material and requires no assumptions about the authenticity of soil parent material. In our discussion of soil acidification we will follow only the proton budget approach. Further details on the processes of soil acidification and methods of quantifying soil acidification are available elsewhere[5].

Proton accounting

We have developed proton budgets using data from several ecosystem studies (Table 2). To facilitate our discussion we have categorized total proton sources and sinks, for each ecosystem into their principal constituents. Proton sources include: (1) atmospheric inputs of free protons and SO_2; (2) the proton loading associated with nitrogen transformations, which is the net ammonium input plus the net nitrate output (this accounts for the protons generated during the net assimilation of atmospheric NH_4^+ by biota and net nitrification); (3) the deprotonation of CO_2 or organic acids; (4) net assimilation of non-nitrogen cations by biomass (or net non-nitrogen anion mineralization); and (5) anion weathering (plus any reverse cation weathering).

We have subdivided proton sinks into: (1) cationic weathering (plus any reverse anionic weathering); (2) biomass assimilation of non-nitrogen anions; (3) proton neutralization associated with nitrogen transformations (which is net nitrate input/net ammonium output); and (4) drainage export of free protons.

We define external proton inputs (which we presume are largely anthrophogenic in origin) as atmospheric inputs of H^+ and SO_2 plus proton sources minus proton sinks due to N transformations or, in other words, net ammonium input minus net nitrate input. The balance of proton sources (3), (4) and (5) will be considered as internal proton loading. It is invariably arbitrary to partition proton loading into internal and external sources. For example, atmospheric NH_4^+ is generally derived from NH_3 volatilization and protonation by acidic atmospheric substances (for example, H_2SO_4 from SO_2, HNO_3). Therefore protons generated from assimilation or oxidation of atmospheric NH_4^+ in the soil clearly reflect an external source. On the other hand in degrading ecosystems mineralization of organic nitrogen followed by nitrification will result in net internal generation of protons. However, nearly all ecosystems considered here increase in biomass or are at steady state, with net ammonium inputs generally exceeding net nitrate outputs. Therefore exported nitrate can be generally assumed to come from atmospheric NH_4^+, so that the proton loading from nitrogen transformations is considered here as an external source. Note that atmospheric N_2 fixation involves no proton transfer. Only after mineralization of organic N to NH_4^+ or NO_3^- will fixed N_2 become involved in proton transfer. This process is accounted for by an input–output budget of NO_3^- and NH_4^+.

To assess the relative importance of external proton inputs to the total sources of an ecosystem, we define the external–internal proton ratio (EIPR) as the ratio of atmospheric inputs of mineral and potentially mineral acids to the internal proton loading.

The rate of soil acidification is equivalent to the proton flux associated with anionic weathering minus the proton flux associated with cationic weathering. The summed proton sources and sinks should, theoretically, be equal. A comparison of the total proton sources and sinks provides an estimate of the accuracy of the budget. Budget discrepancies may be due to errors in measurements or to proton transfer processes that have been overlooked.

To illustrate the nature of soil acidification and its role in regulating drainage water quality, we have also tabulated the constituents contributing to ΔANC (s), ΔANC (aq) and the transport of BNC from the ecosystem (Table 3). The contributions to ΔANC (s) have been subdivided into changes in basic cation components (CaO, MgO, Na_2O, K_2O), aluminium components (Al_2O_3) and anionic components (SO_3, P_2O_5, HCl). Because most soils considered here are oxidized and without extremely low *p*H values, soil acidification due to changes in iron and manganese component content is generally insignificant. Changes in ANC (aq) have been attributed to changes in free proton, bicarbonate, and organic anion concentration. Because of the ecological significance of aqueous acidity, we have also tabulated the quantities of dissolved hydrogen ion and aluminium exported from soils as well as the *p*H of the drainage water.

Soil acidification and proton sinks

There is considerable variation in the extent and nature of proton fluxes in ecosystems (Table 2). Data from the global terrestrial ecosystem (case 1) and sites where precipitation inputs exceed evapotranspiration indicate that soils generally undergo acidification (ΔANC is negative), while water percolating through the soil increases in ANC (aq). However, in arid or semi-arid regions, like Lake Chad (case 2), the supply of solutes exceeds leaching of solutes and reverse weathering occurs (such as $CaCO_3$, or smectite formation), resulting in soil alkalinization.

Although most soils acidify, the extent to which this process occurs is highly variable and essentially depends on soil type, geology and the quantity of drainage water. We have subdivided ecosystems into those with: (1) low rates of soil acidification (<2.5 kmol hectare^{-1} yr^{-1}); (2) intermediate rates of soil acidification (2.5–7.5 kmol hectare^{-1} yr^{-1}) and; (3) high rates of soil acidification (>7.5 kmol hectare^{-1} yr^{-1}).

Ecosystems with low rates of soil acidification often have acidic podzolic soils which are derived from granitic or base poor materials (cases 5–8), strongly weathered volcanic materials (case 9) or are bogs (cases 3, 4). Ecosystems with intermediate rates of soil acidification generally have acidic soils containing appreciable amounts of weatherable silicate minerals. Examples include soils that are derived from young volcanic materials (case 15), decalcified fluvial deposits (cases 12–14) and decalcified loess (cases 10 and 11). Soils with high rates of

Table 3 Constituents contributing to the rate of soil acidification (ΔANC (s)) and to the change in acid neutralizing capacity of water percolating through the soil (ΔANC (aq)) and the transport of BNC (aq) in drainage water

Case	Ecosystem	Ref.	mm yr^{-1}		Δ ANC (s) (kmol ha^{-1} yr^{-1})				Δ ANC (aq) (kmol ha^{-1} yr^{-1})				BNC (aq) (kmol ha^{-1} yr^{-1})			*p*H of drainage water
			Rainfall	Runoff/ percolation	Total	Base cations	Anions	Aluminium	Total	HCO_3^-	A^{n-}	H^+	Total	H^+	Aluminium*	
1	World Average	17	800†	280†	−2.7	−3.2	+0.5	0.0	2.6	2.6	0.0	0.0	0.0	0.0	0.0	8.3
Soil alkalinization																
2	Lake Chad	18	275‡	245	3.4	3.4	0.0	0.0	−3.3	−3.3	0.0	0.0	0.0	0.0	0.0	—
Low rates of soil acidification																
Bogs																
3	Thoreau's bog	19, 20	1,450	240§	−0.1	−0.1	0.0	0.0	0.9	0.0	0.4	0.5	0.5	0.5	0.0	3.7
4	Storgama	21	920	530	−0.4	−0.3	0.0	−0.1	0.4	0.0	0.3	0.1	0.4	0.3	0.1	4.2
Well drained soils																
5	Birkeness	22	1,290	1,030	−1.3	−0.5	−0.1	−0.6	1.4	0.0	0.5	0.9	0.9	0.3	0.6	4.5
6	Lüneburger Heide	23	765	614‖	−0.9	−1.2	0.7	−0.4	0.4	0.0	0.0	0.4	0.6	0.2	0.4	4.4
7	Hubbard Brook	6, 24	1,320	835	−2.1	−1.8	0.1	−0.4	1.3	0.1	0.0	1.2	0.3	0.1	0.2	4.9
8	Gårdsjön	25	1,130	665	−2.1	−1.5	0.2	−0.6	1.2	0.0	0.7	0.6	1.1	0.5	0.6	4.2
9	Mondo	26	3,580	2,955	−2.1	−1.5	0.0	0.0	4.0	4.0	0.0	0.0	0.0	0.0	0.0	6.8
Intermediate rates of soil acidification																
10	Solling (*Fagus*)	27	950	705	−2.9	−0.6	−0.5	−1.5	1.2	0.0	0.0	1.2	2.2	0.5	1.7	4.2
11	Solling (*Picea*)	27	950	480	−5.9	−1.1	−0.2	−4.3	3.3	0.0	0.0	3.3	4.7	0.4	4.3	4.1
12	Hackfort A	28	650	100	−5.0	−3.4	−1.1	−0.4	0.3	0.0	0.0	0.3	0.4	0.0	0.4	4.5
13	Hackfort B	28	650	103	−4.8	−0.7	−1.7	−2.4	0.3	0.0	0.0	0.3	2.5	0.1	2.4	4.2
14	Hackfort C	28	650	149	−3.2	−0.4	−1.6	−1.1	0.3	0.0	0.0	0.3	1.2	0.0	1.1	4.6
15	Andrews	29	2,370	1,545	−3.6	−4.4	0.0	0.0	1.2	0.9	0.0	0.3	0.0	0.0	0.0	6.7
High rates of soil acidification																
16	Northern Cascades	30	3,840	4,090¶	−9.3	−9.9	0.5	0.0	7.6	7.2	0.0	0.4	0.0	0.0	0.0	7.0
17	Hackfort D	28	650	125	−9.2	−7.4	−1.8	0.0	2.1	1.7	0.0	0.4	0.0	0.0	0.0	7.0
18	Castricum I (unvegetated)	31	800	610	−15.6	−15.6	0.0	0.0	14.1	12.6	0.0	1.5	0.0	0.0	0.0	8.0
19	Castricum III (*Quercus*)	31	800	350	−14.3	−14.3	0.0	0.0	14.0	12.4	0.0	1.6	0.0	0.0	0.0	7.8
20	Castricum IV (*Pinus*)	31	800	175	−9.5	−9.5	0.0	0.0	9.5	7.8	0.0	1.7	0.0	0.0	0.0	7.8
Deforested system																
21	Hubbard Brook, deforested	32, 33	1,320	1,075	−2.2	−3.8	−0.4	0.8	0.4	0.0	0.1	0.3	2.6	0.6	2.0	4.2

* For most sites the chemical speciation of Al is unknown, so it was assumed to be trivalent. As a result the contribution of Al (in kmol $\frac{1}{3}$ Al^{3+}) to BNC is an upper limit.

† Except Antarctica, data from ref. 34.

‡ River input 2,020 mm yr^{-1}, evaporation 2,100 mm yr^{-1}.

§ Net annual storage of water in the bog was 190 mm.

‖ High percolation rate associated with a depletion of soil moisture.

¶ Runoff is greater than precipitation inputs due to glacial melting.

acidification are generally calcareous (cases 17–20). An apparent exception to these subdivisions is the Northern Cascades (case 16). Here soils are thin and derived mainly from schists and quartzdiorite, but rates of soil acidification are extremely high (−9.9 kmol hectare^{-1} yr^{-1}). This apparent anomaly may be attributed to glacial activity and the very large amount of water draining the region.

In well drained soils, weathering of cationic components is the principal mechanism of proton neutralization (Table 2). However, bogs (cases 3, 4) are generally rich in organic matter and weathering of minerals is quantitatively unimportant. In the reduced environment of bogs proton removal is predominantly achieved through biomass assimilation of anions (S, N, P), reduction of atmospherically derived sulphate and nitrate, and export of free protons.

Internal proton sources

In ecosystems with relatively high rates of soil acidification, the deprotonation of carbon dioxide is generally the most significant proton source (Table 2; cases 16, 18, 19, 20). This transfer of protons is greatly enhanced when the *p*H values of soil solutions approach or exceed the proton dissociation constant of CO_2 ($pH > pK_a = 6.3$). Dissolved organic acids can deprotonate at low *p*H ($pK = 4$–5) values as is apparent in the two bogs (cases 3 and 4) and two of the four podzols (cases 5 and 8) considered here. Note that transport of organic anions (A^- in Table 3) causes soil acidification only when cations are removed simultaneously with A^- in the drainage water. Transport of partly dissociated organic acids ($HA + A^- + H^+$) may cause relatively low *p*H values in the drainage water but has, in fact, no effect on either ANC (s) or ANC (aq). Contrary to the contention of others[3,4], organic matter in the soil has no direct role in soil acidification, but merely acts as a (cation) exchanger. Effects of cation exchange on the proton budgets are accounted for under the category 'weathering'.

In terrestrial systems with acidic soils and low to intermediate rates of soil acidification, biologically mediated processes can contribute significantly to the internal production of protons. Most vegetation assimilates an excess of non-nitrogen cations over anions. In an aggrading ecosystem this process results in a net production of protons (cases 6–8, 10–14). In a few ecosystems (cases 9 and 21) biomass serves as a net proton sink due to net mineralization processes.

In a deforested watershed at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire (case 21) a herbicide was applied for 3 yr to prevent vegetation regrowth in the deforested area while decomposition processes were allowed to proceed. Decomposition of the forest floor followed by nitrification resulted in excessive proton production. Levels of proton flux, largely resulting from these nitrogen transformations even exceeded some of our values for calcareous systems. Note that only a modest fraction (31%) of this proton loading was neutralized by weathering reactions while the balance of these protons was neutralized through net cationic decomposition of the forest floor and nitrate loss (denitrification?). In ambient conditions at the HBEF (case 7) proton neutralization is largely (88%) achieved by cationic weathering. While the deforestation experiment is an extreme example, it illustrates the nature of mineralization processes in proton transformations.

Biomass also indirectly regulates proton flux by reducing the flow of water through soil. Using data from Castricum, a cal-

careous site in the Netherlands, we computed proton budgets for sites under pine (*Pinus nigra*; case 20), oak (*Quercus robur*; case 19) and with no vegetation (case 18). Although the soils and geology at all three sites are similar, proton flux and rates of soil acidification were very different. Solute concentrations in drainage water were highest at the pine site, intermediate at the oak site and lowest at the unvegetated site. However, these trends in solute concentration were more than compensated for by variations in water flux. High rates of transpiration from the pine stand reduced the flow of water and solutes percolating through the soil and therefore reduced the rate of soil acidification. Drainage fluxes were intermediate at the oak stand and highest at the unvegetated site; the rates of soil acidification at these sites varied accordingly.

External proton sources

As the world average (case 1) and data from more or less pristine ecosystems (cases 2, 9, 15, 16) would attest, atmospheric inputs do not contribute significantly to the proton loading of these ecosystems. However, near centres of anthropogenic activity such as the northeastern portion of the USA (cases 3, 7) and in northwestern Europe (cases 4–6, 8, 10–14, 17–20), atmospheric proton inputs are substantial. For ecosystems with low to intermediate rates of soil acidification, external loading can be a major fraction of the total proton sources. In the USA (cases 3, 7) atmospheric proton inputs largely occur as free protons and to a lesser extent as SO_2. In Central Europe dry deposition is a very significant mechanism of proton loading. At Hackfort (cases 12–14, 17) in the Netherlands transformations of atmospherically derived ammonium ($(NH_4)_2SO_4$), mainly from dry deposition, account for 53–66% of the total proton sources[13]. At Solling in West Germany (case 10, 11), the rates of soil acidification are much greater in a spruce site (-5.9 kmol hectare^{-1} yr^{-1}) than in a beech site (-2.9 kmol hectare^{-1} yr^{-1}). Spruce is more efficient than beech in scavenging sulphur from the atmosphere[11] (inputs due to dry deposition are 2.8 kmol hectare^{-1} yr^{-1} in spruce compared with 0.9 kmol hectare^{-1} yr^{-1} in beech) resulting in higher rates of soil acidification at the coniferous site.

Information on the input of external protons is insufficient to assess ecosystem sensitivity to acid inputs. Rather, a good measure of the capability of an ecosystem to withstand ambient external proton loading is the external/internal proton ratio (EIPR). For example, Castricum (cases 18–20) receives high atmospheric loadings of protons (2.2–2.5 kmol hectare^{-1} yr^{-1}), but this external input is small relative to the internal proton sources in the calcareous soils (EIPR = 0.13–0.26). Such soils can easily accomodate this external proton load because soil acidification proceeds entirely through the dissolution of basic cations, mainly Ca^{2+} from $CaCO_3$ (Table 3).

Systems with low rates of soil acidification such as in Birkeness, Norway (case 5), Lünebürger Heide, West Germany (case 6), the HBEF (case 7), and Gårdsjön, Sweden (case 8) are extremely proton sensitive. These systems receive inputs of acidic and potentially acidic substances (1.1–1.7 kmol hectare^{-1} yr^{-1}) that are comparable to internal proton sources (EIPR = 0.5–2.4). In these conditions soil acidification proceeds partly through the dissolution of aluminium minerals and significant levels of free protons and aluminium are present in the soil solutions and drainage waters.

In forests in central Europe (cases 10–14, 17, 19 and 20) external proton inputs are extremely high (2.2–6.3 kmol hectare^{-1} yr^{-1}) as are EIPR values for ecosystems with low to intermediate rates of soil weathering (cases 10–14). Moreover, in these ecosystems the soil acidification process is characterized by aluminium dissolution as well as strong retention of sulphate. Appreciable quantities of hydrogen ion and aluminium are exported from the soil to the drainage water.

Note that because of very low rates of internal proton production, Thoreau's Bog (case 3) and Storgama (case 4) have high EIPR values. However, these systems may accommodate high loadings of atmospheric protons through anion assimilation by biomass or by microbially-mediated sulphate and nitrate reduction.

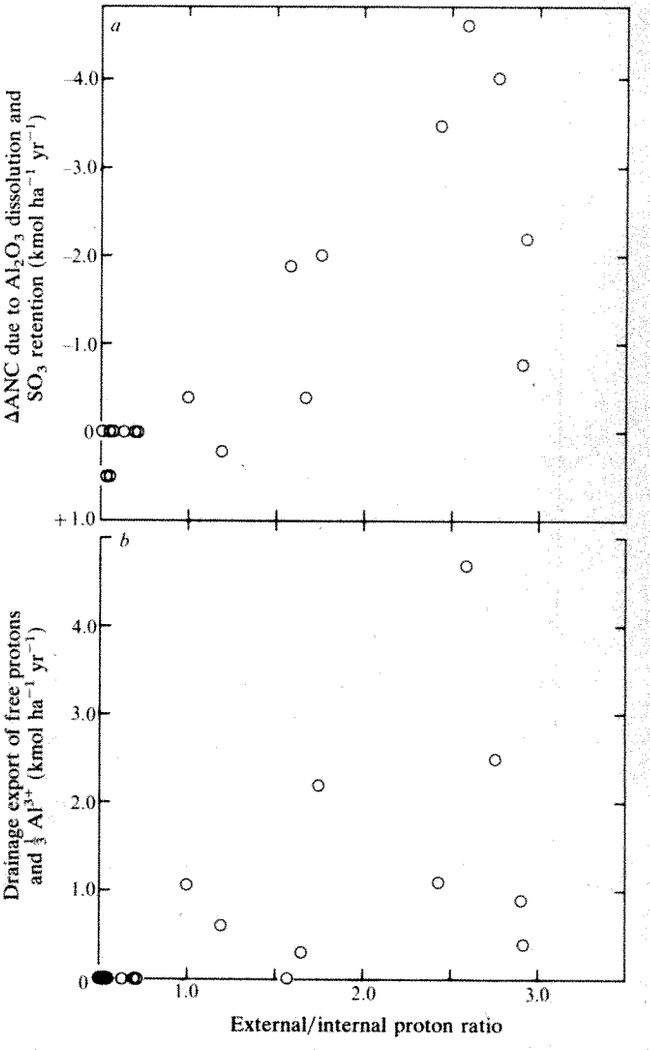


Fig. 1 The rate of soil acidification due to Al_2O_3 dissolution and SO_3 retention (*a*) and the drainage flux of BNC from the soil (*b*) as a function of the external/internal proton ratio of ecosystems. Data from bogs and deforested ecosystems are not included.

To assess the capacity of terrestrial ecosystems to assimilate ambient external acid inputs in an innocuous manner, we have plotted the rate of soil acidification due to Al_2O_3 dissolution and sulphate retention (Fig. 1*a*) and drainage flux of BNC (Fig. 1*b*) as a function of the EIPR. At low (<0.3) EIPR values ecosystems are able to assimilate proton inputs and soil acidification proceeds largely through the dissolution of basic cations. Most ecosystems with EIPR values above 0.5 show significant dissolution of soil aluminium and sulphate retention, resulting in export of free protons and aluminium in drainage waters.

Conclusions

In well drained soils in areas with excess precipitation over evapotranspiration, protons are generated by natural processes (mainly weak acid deprotonation and net assimilation of cations by the vegetation). Soil acidification results from removal of cations and retention of anions. In near-neutral soils rich in weatherable minerals, soil acidification is relatively rapid (7.5–16 kmol hectare^{-1} yr^{-1}) and mainly associated with CO_2 deprotonation. In acidic soils, soil acidification is slower (<2.5 kmol hectare^{-1} yr^{-1}) with cation assimilation and organic acid deprotonation as the principal internal H^+ sources. In the northeastern USA and central and northwestern Europe atmospheric deposition of anthropogenically-derived acidic substances (mainly H^+, SO_2 and NH_4^+) may be 1–5 kmol hectare^{-1} yr^{-1}.

This deposition exceeds internally generated protons in soils with low rates of soil acidification and in many soils with intermediate rates of soil acidification. As a result potentially toxic inorganic aluminium is released into the soil solution and H^+- and aluminium base-neutralizing capacity is transported to drainage waters.

We acknowledge the help of 24 students in calculating H^+ budgets for soil–vegetation systems. This study was funded in part by the EEC environmental programme, EUR contract ENV 650-NL, and the NSF (DEB 82-06980). Partial support for C.T.D. was provided by the Agriculture University, Wageningen.

Received 31 August; accepted 2 November 1983.

1. Ulrich, B. & Matzner, E. *Abiotische Folgewirkungen der weiträumigen Ausbreitung von Luftverunreinigungen* (Luftreinhaltung Forschungsbericht 10402615, Umweltbundesamt, 1983).
2. Overrein, L. N., Seip, H. M. & Tollan, A. (eds) *SNSF Proj. Res. Rep.* FR 19/80 (1980).
3. Rosenqvist, I. Th. *Sur Jord-Surt Vann* (Ingeniør Forlaget A/S, Oslo, 1977).
4. Krug, E. C. & Frink, C. R. *Science* **221,** 520–525 (1983).
5. Van Breemen, N., Mulder, J. & Driscoll, C. T. *Pl. Soil* **75,** 283–308 (1983).
6. Driscoll, C. T. & Likens, G. E. *Tellus* **34,** 283–292 (1982).
7. Stumm, W. & Morgan, J. J. *Aquatic Chemistry* (Wiley, New York, 1970).
8. Van Breemen, N. & Wielemaker, W. G. *Proc. Soil Sci. Soc. Am.* **38,** 61–66 (1974).
9. Johnson, N. M., Driscoll, C. T., Eaton, J. S., Likens, G. E. & McDowell, W. H. *Geochim. cosmochim. Acta* **45,** 1421–1437 (1981).
10. Baker, J. & Schofield, C. *Water, Air Soil Pollut.* **18,** 289–309 (1982).
11. Ulrich, B., Mayer, R. & Khanna, P. K. *Soil Sci.* **130,** 193–199 (1980).
12. Ulrich, B., Mayer, R. & Khanna, P. K. *Deposition von Luftverunreinigungen und ihre Auswirkungen in Waldokosystemen im Solling* ((Sauerländer, Frankfurt a. M., 1979).
13. Van Breemen, N. *et al. Nature* **299,** 548–550 (1982).
14. KNMI/RIV-project *Meetnet voor Bepaling van de Chemische Samenstelling van de Neerslag in Nederland* Jaaroverzicht (KNMI, De Bilt, 1981).
15. Cole, D. W. & Rapp, M. in *Dynamic Properties of Forest Ecosystems* (ed. Reichle, D. E.) 341–409 (Cambridge University Press, 1981).
16. Ovington, J. D. & Madgwick, H. A. I. *For. Sci.* **5,** 344–355 (1959).
17. Garrels, R. M. & MacKenzie, F. T. *Evolution of Sedimentary Rocks* (Norton, New York, 1971).
18. Carmouze, J. P. *La Régulation Hydrogéochimique du Lac Tchad* (ORSTOM, Paris, 1976).
19. Hemond, H. F. *Ecol. Monogr.* **50,** 507–526 (1980).
20. Hemond, H. F. *Ecology* **64,** 99–109 (1983).
21. Braekke, F. H. *Rep. Norwegian Forest Res. Inst.* 36.9 (1981).
22. Braekke, F. H. *Rep. Norwegian Forest Res. Inst.* 36.12 (1981).
23. Matzner, E. & Ulrich, B. *Z. Pfl-Ernähr. Bodenk.* **143,** 666–678 (1980).
24. Likens, G. E., Bormann, F. H., Pierce, R. S., Eaton, J. S. & Johnson, N. M. *Biogeochemistry of a Forested Ecosystem* (Springer, New York, 1977).
25. Hultberg, H. *Ecol. Bull* **36** (in the press).
26. Bruynzeel, L. A. thesis, Free Univ. Amsterdam (1983).
27. Matzner, E. *et al. Elementflüsse in Waldökosystemen im Solling–Datendokumentation* (Göttinger Bodenkundliche Berichte 71, 1982).
28. Van Breemen, N., Van Grinsven, J. J. M. & Jordens, E. R. *Proc. Int. Conf. Acid Precipitation—Origin and Effects* (VDI Düsseldorf, in the press).
29. Sollins, P. *et al. Ecol. Monogr.* **50,** 261–285 (1980).
30. Reynolds, R. C. & Johnson, N. M. *Geochim. cosmochim. Acta* **36,** 537–554 (1972).
31. Minderman, G. & Leeflang, K. W. F. *Pl. Soil* **28,** 61–80 (1968).
32. Likens, G. E., Bormann, F. H., Johnson, N. M., Fischer, D. W. & Pierce, R. S. *Ecol. Monogr.* **40,** 23–47 (1970).
33. Bormann, F. H. & Likens, G. E. *Pattern and Process in a Forested Ecosystem* (Springer, New York, 1979).
34. Holland, H. D. *The Chemistry of the Atmosphere and Oceans* (Wiley, New York, 1978).

Expression of functional acetylcholine receptor from cloned cDNAs

Masayoshi Mishina*, Tomohiro Kurosaki*, Takamasa Tobimatsu*, Yuuki Morimoto*, Masaharu Noda*, Tokuo Yamamoto*, Mineko Terao*, Jon Lindstrom†, Tomoyuki Takahashi‡, Motoy Kuno‡ & Shosaku Numa*

Departments of *Medical Chemistry and ‡Physiology, Kyoto University Faculty of Medicine, Yoshida, Kyoto 606, Japan
† The Salk Institute for Biological Studies, San Diego, California 92138, USA

The cloned cDNAs encoding the four subunits of the Torpedo californica *acetylcholine receptor, each carried by a simian virus 40 vector, direct the synthesis of the functional receptor in a combined expression system consisting of COS monkey cells and* Xenopus *oocytes. Our results suggest that all four subunits are required to elicit a normal nicotinic response to acetylcholine, whereas only the α-subunit is indispensable for α-bungarotoxin binding activity.*

THE nicotinic acetylcholine receptor (AChR) is a postsynaptic membrane protein complex that alters the ionic permeability of the membrane as a consequence of binding acetylcholine. The AChR from the electric organ of the ray *Torpedo californica* is composed of five subunits present in a molar stoichiometry of $\alpha_2\beta\gamma\delta$ and contains both the binding site for the neurotransmitter and the ionic channel (reviewed in refs 1–3). Recently, the primary structures of all four subunits of the *T. californica* AChR[4–6] and the α-subunit of the calf and human muscle AChR[7] have been elucidated in our laboratory by cloning and sequencing cDNAs or genomic DNA encoding these polypeptides; cDNA sequences for the γ-subunit of the *T. californica* AChR[8] and the α-subunit of the *Torpedo marmorata* AChR[9,10] have also been reported by other groups. On the basis of the amino acid sequences of the four subunits, the locations of functionally and immunologically important regions of the AChR molecule have been proposed. These include the acetylcholine binding site[4–7], the transmembrane segments[4–8,10] presumably involved in the ionic channel, and the 'main immunogenic region'[4,6,7] related causally to myasthenia gravis (reviewed in ref. 11).

One approach to verifying these proposed sites would be to introduce amino acid alterations into the respective regions of the AChR molecule and to analyse the resultant effects on the receptor function and the antigenic determinants. As a first step towards this goal, we have now developed a combined system consisting of COS monkey cells and *Xenopus* oocytes in which the synthesis of the functional AChR is directed by the cloned cDNAs encoding the four subunits of the *T. californica* receptor, each carried by a simian virus 40 (SV40) vector. This expression system has allowed us to study the effect of deletion of each subunit on AChR function.

Construction of vectors

The expression vector used (pKCRH2), a derivative of the plasmid pKCR constructed by O'Hare *et al.*[12], contained the SV40 early gene promoter, donor and acceptor splice sites derived from the rabbit β-globin gene, and polyadenylation sites derived from the rabbit β-globin gene and the SV40 early gene. The entire protein-coding sequence of the cDNA for each subunit of the *T. californica* AChR[4–6], cloned into the Okayama–Berg vector[13], was excised by cleavage at the *Hin*dIII or *Eco*RI site, which was introduced into both the 5′- and 3′-noncoding regions of each cDNA by using synthetic linkers (Fig. 1*a*). The excised cDNA fragment was inserted into the vector pKCRH2 at the *Hin*dIII site and/or the *Eco*RI site located between the acceptor splice site and the polyadenylation site derived from the rabbit β-globin gene. The structure of the

0028–0836/84/070604—05$01.00

© 1984 Macmillan Journals Ltd

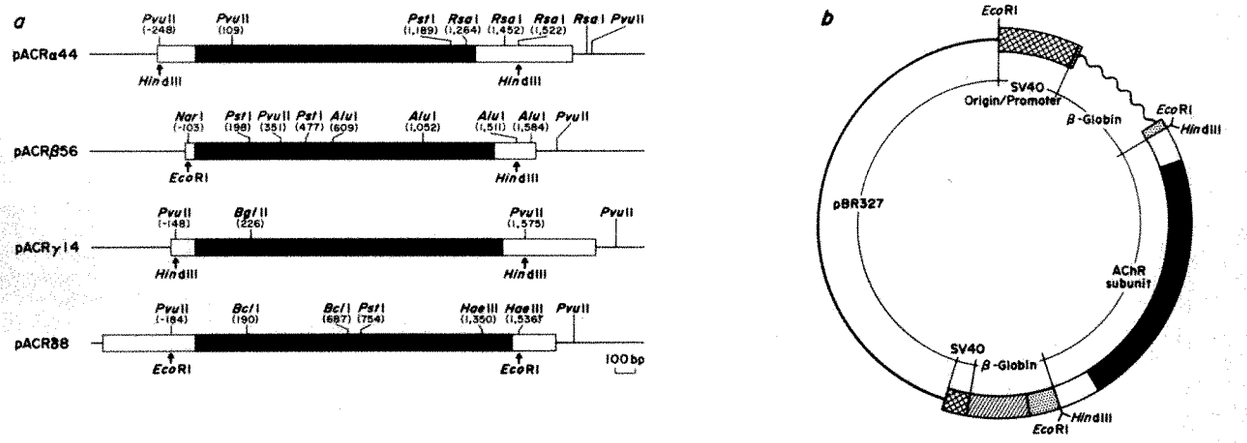


Fig. 1 Recombinant plasmids used for the expression of AChR. ***a***, Restriction maps of the cloned AChR subunit cDNAs. The plasmids pACRα44, pACRβ56, pACRγ14 and pACRδ8, carrying cDNAs for the α-, β-, γ- and δ-subunits of the *T. californica* AChR, respectively, have been described previously[4–6]. The protein-coding regions are indicated by closed boxes, the 5′- and 3′-noncoding regions by open boxes, and the vector sequence including the tails by lines. Only the relevant restriction endonuclease sites are shown and identified by numbers indicating the 5′-terminal nucleotide generated by cleavage; for numbering of the nucleotide residues, see refs 4–6. The *Hin*dIII and *Eco*RI sites introduced by the use of synthetic linkers are shown by arrows beneath the noncoding regions. ***b***, Schematic representation of pKCRH2 recombinant plasmids containing the respective AChR subunit cDNAs (pKCRα2, pKCRβ2, pKCRγ2 and pKCRδ2). The origin of the segments is shown on the inner circle. The protein-coding region of the AChR subunit cDNA is indicated by a closed box, and its 5′- and 3′-noncoding regions by open boxes. The SV40 sequences are shown by cross-hatched boxes; the orientation of the early gene promoter is such that transcription occurs clockwise. The exonic sequences of the rabbit β-globin gene are indicated by stippled boxes, its intronic sequence by a wavy line, and its 3′-flanking sequence by a hatched box. The sequence of the plasmid pBR327 is represented by a line. Only the relevant restriction endonuclease sites are shown.

Methods: The plasmid pKCRH2 was constructed as follows. The plasmid pKCR327 (given by C. Weissmann), in which the pBR322 sequence of pKCR (ref. 12) was replaced by the corresponding sequence of pBR327 (ref. 31), was partially digested with *Eco*RI, treated successively by alkaline phosphatase and by T4 DNA polymerase with the four deoxyribonucleotide triphosphates (dNTPs), ligated to the *Hin*dIII linker dCAAGCTTG, cleaved with *Hin*dIII and ligated. One of the resulting plasmids, harbouring the *Hin*dIII site in the rabbit β-globin gene sequence, was designated pKCRH2. The pKCRH2 recombinants containing the respective AChR subunit cDNAs were constructed as follows. pACRα44 was partially digested with *Pvu*II, ligated to the *Hin*dIII linker and cleaved with *Hin*dIII and *Eco*RI, and the 4.4-kilobase pair (kb) fragment formed was isolated. The plasmid pSV2-gpt[32] was cleaved with *Pvu*II, ligated to the *Eco*RI linker dGGAATTCC and cleaved with *Eco*RI and *Hin*dIII, and the resulting 0.34-kb fragment containing the SV40 early gene promoter was isolated. The two DNA fragments thus obtained were ligated to yield the plasmid pSVACRα. pSVACRα was partially digested with *Pvu*II, ligated to the *Hin*dIII linker and cleaved with *Hin*dIII. The resulting 2.1-kb fragment containing the entire coding sequence for the AChR α-subunit was inserted into the *Hin*dIII site of pKCRH2 in the same orientation with respect to SV40 early gene transcription to generate the plasmid pKCRα1. The 0.70-kb *Pst*I–*Hin*dIII fragment derived from the 2.1-kb fragment described above was partially digested with *Rsa*I, ligated to the *Hin*dIII linker and cleaved with *Hin*dIII and *Pst*I. The resulting 0.34-kb fragment was isolated and ligated to the 4.1-kb *Hin*dIII–*Bam*HI fragment from pKCRH2 and the 2.1-kb *Bam*HI–*Pst*I fragment from pKCRα1, to yield pKCRα2. pACRβ56 was cleaved by *Nar*I, treated successively with alkaline phosphatase and with T4 DNA polymerase with the four dNTPs and ligated to the *Eco*RI linker. After digestion with *Eco*RI, the 4.1-kb fragment formed was isolated and ligated. The resulting plasmid was partially cleaved with *Pvu*II, ligated to the *Eco*RI linker and cleaved with *Eco*RI. The resulting 1.8-kb fragment containing the entire coding sequence for the AChR β-subunit was inserted into the *Eco*RI site in the β-globin gene sequence of pKCR327 in the same orientation with respect to SV40 early gene transcription to produce the plasmid pKCRβ1. The 1.2-kb *Eco*RI–*Pst*I fragment derived from the 1.8-kb fragment described above was partially digested with *Alu*I, ligated to the *Hin*dIII linker and cleaved with *Hin*dIII and *Pst*I. The 1.0-kb fragment formed was isolated and ligated to the 4.1-kb *Bam*HI–*Hin*dIII fragment from pKCRH2 and the 1.2-kb *Bam*HI–*Pst*I fragment from pKCRβ1, to yield pKCRβ2. pACRγ14 was partially digested with *Pvu*II, ligated to the *Hin*dIII linker, cleaved with *Hin*dIII and partially digested with *Eco*RI. The resulting 4.5-kb fragment was ligated to the 0.34-kb *Eco*RI–*Hin*dIII fragment of pSVACRα to yield the plasmid pSVACRγ. pSVACRγ was partially digested with *Pvu*II, ligated to the *Hin*dIII linker and cleaved with *Hin*dIII. The resulting 2.2-kb fragment containing the entire coding sequence for the AChR γ-subunit was inserted into the *Hin*dIII site of pKCRH2 in the same orientation with respect to SV40 early gene transcription to generate the plasmid pKCRγ1. The 1.4-kb *Bgl*II–*Pvu*II fragment derived from the 2.2-kb fragment described above was ligated to the *Hin*dIII linker, digested with *Hin*dIII and *Bgl*II and ligated to the 2.9-kb *Hin*dIII–*Pst*I fragment from pKCRH2 and the 2.2-kb *Pst*I–*Bgl*II fragment from pKCRγ1, to yield pKCRγ2. pACRδ8 was digested with *Pvu*II, ligated to the *Eco*RI linker and cleaved with *Eco*RI. The resulting 2.0-kb fragment containing the entire coding sequence for the AChR δ-subunit was inserted into the *Eco*RI site in the β-globin sequence of pKCR327 in the same orientation with respect to SV40 early gene transcription, to produce the plasmid pKCRδ1. The 1.1-kb fragment formed by cleaving the 2.0-kb fragment described above with *Eco*RI, *Pst*I and *Bcl*I was isolated, partially digested with *Hae*III, ligated to the *Eco*RI linker and cleaved with *Eco*RI and *Pst*I. The resulting 0.79-kb fragment was isolated and ligated to the 4.1-kb *Eco*RI–*Bam*HI fragment from pKCR327 and the 1.6-kb *Bam*HI–*Pst*I fragment from pKCRδ1, to yield pKCRδ2. For further experimental details, see ref. 33.

recombinant plasmids thus constructed is schematically illustrated in Fig. 1*b*; the plasmids containing the α-, β-, γ- and δ-subunit cDNAs are designated pKCRα2, pKCRβ2, pKCRγ2 and pKCRδ2, respectively (see Fig. 1 legend for experimental details).

Expression of functional AChR

Because the recombinant plasmids described above contain the replication origin of SV40, they are expected to propagate to high copy numbers in COS monkey cells[14], which produce SV40 T-antigen and are permissive for the replication of these recombinants. Therefore, each recombinant plasmid was introduced into COS cells by transfection[15]. S_1 nuclease mapping[16] of poly(A) RNA isolated from the transfected cells indicated that the 5′ end of the AChR subunit-specific mRNAs produced from the recombinant plasmids corresponded to the heterogeneous start sites of the transcripts formed under the control of the SV40 early gene promoter[12,17–19] and that the intronic sequence of the rabbit β-globin gene was eliminated. Thus, the AChR subunit-specific mRNAs produced in the transfected COS cells contained no AUG triplet upstream of the translation initiation site for the AChR subunit precursors[4–6]. The contents of the α-, β-, γ- and δ-subunit-specific mRNAs in poly(A) RNA from the transfected cells, as quantified by S_1 nuclease analysis, were respectively, 2.3–5.6 times, 2.6–6.8 times, 1.6–4.6 times and 0.9–1.4 times the level in *T. californica* electroplax poly(A) RNA.

To examine whether the AChR subunit-specific mRNAs produced from the recombinant plasmids could direct the synthesis of the functional AChR, we used the *Xenopus* oocyte translation system[20–22]. The mRNAs specific for the α-, β-, γ- and δ-subunits of the AChR, derived from transfected COS cells, were combined in the same ratio as in *T. californica* electroplax RNA and were introduced into oocytes by microinjection. Extracts from the injected oocytes, prepared 3 days later, exhibited ^{125}I-α-bungarotoxin binding activity ranging between 0.3 and 3 fmol per oocyte (see Table 1 and Fig. 4 legend for experimental details).

The injected oocytes, after incubation for 3 days, were studied electrophysiologically by recording the intracellular potentials or the membrane currents under voltage clamp[22]. The response to acetylcholine applied ionophoretically was examined in the presence of atropine which blocks the native muscarinic AChR present in the membrane of *Xenopus* oocytes[23,24]. Figure 2*a* shows that an inward current with a short latency was observed in response to acetylcholine. This response was virtually abolished within 3 min after the application of (+)tubocurarine (Fig. 2*b*), and the effect was reversible (Fig. 2*c*). The magnitude of the acetylcholine response depended on the membrane potential. The reversal potential of the acetylcholine response, measured in six injected oocytes under voltage clamp, was −5 to −1 mV. The reversal potential was not altered in a Cl^--free solution. When the ionophoretic application of acetylcholine was repeated at short intervals (10–20 s), the response was progressively diminished because of desensitization of the AChR. These results indicate that *Xenopus* oocytes injected with the four AChR subunit-specific mRNAs from transfected COS cells synthesize the functional AChR of the nicotinic type which increases the cationic conductances in response to acetylcholine. The electrophysiological properties of the AChR formed by translation of the mRNAs derived from the recombinant plasmids are in good agreement with those of AChR synthesized in oocytes injected with *Torpedo* electroplax poly(A) RNA[22].

The translation products encoded by the AChR subunit-specific mRNAs from transfected COS cells were identified by labelling them in oocytes with ^{35}S-methionine and analysing them by SDS-polyacrylamide gel electrophoresis after α-toxin affinity chromatography and immunoprecipitation. Figure 3*a* shows that oocytes injected with these mRNAs (lanes 6–8), like oocytes injected with *T. californica* electroplax poly(A) RNA (lanes 3–5), contained four polypeptides that were specifically precipitated with antiserum to the *T. californica* AChR and that were indistinguishable with respect to mobility from the subunits of the purified native AChR (lane 2) except for the γ-subunit. The γ-subunit synthesized in both groups of oocytes migrated somewhat faster than that of the purified native AChR; this may be due to a difference in glycosylation or other processing events *in vivo* or to proteolytic modification during the isolation procedure[1].

Effect of deletion of each subunit

In view of the conspicuous amino acid sequence homology found among the four subunits of the AChR and of their pseudosymmetric orientation across the membrane, proposed on the basis of their similarity in hydrophilicity profile and predicted secondary structure[4–6], the question arises as to whether or not all four subunits are required for receptor function. The expression system described above allowed us to examine this issue.

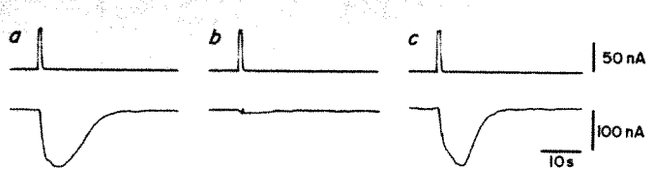


Fig. 2 Acetylcholine responses recorded for a *Xenopus* oocyte injected with the four AChR subunit-specific mRNAs produced from the recombinant plasmids in COS cells. The recordings were made at room temperature in a frog Ringer's solution containing 1 μM atropine: upper traces, currents used for ionophoresis of acetylcholine (backing current, 12 nA); lower traces, acetylcholine currents recorded under voltage clamp (downward deflection indicates inward current). The membrane potential was held at −60 mV, and acetylcholine was applied to the vegetal pole. Responses to acetylcholine before (*a*) and 2.5 min after perfusion with 2.5 μM (+)tubocurarine (*b*), and 15 min after washing out the antagonist (*c*).

Methods: COS monkey cells[14] were transfected with pKCRα2, pKCRβ2, pKCRγ2 or pKCRδ2 (20 μg of each recombinant DNA per 90-mm dish without carrier DNA) as described previously[33]. After 42–48 h, the cells were collected and total RNA was extracted as described elsewhere[34], except that the homogenization buffer contained 0.5% Nonidet P-40. Poly(A)-containing RNA was isolated by oligo(dT)-cellulose chromatography[35]. About 20 ng of poly(A) RNA, containing the α-, β-, γ- and δ-subunit-specific mRNAs, were injected into each oocyte of *Xenopus laevis*. The injected oocytes were incubated at 19 °C for 3 days in modified Barth's medium[20] containing gentamicin (0.1 mg ml^{-1}) and nystatin (50 U ml^{-1}). Oocytes having a healthy appearance were selected for subsequent studies.

Oocytes were injected with various combinations of three of the AChR subunit-specific mRNAs, and the resulting translation products were first analysed to ensure that the subunit polypeptides encoded by the three mRNAs injected were actually formed. For this purpose, the translation products, labelled in oocytes with ^{35}S-methionine, were subjected to immunoprecipitation with a mixture of four monoclonal antibodies[25], each specific for the individual subunits, and then to electrophoretic analysis. The results showed that every combination of three of the mRNAs introduced into oocytes directed the synthesis of the corresponding subunits, although the amounts of the polypeptides found were variable, depending on the particular mRNA combinations (Fig. 3*b*). In comparison with a control experiment in which all four mRNAs were injected (lane 2), smaller amounts of the respective subunits were generally present in oocytes when only three of the mRNAs were injected. Densitometric scanning of the fluorogram indicated that the levels of the α- and δ-subunits were especially low, being ~4%

Table 1 Effect of deletion of each subunit on AChR function

AChR subunit-specific mRNAs injected	^{125}I-α-bungarotoxin binding† (fmol per oocyte)	Response to acetylcholine* No. of responsive oocytes/no. of oocytes tested	Mean acetylcholine sensitivity‡ (mV μC^{-1})
α, β, γ, δ	2.06	126/170	37 (0.4–460)
β, γ, δ	<0.01	0/82	—
α, γ, δ	0.06	0/82	—
α, β, δ	0.09	2/82	1 (0.9–1)
α, β, γ	0.33	3/105	2 (0.2–4)
None	<0.01	0/80	—

Oocytes were injected with various combinations of the AChR subunit-specific mRNAs as indicated and were incubated for 3 days as described in Fig. 2 legend. The ^{125}I-α-bungarotoxin binding activity in the cell extract from each group of oocytes (24–110 oocytes per assay) was determined by the antibody precipitation assay[30] using a mixture of the four subunit-specific monoclonal antibodies (see Fig. 3 legend); incubation was at room temperature for 3 h in the presence of 5 nM ^{125}I-α-bungarotoxin (230 Ci $mmol^{-1}$). Acetylcholine potentials were recorded at a holding potential of −−70 mV; see Fig. 2 legend for further details.

* Our detectable limit of acetylcholine sensitivity was ~0.2 mV μC^{-1}.

† Means from assays of 106–343 oocytes.

‡ The means were calculated only for the responsive oocytes; the ranges of acetylcholine sensitivity are given in parentheses.

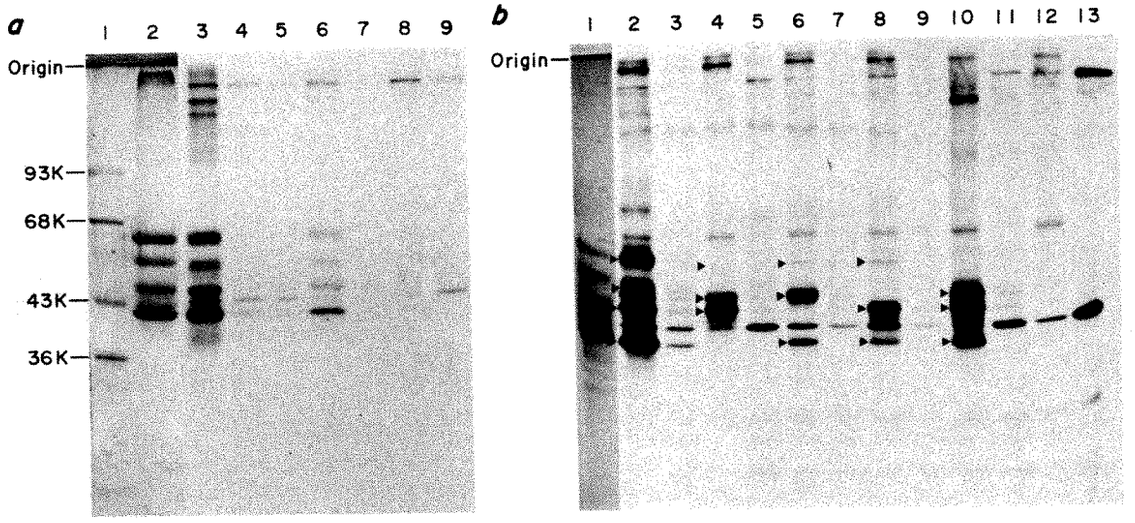


Fig. 3 Electrophoretic analysis of the translation products encoded by AChR subunit-specific mRNAs produced from recombinant plasmids in COS cells. Oocytes were injected with various combinations of the AChR subunit-specific mRNAs from transfected COS cells or with *T. californica* electroplax poly(A) RNA[35,36], then were incubated for 3 (*a*) or 2 (*b*) days in the presence of L-[^{35}S]methionine (1,440 Ci mmol^{-1}; final concentration 1 mCi ml^{-1}). The homogenate[21] of the oocytes was centrifuged at 100,000*g* for 60 min, and the supernatant (cell extract) was subjected to the following procedures. In ***a***, each cell extract was shaken with *Laticauda semifasciata* III–Sepharose. After washing, the AChR was eluted with 1 M carbamylcholine, immunoprecipitated with rabbit antiserum to intact *T. californica* AChR and protein A–Sepharose, and electrophoresed on a SDS/10% polyacrylamide gel[37]. The fluorograms[38] of the electrophoretic analyses (lanes 3–9), together with the standard polypeptides stained with Coomassie brilliant blue (lanes 1 and 2), are shown. Lane 1, size markers: rabbit lactate dehydrogenase [molecular weight (M_r) 36,000 (36K)], chicken ovalbumin (M_r 43K), bovine serum albumin (M_r 68K) and rabbit muscle phosphorylase *b* (M_r 93K). Lane 2, α-, β-, γ- and δ-subunits (from bottom to top) of native *T. californica* AChR purified by α-toxin affinity chromatography[39]. Lane 3, AChR preparation derived from 33 oocytes injected with *T. californica* electroplax poly(A) RNA. Lane 4, a control for lane 3 (immunoprecipitate obtained in the presence of a large excess of native *T. californica* AChR). Lane 5, another control for lane 3 (immunoprecipitate obtained with non-immunized rabbit serum). Lane 6, AChR preparation derived from 100 oocytes injected with the α-, β-, γ- and δ-subunit-specific mRNAs. Lane 7, a control for lane 6 (like lane 4). Lane 8, another control for lane 6 (like lane 5). Lane 9, immunoprecipitate derived from 100 oocytes injected with poly(A) RNA from non-transfected COS cells. In ***b***, 51 ng of purified native *T. californica* AChR as carrier was added to each homogenate derived from 80 injected oocytes, and each cell extract was subjected to prior treatment with non-immunized rabbit serum and protein A–Sepharose to diminish co-precipitation of nonspecific proteins. Immunoprecipitation was done using a mixture of the four subunit-specific monoclonal antibodies[25] (2.6 pmol each of monoclonal antibodies (against intact AChR) mAb5, mAb111, mAb154 and mAb137 specific for the α-, β-, γ- and δ-subunits of the *T. californica* AChR, respectively) and anti-rat immunoglobulin–Sepharose. The resulting immunoprecipitates were electrophoresed on a SDS/10% polyacrylamide gel: lane 1, subunits of native *T. californica* AChR stained; lanes 2, 4, 6, 8, 10 and 12, fluorograms of the electrophoretic analyses of the immunoprecipitates derived from oocytes injected with the α-, β-, γ- and δ-subunit-specific mRNAs (lane 2), the β-, γ- and δ-subunit-specific mRNAs (lane 4), the α-, γ- and δ-subunit-specific mRNAs (lane 6), the α-, β- and δ-subunit-specific mRNAs (lane 8), the α-, β- and γ-subunit-specific mRNAs (lane 10) and poly(A) RNA from non-transfected COS cells (lane 12). Lanes 3, 5, 7, 9, 11 and 13 are controls for lanes 2, 4, 6, 8, 10 and 12, respectively (immunoprecipitates obtained in the presence of a large excess of native *T. californica* AChR). The AChR subunits derived from the oocytes are indicated by arrowheads.

(lanes 6 and 8) and ~1% (lane 4) or ~3% (lanes 6 and 8) of the control value, respectively, except for the α-subunit (~20%) found in the absence of the δ-subunit (lane 10). On the other hand, the levels of the β-subunit (lanes 4, 8 and 10) and the γ-subunit (lanes 4, 6 and 10) were relatively high, being ~30 to ~80% of the control value. These observations suggest that the subunit polypeptides, when one of them is lacking, are more readily degraded in oocytes or during the isolation procedure, although the possibility of different rates of their synthesis cannot be excluded. The absence of one subunit may hinder the assembly of the remaining subunits or lead to the formation of an aberrant complex, thus reducing the stability of the constituent polypeptides. It is interesting in this context that, in the absence of the δ-subunit, fairly large amounts of the remaining three subunits, including the otherwise unstable α-subunit, were found. Thus, the coexistence of the β- and γ-subunits seems to stabilize the α-subunit, presumably by forming a complex of these three subunits with a relatively correct conformation. On the other hand, the δ-subunit seems to be stabilized only when all of the three other subunits are present.

In the experiments shown in Table 1, oocytes injected with various combinations of three of the AChR subunit-specific mRNAs were tested for ^{125}I-α-bungarotoxin binding activity in the cell extract as well as for the response of the membrane potential to acetylcholine. The cell extracts containing all but the β-, γ- or δ-subunit exhibited 3, 4 and 16%, respectively, of the α-bungarotoxin binding activity of the complete AChR synthesized in oocytes, whereas the cell extract containing all but the α-subunit showed no detectable activity. The extent of α-bungarotoxin binding seems to correlate with the amount of the α-subunit present (see above and Fig. 3*b*). This is consistent with the finding that the isolated α-subunit alone is capable of binding α-bungarotoxin[26,27].

The cell extract containing all four subunits and that containing all but the δ-subunit, which exhibited a relatively high level of α-bungarotoxin binding activity, were tested for the protective effect of an agonist on α-toxin binding[28]. Figure 4 shows that, in the presence of carbamylcholine at increasing concentrations, the ^{125}I-α-bungarotoxin binding activity in both of the cell extracts decreased gradually. The carbamylcholine concentration required for 50% protection of the complete AChR was essentially the same as that observed for the native *T. californica* AChR, suggesting that the AChR encoded by the mRNAs produced from the recombinant plasmids possesses an agonist binding site having normal affinity. An approximately fivefold higher concentration of carbamylcholine was required for 50% protection of the AChR lacking the δ-subunit; the apparent binding affinity of α-bungarotoxin was lower for the

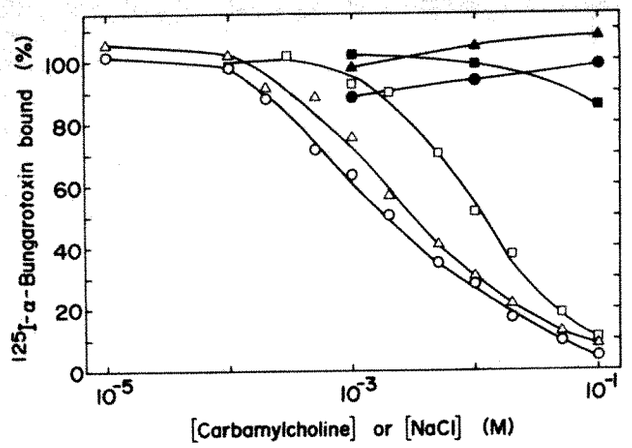


Fig. 4 Effect of carbamylcholine on ^{125}I-α-bungarotoxin binding. Cell extracts prepared from oocytes injected as indicated, or purified native *T. californica* AChR dissolved in the homogenization buffer[21], were preincubated at room temperature for 10 min with carbamylcholine at the specified concentrations and were then mixed with ^{125}I-α-bungarotoxin (230 Ci mmol^{-1}; final concentration 7 nM); the concentration of the toxin-binding site was 63–78 pM. After incubation at room temperature for 3 h, the amount of the toxin bound was determined by the antibody precipitation method[30] using rabbit antiserum to native *T. californica* AChR. ○, Extract from oocytes injected with the α-, β-, γ- and δ-subunit-specific mRNAs from transfected COS cells (●, control experiment in which carbamylcholine was replaced by NaCl at the indicated concentrations). □, Extract from oocytes injected with the α-, β- and γ-subunit-specific mRNAs from transfected COS cells (■, control experiment as above). △, Purified native AChR (▲, control experiment as above). The apparent K_D value for ^{125}I-α-bungarotoxin, estimated by varying the toxin concentration at a fixed concentration (20–43 pM) of the toxin-binding site[40], was 0.1 nM for the native AChR, 0.2 nM for the AChR from oocytes injected with the four subunit-specific mRNAs and 0.7 nM for the AChR from oocytes injected with the α-, β- and γ-subunit-specific mRNAs.

AChR lacking the δ-subunit than for the complete AChR (see Fig. 4 legend). These results imply that the agonist binding site is formed even in the absence of the δ-subunit, but its binding affinity is lower than in the intact AChR molecule.

Of the oocytes injected with all four AChR subunit-specific mRNAs, 74% responded to acetylcholine, when tested electrophysiologically (Table 1). In contrast, the oocytes injected with three of the mRNAs exhibited no acetylcholine potential, except that 2–3% of the oocytes injected with all but the γ- or δ-subunit-specific mRNA showed a weak (+)tubocurarine-sensitive response. The mean acetylcholine sensitivity[29] of the responsive oocytes, expressed as the acetylcholine potential amplitude (mV) divided by the quantity of electricity (μC) used for ionophoresis of acetylcholine, was 37 mV μC^{-1} for the oocytes with the complete AChR, but was 1–2 mV μC^{-1} for those with AChR lacking the γ- or δ-subunit.

In view of the low levels of the α- and δ-subunits found in the oocytes injected with three of the AChR subunit-specific mRNAs (see above and Fig. 3*b*), we examined the effect of reducing the amount of a mixture of the four mRNAs injected into oocytes on their responsiveness to acetylcholine. When oocytes were injected with a mixture of the four mRNAs diluted 10-fold with poly(A) RNA from non-transfected COS cells, 59% of the oocytes (22 out of 37) still responded to acetylcholine with a mean sensitivity of 3 mV μC^{-1} (range 0.3–14 mV μC^{-1}), but the ^{125}I-α-bungarotoxin binding activity as well as the amounts of the α- and δ-subunits found in the oocytes treated in the same manner ranged between barely detectable levels and ~4% of the values obtained for the oocytes injected with a normal mixture of the four mRNAs. Therefore, the amounts of the individual subunits present in the oocytes injected with all but the β-, γ- or δ-subunit-specific mRNA (~3 to ~40%, ~3 to ~30% and ~20 to ~80% of the control value, respectively) would be sufficient for many of these oocytes to respond to acetylcholine. The amount of the δ-subunit present in the oocytes injected with all but the α-subunit-specific mRNA (~1% of the control value) might be limiting, but it is hardly conceivable that the AChR lacking the α-subunit can be functional because this subunit is principally responsible for binding acetylcholine[1].

Thus, our results suggest that all four subunits are required for normal AChR function. The possibility remains that the γ- and δ-subunits can replace each other to some extent for the receptor function because 2–3% of the oocytes injected with all but the γ- or δ-subunit-specific mRNA responded to acetylcholine, albeit slightly. However, the aberrant presence of the nicotinic AChR in non-injected oocytes cannot completely be excluded. The expression system described here will be useful for studying the structure–function relationship as well as the antigenic determinants of the AChR by introducing mutations into this molecule.

We thank Dr Charles Weissmann for the plasmid pKCR327, Dr Paul Berg for pSV2-gpt, Dr Yakov Gluzman for COS cells, Dr Michiaki Kohno for the purified *T. californica* AChR and Dr Katumi Sumikawa for helpful advice. This investigation was supported in part by research grants from the Ministry of Education, Science and Culture of Japan, the Mitsubishi Foundation and the Japanese Foundation of Metabolism and Diseases.

Received 30 September; accepted 25 November 1983.

1. Karlin, A. in *Cell Surface Reviews* Vol. 6 (eds Cotman, C. W., Poste, G. & Nicolson, G. L.) 191–260 (North-Holland, Amsterdam, 1980).
2. Changeux, J.-P. *Harvey Lect.* **75,** 85–254 (1981).
3. Conti-Tronconi, B. M. & Raftery, M. A. *A. Rev. Biochem.* **51,** 491–530 (1982).
4. Noda, M. *et al. Nature* **299,** 793–797 (1982).
5. Noda, M. *et al. Nature* **301,** 251–255 (1983).
6. Noda, M. *et al. Nature* **302,** 528–532 (1983).
7. Noda, M. *et al. Nature* **305,** 818–823 (1983).
8. Claudio, T., Ballivet, M., Patrick, J. & Heinemann, S. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1111–1115 (1983).
9. Sumikawa, K. *et al. Nucleic Acids Res.* **10,** 5809–5822 (1982).
10. Devillers-Thiery, A., Giraudat, J., Bentaboulet, M. & Changeux, J.-P. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2067–2071 (1983).
11. Lindstrom, J. & Dau, P. *A. Rev. Pharmac. Tox.* **20,** 337–362 (1980).
12. O'Hare, K., Benoist, C. & Breathnach, R. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1527–1531 (1981).
13. Okayama, H. & Berg, P. *Molec. cell. Biol.* **2,** 161–170 (1982).
14. Gluzman, Y. *Cell* **23,** 175–182 (1981).
15. Graham, F. L. & van der Eb, A. J. *Virology* **52,** 456–467 (1973).
16. Berk, A. J. & Sharp, P. A. *Cell* **12,** 721–732 (1977).
17. Haegeman, G. & Fiers, W. *J. Virol.* **35,** 955–961 (1980).
18. Benoist, C. & Chambon, P. *Nature* **290,** 304–310 (1981).
19. Gruss, P. & Khoury, G. *Proc. natn. Acad. Sci. U.S.A.* **78,** 133–137 (1981).
20. Gurdon, J. B. *The Control of Gene Expression in Animal Development* (Clarendon, Oxford, 1974).
21. Sumikawa, K., Houghton, M., Emtage, J. S., Richards, B. M. & Barnard, E. A. *Nature* **292,** 862–864 (1981).
22. Barnard, E. A., Miledi, R. & Sumikawa, K. *Proc. R. Soc.* **215,** 241–246 (1982).
23. Kusano, K., Miledi, R. & Stinnakre, J. *Nature* **270,** 739–741 (1977).
24. Kusano, K., Miledi, R. & Stinnakre, J. *J. Physiol., Lond.* **328,** 143–170 (1982).
25. Gullick, W. J. & Lindstrom, J. M. *Biochemistry* **22,** 3312–3320 (1983).
26. Haggerty, J. G. & Froehner, S. C. *J. biol. Chem.* **256,** 8294–8297 (1981).
27. Tzartos, S. J. & Changeux, J.-P. *EMBO J.* **2,** 381–387 (1983).
28. Weber, M. & Changeux, J.-P. *Molec. Pharmac.* **10,** 15–34 (1974).
29. Miledi, R. *J. Physiol., Lond.* **151,** 1–23 (1960).
30. Weinberg, C. B. & Hall, Z. W. *Proc. natn. Acad. Sci. U.S.A.* **76,** 504–508 (1979).
31. Soberon, X., Covarrubias, L. & Bolivar, F. *Gene* **9,** 287–305 (1980).
32. Mulligan, R. C. & Berg, P. *Science* **209,** 1422–1427 (1980).
33. Mishina, M. *et al. EMBO J.* **1,** 1533–1538 (1982).
34. Nakamura, M., Nakanishi, S., Sueoka, S., Imura, H. & Numa, S. *Eur. J. Biochem.* **86,** 61–66 (1978).
35. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69,** 1408–1412 (1972).
36. Nakanishi, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **73,** 4319–4323 (1976).
37. King, J. & Laemmli, U. K. *J. molec. Biol.* **62,** 465–477 (1971).
38. Chamberlain, J. P. *Analyt. Biochem.* **98,** 132–135 (1979).
39. Kaneda, N., Tanaka, F., Kohno, M., Hayashi, K. & Yagi, K. *Archs Biochem. Biophys.* **218,** 376–383 (1982).
40. Weber, M. & Changeux, J.-P. *Molec. Pharmac.* **10,** 1–14 (1974).

Two configurations of a channel-forming membrane protein

P. N. T. Unwin & P. D. Ennis

Department of Structural Biology, Stanford University School of Medicine, Stanford, California 94305, USA

The protein oligomer forming the gap junction channel has been analysed in two Ca^{2+}-sensitive states by electron microscopy of membranes in frozen aqueous solutions. Switching between states occurs by a small cooperative rearrangement involving tilting of the subunits, which may be responsible for the effect of Ca^{2+} on channel permeability in vivo.

FUNDAMENTAL biological processes such as the transmission of nerve impulses, visual excitation and cell-to-cell communication depend on the fact that membranes can regulate the passage of ions and/or small molecules across their surfaces. Proteins create channels in the membranes for this purpose and regulation is achieved by conformational changes of the protein in response to chemical or electrical stimuli. An example of such a protein is the one which forms the gap junction (or cell-to-cell) channel. We report here on its structure and action when stimulated by Ca^{2+}, an ion which induces this channel to close[1,2].

The protein is a cylindrical oligomer composed of six identical, rod-shaped subunits[3,4]. It spans the lipid bilayer, creating a channel along its central, hexad, axis. The channel traverses the plasma membranes of two apposed cells in consequence of a hexamer in one membrane being joined symmetrically to an oppositely facing hexamer in the other. These 12-subunit assemblies accumulate at specialized regions of contact (gap junctions), where they regulate the passage of small molecules and ions between cell interiors (reviewed in refs 5, 6).

Based on the appearance of gap junction surfaces in negative stain, we speculated that the channel might open and close, given the appropriate stimulus, by a coordinated tilting and sliding motion of the protein subunits along their lines of contact[4]. Subsequent X-ray diffraction experiments showed that a reversible transition of this nature could be induced by the action of Ca^{2+} *in vitro*[7]. Now we describe the two Ca^{2+}-sensitive configurations seen more directly in electron micrographs of junctions in frozen aqueous solutions. The three-dimensional maps obtained by this technique[8,9] reveal features of the subunits extending over their entire length, both within the lipid bilayer and in the aqueous spaces on either side. Differences between these maps demonstrate that Ca^{2+} does induce a small coordinated rearrangement of the subunits around the channel and affect its dimensions in a manner consistent with the original proposal.

Imaging and diffraction

We made one preparation of rat liver junctions for these experiments and produced two alternative structures by dialysing separate portions against Ca^{2+}-free (5 mM EGTA, 0.5 mM $MgCl_2$, 5 mM HEPES *p*H 8.0) and Ca^{2+}-containing (0.05 mM $CaCl_2$, 5 mM HEPES *p*H 8.0) solutions. Methods were as described previously[7]. Such junctions are double membrane plaques, 0.5–1.5 μm in diameter, composed of ordered domains of oppositely facing hexamers and intervening lipid molecules. The hexamers pack within the domains on a hexagonal lattice of dimension 80–85 Å and protrude into the space between the two membranes, making a gap between them of ~30 Å.

Electron microscopy was used to derive a set of three-dimensional Fourier terms corresponding to each structure. The junctions were frozen in their respective solutions and examined with a cold stage at −120 °C. Images were recorded of specimens tilted at angles of up to 53° to the incident 100-kV electron beam (Fig. 1). Densitometry and computer processing were carried out as described previously[4,10] and the Fourier terms to 20 Å resolution from each image were combined according to the symmetry of the space group P6. The average phase errors in combining the data were 16.7° and 15° for the $-Ca^{2+}$ and $+Ca^{2+}$ structures, respectively, based on comparisons between each new phase and all previously accumulated values related by symmetry. For both structures, 18 images provided sufficient values of amplitude and phase to map out the continuous variations along each of the five crystallographically independent reciprocal lattice lines. Curves were fitted to the data along the lattice lines by a least-squares method[11], with the amplitude curves weighted according to the reliability of the corresponding phases (Fig. 2). These curves, sampled at regular intervals (0.002 $Å^{-1}$), provided the Fourier terms for calculating three-dimensional maps.

There were cones of missing information due to the restricted ranges of tilt. By far the most significant omissions, according to X rays[7], were the terms from the central (0, 0) lattice line. These terms determine only the absolute levels of the contours in sections parallel to the membrane and therefore have no influence on the positions of peaks identifying the paths taken by the protein subunits, described below. They do, however, affect the dimensions of features bound by shallow gradients, for example the channel. Such dimensions were obtained more accurately previously[4] with complete sets of Fourier terms.

X-ray diffraction was used (as in ref. 7) to provide an independent evaluation of the structures. The partially oriented gap junction pellets gave rise to intensities along the lattice lines which were smeared into concentric arcs. Traces across these arcs, obtained by circular integration, were very similar to analogous profiles calculated from the electron microscopic data (Fig. 2, panel 6). Moreover, in comparing the two structures,

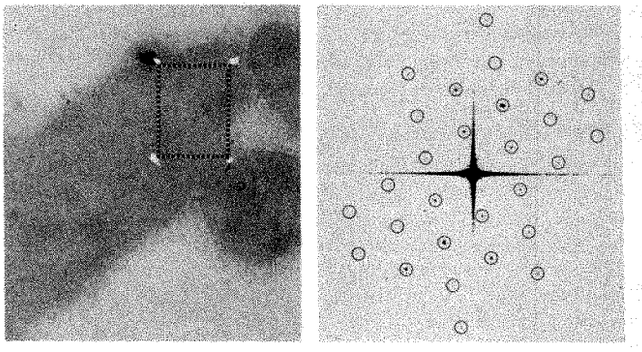


Fig. 1 Image (×32,000) and optical diffraction pattern (from the boxed area) of a gap junction frozen in Ca^{2+}-free solution and tilted 31.5° to the incident electron beam. The regular hexagonal lattice is evident from the array of diffraction peaks encircled, but is barely visible in the image because of the small differences in electron scattering properties between ice, lipid and protein.

0028-0836/84/070609—05$01.00

© 1984 Macmillan Journals Ltd

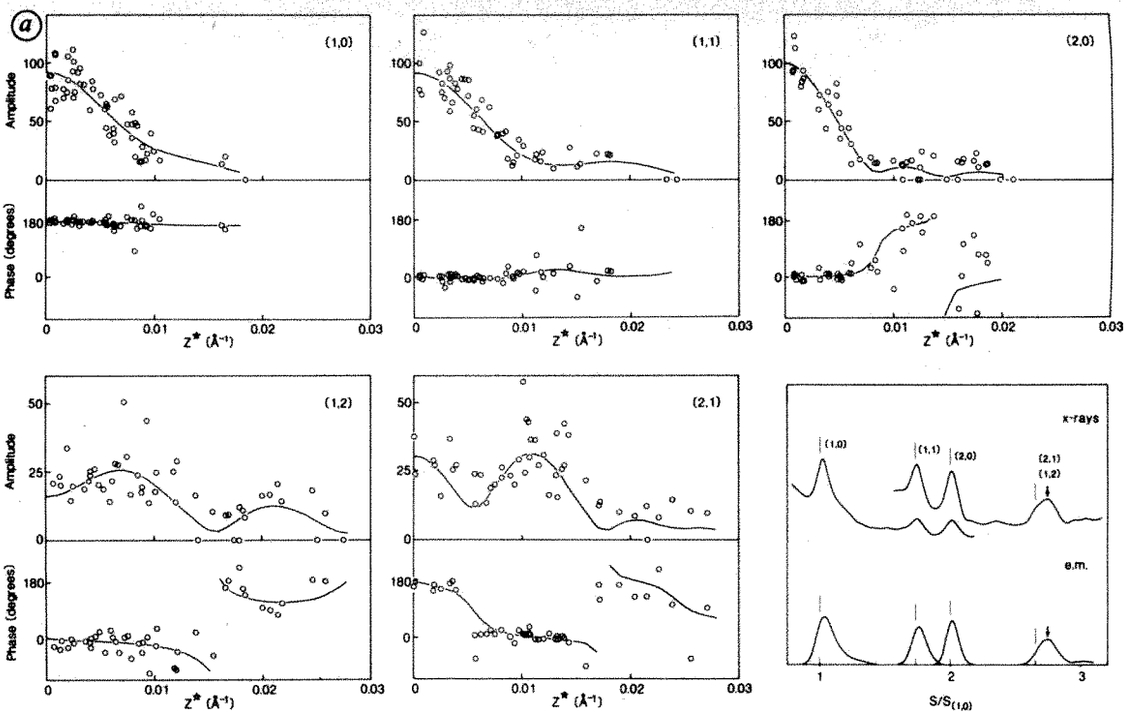


Fig. 2 Diffraction data from gap junctions in: *a*, Ca^{2+}-free solution; *b* (right), Ca^{2+}-containing solution. The values of amplitude and phase along the reciprocal lattice lines (first five panels) were derived from images of junctions tilted to the incident electron beam by angles of up to 53° in *a* and 50° in *b*. Z^* is the distance from the midpoint along the lattice line; the Friedel relationship gives the values for negative Z^*. The sixth panel shows X-ray traces.

Methods: Specimens were prepared by the double carbon film technique[8,20]. The carbon support grid containing the junctions was immersed in 10× diluted Ca^{2+}-free or Ca^{2+}-containing solution, raised through a second carbon film floated on the surface, pressed firmly onto Millipore filter paper, held away from the filter paper for several seconds and then plunged into liquid nitrogen. Holes in the support film were important for efficient withdrawal of excess solution onto the filter paper. After transfer to the microscope, the grids were maintained at ~−120 °C using a modified Philips EM300 high-resolution stage[21]. Images were focused using an off-axis viewing screen[22], and recorded at a magnification of ×28,000 on Kodak 4463 film with low doses (2–5 electrons $Å^2$) to minimize radiation damage. Films were developed in full strength Kodak D19 developer for 8 min. Tilting was accomplished by bending the microscope grids under liquid nitrogen before inserting them into the stage. Preliminary selection of images was by optical diffraction evaluation of the strength of the Fourier terms and of the focus levels indicated by the positions of the zeros between the Thon rings[19]. Images only gave suitable diffraction patterns if they were of specimens surrounded by visible films of ice. Those images selected for subsequent processing and incorporation into the three-dimensional data sets all had underfocus values within the range: 4,000–12,000 Å; thus, variations in strengths of the Fourier terms due to different contrast transfer functions were small. In the processing, the densitometer step and sampling sizes were 20 μm, equivalent to 7 Å at the specimen, and the array size for Fourier transformation was 512×512. An analysis of images very close to focus indicated that the poorer contrast transfer at low spatial frequencies, produced by underfocusing, weakened the amplitude of the (1, 0) peak by up to 30% relative to values for the other peaks; the same correction was applied to all images for this effect. Tilt angles and axes were estimated from the geometry of the peaks in the computed Fourier transforms and from focus changes across the film. In combining the Fourier terms from different images, the comparison range used along Z^* was 0.0022 $Å^{-1}$. Images were not incorporated into the three-dimensional data sets if the phase residuals, based on comparisons with the previously accumulated phases, were greater than 25°. With specimens tilted by >45°, some of the terms were omitted from the refinement. Unlike previously[4], junctions from this preparation were strongly chiral (see also ref. 23), a feature most obvious from the opposite phases of the (1, 2) and (2, 1) peaks at low values of Z^*. Seven of the 18 images for *a* and 8 of the 18 images for *b* were of junctions with their positive crystallographic direction pointing towards the electron source. The X-ray traces (panel 6) were obtained from partially oriented pellets, as described previously[7]. The dimensionless parameter, $S/S_{(1,0)}$, provides a linear reciprocal scale such that the hexagonal lattice positions, marked by vertical lines, are at 1, $\sqrt{3}$, 2 and $\sqrt{7}$. Peak values depart from these positions depending on the strength of the Fourier terms along the lattice lines away from the point, $Z^*=0$. The electron microscopy-derived curves compared were obtained by plotting the amplitudes against reciprocal spacing, rather than Z^*, squaring to obtain intensities and convolving these intensities with a gaussian distribution to simulate the broadening of the X-ray peaks due to finite beam size and disorder. Changes in the position of the overlapped (2, 1) and (1, 2) peak (arrows) and in the intensity of the (1, 0) peak represent the most significant differences between the two structures. These changes are consistently observed in the X-ray patterns[7].

the same differences (displacement of the overlapped (2, 1) and (1, 2) peak; change in intensity of the (1, 0) peak) were indicated by X rays as by electrons. Therefore, we presume that the protein configurations being visualized at −120 °C are the same as those at more natural temperatures.

Three-dimensional maps

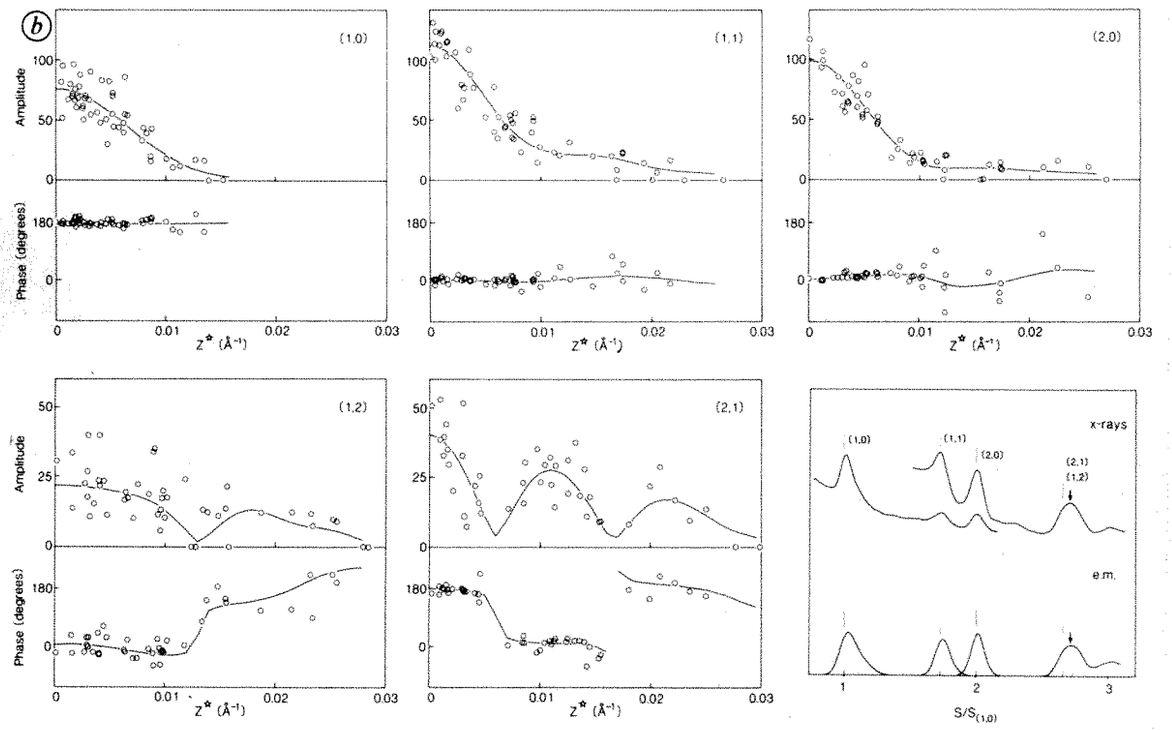
The maps, synthesized from the two sets of Fourier terms, depict the protein subunits surrounding the channels and extending through the lipid bilayer into the aqueous spaces on either side (Fig. 3). The subunits have several features of interest. They are rods which in cross-section must taper significantly towards the hexad axis. They are tilted tangentially and radially around the channel (see below). They appear more distinct and slightly thicker in the bilayer than in the extracellular region, as if their secondary structures in the two environments are quite different.

Ice and lipid are not separately resolved because of their similar mean electron scattering densities. Thus, the maps do not reveal accurately the extents to which the subunits protrude from the bilayer. However, the surface details seen with negative stain[4] correspond to features in Fig. 3 and suggest that the subunits protrude from the bilayer only slightly on the cytoplasmic side (5–10 Å) compared with the extracellular side (15–20 Å).

The hexamers come closest to each other inside the bilayer and the way they fit into the crystalline lattice depends on the bilayer in question. In Fig. 3 the ridges on the sides of the hexamers interlock, forming the same neighbour-to-neighbour relationship in both maps, while in the other bilayer they abut. The latter hexamers are less well preserved, and probably distorted, as the density fluctuations associated with their subunits are weaker (by 45% and 15% for the $-Ca^{2+}$ and $+Ca^{2+}$ structures, respectively). These effects may reflect constraints on the potentially more open packing due to the restricted volume of lipid present or due to the curved membranes of isolated junctions[12] being forced to lie flat. More symmetrical packing[4] presumably occurs when more lipid is available to fill intervening spaces.

Differences

The hexamers look different in the two maps because of the different subunit configurations. They also have a slightly smaller



diameter in the $+Ca^{2+}$ structure, where the lattice dimension is almost 2 Å less[7]. We analysed the differences by comparing the tangential and the radial displacements of the peaks around the channel at various levels through the two structures. In projection down the hexad axis (Fig. 4) these peaks follow curved paths which are more foreshortened for the $+Ca^{2+}$ than for the $-Ca^{2+}$ subunits. The $+Ca^{2+}$ subunits, then, are aligned more nearly parallel to the hexad axis. From the direction and magnitude of foreshortening we find that the two paths of the subunits are related, approximately, by a tilt of ~7.5° tangential to the hexad axis and about the extracellular end (compare Fig. 4*b* and *c*).

The radial path changes of the subunits in going to the $+Ca^{2+}$ configuration are small: about 2 Å inwards towards the cytoplasmic end, but outwards towards the extracellular end (Fig. 5). As shown, the constricting action at the cytoplasmic end would be expected from a tilt strictly tangential to the hexad axis. However, the opposite action at the other end is an additional feature, increasing slightly the separation between neighbouring subunits in the part of the structure outside the cell. This could be needed for the subunits to fit into the 'tighter' configuration without being deformed.

The significance of the small radial changes was checked by calculating difference Fourier maps between the two structures. In these maps the displacement of matter into and away from the channel is seen as two peaks of opposite sign, centred on the hexad axis and near the two ends of the subunits. The peaks were present in both membranes and did not depend on which way up one structure was with respect to the other. Further, the major difference terms responsible for the peaks were significant at the 5% level, based on the standard deviations of the observed amplitudes along the contributing reciprocal lattice lines.

Switching between the two structures

The three-dimensional maps revealed several basic features of this channel-forming protein, It has a length of ~70 Å perpendicular to the plane of the membrane and an average diameter of ~64 Å within this plane. It occupies a space of ~225,000 $Å^3$ (from the zero-level contour), consistent with the value estimated from the subunit molecular weight, 28,000 daltons (refs 13, 14), assuming 1.3 $Å^3$ per dalton for the partial specific volume. It is composed of two main portions, one within and the other outside the membrane, which differ distinctly in

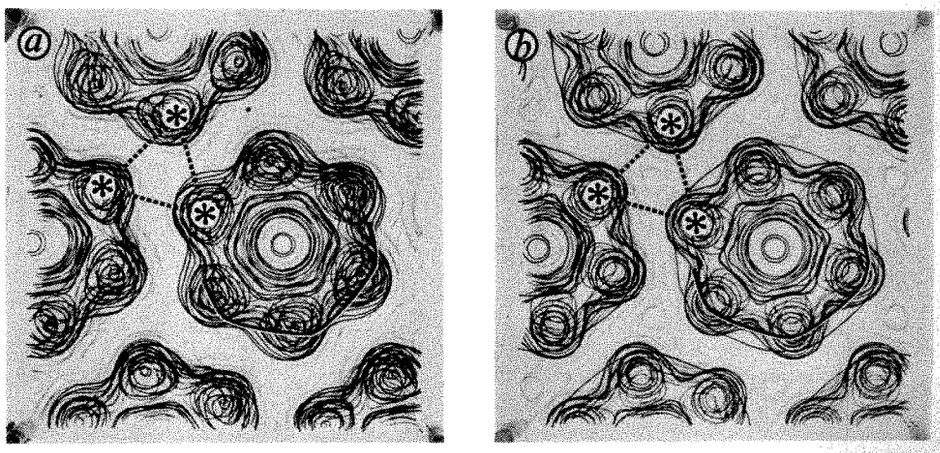


Fig. 3 Maps of the $-Ca^{2+}$ (*a*) and $+Ca^{2+}$ (*b*) structures in one of the membranes, as they would appear viewed from the inside of the cell. The distance between channels in *a* is 82.4 Å and in *b* it is 80.6 Å, from X rays[7]. The six surrounding protein subunits create strong fluctuations in density within the lipid bilayer, but weaker fluctuations in the aqueous extracellular space (furthermost portion of hexamer, outer surface partly outlined). All hexamers have the same neighbour-to-neighbour relationship where they come closest to each other, near the middle of the bilayer (indicated by equivalent triangles connecting equivalent points in the two maps). The positive and zero-level contours (darker) correspond to regions of higher electron scattering density. Detail extends over a distance of 68 Å perpendicular to the sections; fluctuations in density outside the maps on the cytoplasmic side are less than one contour level.

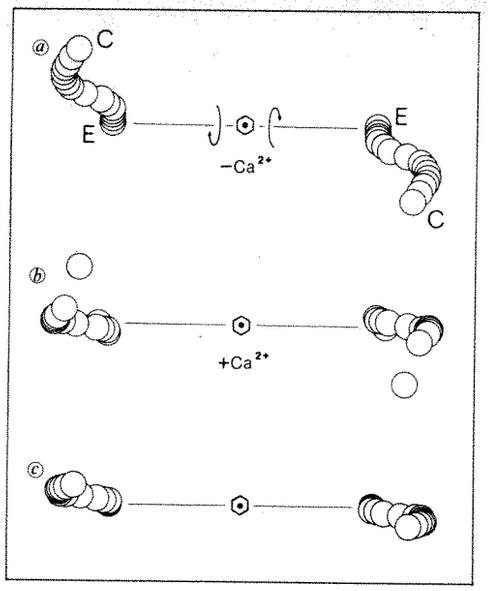


Fig. 4 Projection down the hexad axis of paths taken by subunits on opposite sides of the channel: *a*, $-Ca^{2+}$ and *b*, $+Ca^{2+}$ structures; *c*, path derived from *a* assuming a tilt of 7.5° about the radial axis is indicated. The circles mark the peak positions in sections parallel to the membrane through the three-dimensional maps. E and C are the extracellular and cytoplasmic ends of the subunits, respectively, corresponding to the top and bottom sections in Fig. 3. Correspondence between *c* and *b* indicates that the paths in *a* and *b* are related, approximately, by a small tilt tangential to the hexad axis.

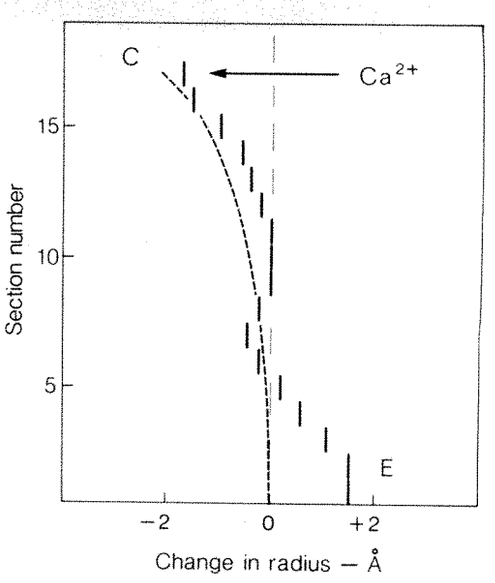


Fig. 5 Radial displacements of the subunits relative to the hexad axis (vertical) on addition of Ca^{2+}, determined from the positions of peaks around the channel in sections through the three-dimensional maps. The broken line shows the radial displacement corresponding to the tilt *a* to *c* in Fig. 4. E and C are as in Fig. 4. Similar radial displacements are suggested by the negative stain maps (ref. 4, Fig. 4), which show a decrease and an increase in stain accumulation in the cytoplasmic and extracellular parts of the channel, respectively.

appearance. In detail, the structure appears rather different from that determined from mouse liver gap junctions[3,12].

Calcium acts to produce small variations in these features by changing, or switching, the configurations of the subunits. From the differences in configuration investigated above, the switch must entail motion of essentially rigid rods having surfaces which do not rotate about the rod axes, but continue to point in the same directions. At low resolution, therefore, it consists simply of a rearrangement of the subunits by tilting and sliding along their lines of contact. This observed mechanism by which the protein changes its structure confirms the one proposed[4], based on the appearance of gap junction surfaces in negative stain.

What is the effect of calcium on the dimensions of the channel? The rearrangement is depicted by wooden models (Fig. 6). We found that subunits on either side of the channel become aligned more nearly parallel to it on exposure to Ca^{2+}. They tilt in opposite senses about a radial axis at the extracellular end (bottom of model) and consequently are displaced tangentially towards each other at the cytoplasmic end (top of model). The change in tilt of each subunit is only ~7.5°, but the protein is long, so that its displacement at the cytoplasmic end is quite large, that is, $\sim 70 \sin 7.5 = 9$ Å. Thus the channel-lining faces of each pair of subunits (not visualized at this resolution) could, in principle, move towards each other by up to ~18 Å in the cytoplasmic region of the structure. Such a constricting action may provide the means of controlling channel permeability, as it occurs in the part of the protein where the structure changes most and where the channel appears to close[4]. A restrictive opening of 16–20 Å diameter is predicted by permeability measurements made on mammalian cells[15,16].

The switching between configurations seen here appears able to accomplish a large change in channel dimensions without the individual polypeptide chains becoming grossly distorted, losing contact or moving over each other by distances of more than a few angstroms. Tilting of the subunits tangential to the channel has a crucial role in mediating this action. Energetically unfavourable interactions are avoided by the subunits moving predominantly parallel to the plane of the bilayer and keeping the same polar and hydrophobic surfaces exposed to the solvent

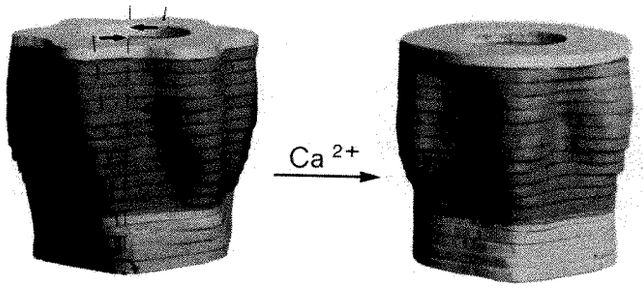


Fig. 6 Models of the two protein configurations, constructed from the maps in Fig. 3. The lighter shaded portions correspond to parts of the protein contacting fluid inside (top) and outside (bottom) the cell; the darker shaded portion would be in contact with lipid. The configurations are related to each other by tilt axes passing radially through the bases of the subunits (as depicted on the left-hand model). Subunits on either side of the channel tilt in opposite directions about these axes, and the resulting tangential displacements (pair of arrows) are greatest at the upper, cytoplasmic face.

and lipid. It is a cooperative mechanism, as the subunits must change their mode of packing together, and may be analogous to the transitions which occur in spherical and helical virus particles[17,18], controlled by divalent cation concentration or *p*H. Other oligomeric membrane proteins may respond to chemical or electrical stimuli by undergoing similar rearrangements predominantly within the plane of the bilayer.

We thank Chris Raeburn for constructing the electron microscope cold stage used in this work. We also thank David Agard, David Grano, Ron Milligan and Guido Zampighi for their help. Computing facilities were generously provided by Dr L. Herzenberg. The research was supported by grants from the NIH and American Cancer Society.

Received 27 June; accepted 31 October 1983.

1. Rose, B. & Loewenstein, W. R. *Nature* **254**, 250–252 (1975).
2. Spray, D. C., Stern, J. H., Harris, A. L. & Bennett, M. V. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 441–445 (1981).

3. Makowski, L., Caspar, D. L. D., Phillips, W. C. & Goodenough, D. A. *J. Cell Biol.* **74,** 629–645 (1977).
4. Unwin, P. N. T. & Zampighi, G. *Nature* **283,** 545–549 (1980).
5. Hertzberg, E. G., Lawrence, T. S. & Gilula, N. B. *A. Rev. Physiol.* **43,** 479–491 (1981).
6. Loewenstein, W. R. *Physiol. Rev.* **61,** 829–913 (1981).
7. Unwin, P. N. T. & Ennis, P. D. *J. Cell Biol.* **97,** 1459–1466 (1983).
8. Taylor, K. A. & Glaeser, R. M. *Science* **186,** 1036–1037 (1974).
9. Dubochet, J., Lepault, J., Freeman, R., Berriman, J. A. & Homo, J.-C. *J. Microsc.* **128,** 219–237 (1982).
10. Henderson, R. & Unwin, P. N. T. *Nature* **257,** 28–32 (1975).
11. Agard, D. A. *J. molec. Biol.* **167,** 849–852 (1983).
12. Caspar, D. L. D., Goodenough, D. A., Makowski, L. & Phillips, W. C. *J. Cell Biol.* **74,** 605–628 (1977).
13. Hertzberg, E. L. & Gilula, N. B. *J. biol. Chem.* **254,** 2138–2147 (1979).
14. Nicholson, B. J., Hunkapiller, M. W., Grim, L. B., Hood, L. E. & Revel, J.-P. *Proc. natn. Acad. Sci. U.S.A.* **78,** 7594–7598 (1981).
15. Flagg-Newton, J., Simpson, I. & Loewenstein, W. R. *Science* **205,** 404–407 (1979).
16. Schwarzmann, G. *et al. Science* **213,** 551–553 (1981).
17. Durham, A. C. H. & Klug, A. *Nature new Biol.* **229,** 6–10 (1971).
18. Robinson, I. K. & Harrison, S. C. *Nature* **297,** 563–568 (1982).
19. Thon, F. *Z. Naturforsch.* **219,** 476–478 (1966).
20. Jaffe, J. & Glaeser, R. M. *Proc. 40th an. EMSA Meet.*, 72–73 (San Francisco, 1982).
21. Taylor, K. A., Milligan, R. A., Raeburn, C. & Unwin, P. N. T. *Ultramicroscopy* (submitted).
22. Unwin, P. N. T. & Henderson, R. *J. molec. Biol.* **95,** 425–440 (1975).
23. Baker, T. S., Caspar, D. L. D., Hollingshead, C. J. & Goodenough, D. A. *J. Cell Biol.* **96,** 204–216 (1983).

LETTERS TO NATURE

Implications of 10^{16} eV γ rays from Cyg X-3

David Eichler

Astronomy Program, University of Maryland, College Park, Maryland 20742, USA

W. Thomas Vestrand

Physics Department, University of New Hampshire, Durham, New Hampshire 03824, USA

Samorski and Stamm[1] have reported and Lloyd-Evans *et al.*[2] have recently confirmed the detection of high-energy quanta, presumably γ rays, with energies $E > 2\times10^{15}$ eV from Cygnus X-3. These ultra-high energy (UHE) γ rays were detected with extensive air shower arrays and included four events with $E > 10^{16}$ eV. Temporal analyses[1,2] of the events indicate that the flux is modulated with a 4.8-h period and is sharply pulsed. Here (1) we discuss the implications of these γ-ray detections and suggest that autocorrelating the air shower data may be the best way to determine the intrinsic width of the γ-ray pulses; (2) we argue that the radiating particles are accelerated by a pulsar and that if they are accelerated according to any pulsar mechanism we know of, then they must be ions; (3) we note that if the ions are accelerated to 10^{16} eV by a large amplitude Deutsch wave, then the gravitational wave luminosity L_g should exceed that of the Crab pulsar by a factor of $\sim5\times10^5$, and the spin-down time should be ~80 yr (requiring a truly remarkable object); and (4) we show that the ions can be accelerated in the near zone but only if, contrary to the standard view, pair production does not greatly reduce the vacuum potential drop in the near zone. We note that near-zone acceleration could be confirmed by detection of curvature radiation.

Several groups have detected γ rays from the Cyg X-3 system at energies $<10^{15}$ eV using atmospheric Cerenkov techniques. The system is known to be a luminous ($L_{peak} > 10^{37}$ erg s^{-1}) source of TeV γ rays[3–5]. The flux at TeV energies is periodic and has the same 4.8-h periodicity seen in medium energy γ rays[6] (~10^2 MeV), X rays[7], and at IR energies[8] and is generally identified as the orbital period. The TeV light curve is composed of two narrow pulses separated by ~1.9 h (ref. 3), and roughly centred about the X-ray minimum. A possible detection of γ rays having energies in the range 10^{14}–10^{15} eV is reported by Bhat *et al.*[9], who studied the arrival times of atmospheric Cerenkov pulses produced by air showers with energies $E > 10^{14}$ eV. By autocorrelating their data they found a significant excess of events with time separations $\Delta t < 40$ s that had a sidereal association. The width of the directional enhancement peak in right ascension is comparable to the angular response function of the detection system. Their data is consistent with a point source at a right ascension of 20 ± 1 h. The detection system was stationed at Gulmarg, India (latitude 34° N) and pointed at the zenith. It is tempting to identify this nonrandom air shower component with γ rays from Cyg X-3 ($\alpha = 20$ h 31 min, $\delta = 40°\,47'$). However, the required flux would have to be roughly a factor of three larger than the upper limit of Fegan and Danaher[10] and several orders of magnitude higher than the value obtained by interpolating between measurements at 10^{15} eV and below 10^{13} eV. Nevertheless, we feel that autocorrelating the data may be the best way to determine the intrinsic width of the γ-ray pulses since small errors in the ephemeris will broaden pulses in phase histograms that are generated using data obtained over long periods of time. Taken at face value, the observations of Bhat *et al.*[9] indicate an excess only for $\Delta t < 40$ s (the autocorrelation was done only up to $\Delta t = 450$ s) and require the pulses to be extremely narrow ($\Delta\phi = 2\times10^{-3}$). We feel that an autocorrelation analysis of data obtained by a detection system with better angular resolution could be useful for determining whether or not the pulses are actually this narrow.

A natural interpretation of the narrow pulses is that Cyg X-3 is a young pulsar in a binary system[11] that is emitting UHE particles which interact with the corona of the companion star[12]. Most of the UHE emission should come from a translucent 'window' (optical depth $\tau \sim 1$) in the companion star's corona, which may be extremely variable and complex due to its interaction with the pulsar wind. Higher τ would obscure the photons, and lower τ would be inefficient as a scintillation screen. It seems reasonable that the translucency condition is fulfilled, during any given orbital period, for 40 s which would account for the autocorrelation time scale. If the pulse width is actually this narrow, the intrinsic luminosity of the system must be $L > 1.3\times10^{39}$ erg s^{-1}; this would rank the pulsar as the youngest known.

The production of γ rays with $E > 10^{16}$ eV requires, of course, the acceleration of particles to even higher energies. We have argued elsewhere[12] that shock-accelerated particles could not generate narrow γ-ray pulses at energies $E > 10^{14}$ eV. The basic point is that shock acceleration requires scattering, whereas the narrow pulses at the observed phases imply straight-line trajectories. Additional arguments rule out shock acceleration of electrons beyond ~10^{14} eV (ref. 12).

We now consider whether acceleration by a large amplitude Deutsch wave, as proposed by Gunn and Ostriker[13], could account for particles whose energy exceeds 10^{16} eV. The maximum energy that a particle with mass Am_p and charge Ze can attain by this mechanism is

$$E_{max} \simeq 6\times10^{14} A^{1/3} Z^{2/3} \times \left(\frac{B_{surf}}{10^{12}\,G}\right)^{2/3} \left(\frac{P}{10\,ms}\right)^{-4/3} \left[\ln\left(\frac{R_B}{R_L}\right)\right]^{2/3} \quad eV \quad (1)$$

where B_{surf} is the surface magnetic field, P is the pulsar's period, and R_L and R_B are the radii of the light cylinder and binary orbit, respectively. The luminosity in magnetic dipole radiation would be (magnetic moment assumed $\perp$ to $\vec{\Omega}$):

$$L_{MD} \simeq 6.5\times10^{40} \left(\frac{P}{10\,ms}\right)^{-4} \left(\frac{B_{surf}}{10^{12}\,G}\right)^{2} \quad erg\ s^{-1} \quad (2)$$

0028-0836/84/070613—02$01.00

© 1984 Macmillan Journals Ltd

so the maximum particle energy is given by

$$E_{max} \simeq 6.0\times10^{14}A^{1/3}Z^{2/3} \times\left(\frac{L_{MD}}{6.5\times10^{40}\ \mathrm{erg\ s^{-1}}}\right)^{1/3}\left(\ln\left(\frac{R_B}{R_N}\right)\right)^{2/3}\ \mathrm{eV} \qquad (3)$$

If the 10^{16} eV particles are electrons or positrons, then equation (3) implies that $L_{MD} > 2\times10^{46}$ erg s^{-1}, and the gravitational wave luminosity L_g ($\propto\Omega^6$), is even larger for any reasonable estimate of the quadrupole moment. However, the maximum rotational energy is $\sim10^{52}$ erg, and Cyg X-3 has been observed for over a decade with no obvious change in any of its behaviour, so the total luminosity must be $<10^{43}$ erg s^{-1}. This implies that particles accelerated to 10^{16} eV would have to be ions.

If ions are accelerated to $E > 10^{16}$ eV by a large amplitude Deutsch wave, the pulsar should be a strong source of gravitational radiation. Using equations (1) and (3) we find that if the accelerated ions are protons, the required period is $\sim3.7\,(B_{surf}/10^{12}\,\mathrm{G})^{1/2}$ ms and the corresponding magnetic dipole luminosity is $L_{MD} > 3\times10^{14}$ erg s^{-1}. Defining the quadrupole moment D as being equal to $q(6\times10^{41})\,(B_{surf}/10^{12}\,\mathrm{G})^2$, for easy comparison with Gunn and Ostriker[13], the spin-down rate due to gravitational radiation is $5q^2(B_{surf}/10^{12}\,\mathrm{G})^{-1}\times10^{-8}$ s^{-1}. Requiring the spin-down rate to be comfortably less than $(10\,\mathrm{yr})^{-1}$ limits q to less than $\sim0.25\ (B_{surf}/10^{12}\,\mathrm{G})^{-1}$. The corresponding gravitational wave luminosity would be $L_g \simeq 5\times10^{43}\,q^2(B_{surf}/10^{12}\,\mathrm{G})^{-1}$ erg s^{-1}, larger than that from the Crab pulsar by 5×10^5. This may be detectable, even without knowing the precise period, with a relatively low Q bar antenna. A young luminous pulsar in a close binary system like Cyg X-3 would probably drive a wind off the companion star that would enshroud the entire system; to this we attribute the lack of any detected pulses thus far. Indeed, X-ray observations[14] and radio observations[15] indicate that the system is embedded in optically thick material. In any case, the extreme youth of the pulsar required by the mechanism's production of 10^{16} eV ions could be considered an uncomfortably strong assumption.

An alternative view, which may be slightly less radical, is that the $>10^{16}$ eV particles are accelerated in the near zone along polar field lines where charge-separated zones can generate large potential drops[16–18]. A very liberal estimate of the maximum potential drop Φ_{max} is found by assuming that the entire polar zone is evacuated and the full vacuum potential is available[16]. In this case,

$$\Phi_{max} \simeq 7\times10^{16}\left(\frac{B_{surf}}{10^{12}\,\mathrm{G}}\right)\left(\frac{P}{10\ \mathrm{ms}}\right)^2\left(\frac{R}{10\ \mathrm{km}}\right)^3\ \mathrm{V} \qquad (4)$$

A pulsar in the Cyg X-3 system comparable to (perhaps slightly younger than) the Crab could account for 10^{16} eV particles if the full vacuum potential were available. However, near-zone acceleration requires that the particles be ions, as curvature radiation from electrons travelling along field lines (with radius of curvature R_c) limits the electron energy to

$$E_{max} \simeq 1.5\times10^{13}\left(\frac{P}{10\ \mathrm{ms}}\right)^{-1/4} \times\left(\frac{R_c}{10\ \mathrm{km}}\right)^{1/2}\left(\frac{B_{surf}}{10^{12}\,\mathrm{G}}\right)^{1/4}\ \mathrm{eV} \qquad (5)$$

Moreover, the UHE observations imply that pair production does not significantly short out electric fields in the near zone. Neither of these conclusions is expected from standard pulsar theory.

The possible detection[19,20] of $E > 10^{15}$ eV γ rays from the Crab could be explained as being curvature radiation from 10^{16} eV ions that are accelerated in the near zone. A better detection of this source—and similarly, the detection of a steady component of 10^{16} eV γ rays from Cyg X-3—would independently support the model of ion acceleration in the near zone. This would seem at present to be the least radical account of the 10^{16} eV γ rays that have been reported.

The more recent reports (ref. 21) of 10^{17} eV air showers from the direction of Cygnus cannot be accounted for by either of the pulsar mechanisms discussed here, when parameters that are consistent with a spin-down time >10 yr are used. If this directional excess is real and is found to have a 4.8-h period, more exotic models for particle acceleration in the Cyg X-3 system are required.

In conclusion, the detections of $>10^{15}$ eV γ rays from Cyg X-3 and their possible detection from the Crab have important implications for the theory of pulsars.

Received 3 October; accepted 17 November 1983.

1. Samorski, M. & Stamm, W. *Astrophys. J. Lett.* **268,** L17–L22 (1983).
2. Lloyd-Evans, J. *et al. Nature* **305,** 784–787 (1983).
3. Nesphore, Yu I. *et al. Astrophys. Space Sci.* **61,** 349–355 (1979).
4. Danaher, S., Fegan, D., Porter, N. & Weekes, T. *Nature* **289,** 568–569 (1981).
5. Lamb, R., Godfrey, C., Wheaton, W. & Tumer, T. *Nature* **296,** 543–544 (1982).
6. Lamb, R., Fichtel, C., Hartman, R., Kniffen, D. & Thomson, D. *Astrophys. J. Lett.* **212,** L63-L66 (1977).
7. Parsignault, D., Grindlay, J., Gursky, H. & Tucker, W. *Astrophys. J.* **218,** 232–242 (1977).
8. Becklin, E. *et al. Nature* **245,** 302–304 (1973).
9. Bhat, C., Sparu, M. & Kaul, C. *Nature* **288,** 146–149 (Corrigendum: **291,** 168) (1980).
10. Fegan, D. J. & Danaher, S. *Proc. 17th int. Cosmic Ray Conf.* **1,** 31–33 (1981).
11. Milgrom, M. & Pines, D. *Astrophys. J.* **220,** 272–278 (1982).
12. Vestrand, W. T. & Eichler, D. *Astrophys. J.* **261,** 251–258 (1982).
13. Gunn, J. E. & Ostriker, J. P. *Phys. Rev. Lett.* **22,** 728–731 (1969).
14. Hertz, P., Joss, P. & Rappaport, S. *Astrophys. J.* **224,** 614–624 (1978).
15. Vestrand, W. T. *Astrophys. J.* **271,** 304–314 (1983).
16. Arons, J. in *IAU Conf.* No. 94, 175–204 (Reidel, Dordrecht, 1981).
17. Ruderman, M. A. & Sutherland, P. G. *Astrophys. J.* **196,** 51–72 (1975).
18. Sturrock, P. A. *Astrophys. J.* **164,** 529–556 (1971).
19. Boone, J. *et al.* preprint University of Utah (1983).
20. Dzikowski, T., Gawin, J., Grochalska, B., Wasilewski, A. & Wdowczych, J. *Proc. 17th int. Cosmic Ray Conf.* **1,** 8–12 (1981).
21. Thomson, D. E. *Sci. News* **123,** 405 (1983).

A simple non-markovian equation for a magnetoplasma

L. C. Woods

University of Oxford, Mathematical Institute, 24–29 St Giles, Oxford OX1 3LB, UK

A new kinetic equation for the velocity distribution function f is proposed here, with the innovation that the term $(\partial f/\partial t)_c$ representing the effect of particle collisions, incorporates time delays extending over the interval between successive 90° collisions. When this theory is applied to magnetoplasmas of the type found in tokamaks, it is found that the time delay has a dramatic effect on the heat flux across the magnetic field, giving results orders of magnitude larger than the classical values, and in fair agreement with observations. It appears that non-markovian effects are of great importance in magnetoplasmas, and that they provide answers to some unsolved problems in fusion research.

If $\mathbf{w}$ is the velocity of a cloud of particles at position $\mathbf{r}$, then the collision term in the kinetic equation for the one-particle phase-space density f,

$$\mathbb{D}f \equiv \frac{\partial f}{\partial t} + \mathbf{w}\cdot\frac{\partial f}{\partial \mathbf{r}} + \mathbf{F}\cdot\frac{\partial f}{\partial \mathbf{w}} = \left(\frac{\partial f}{\partial t}\right)_c \qquad (\mathbf{F} = \dot{\mathbf{w}}) \qquad (1)$$

may depend on the history of f. In general we should assume that it does, but Bogoliubov's[1] hypothesis permits us to ignore time delays in $(\partial f/\partial t)_c$, and while doubts are sometimes expressed, it is usually accepted that the timescale arguments that he devised for neutral gases also apply to fully ionized plasmas.

In a neutral gas the time τ_0 during which particles interact is very much shorter than the time interval τ between successive collisions. This means that the two-particle distribution function implicit in $(\partial f/\partial t)_c$ relaxes to its equilibrium state at a rate τ/τ_0 times more rapidly than f relaxes to its equilibrium state, namely

0028-0836/84/070614—02$01.00

© 1984 Macmillan Journals Ltd

the maxwellian distribution

$$f_0 = n\left(\frac{m}{2\pi kT}\right)^{3/2} e^{-u^2} \qquad \left(\mathbf{u} = \mathbf{c}/C,\ C^2 = \frac{2kT}{m}\right) \tag{2}$$

Here n is the number density, T is the temperature and $\mathbf{c}$ is the peculiar velocity, that is the difference between $\mathbf{w}$ and its average, $\bar{w} = \mathbf{v}$.

To take a simple example, the usual relaxation model for $(\partial f/\partial t)_c$ is

$$\left(\frac{\partial f}{\partial t}\right)_c = \tau^{-1}\{f_0(t) - f(t)\} \tag{3}$$

where f_0 and f are evaluated in the volume element $d\nu = d\mathbf{r}\, d\mathbf{w}$ of the cloud at the current instant. With this expression, equation (1) is 'markovian', that is, independent of the history of either f_0 or f. But $f_0(t)/\tau$ represents the rate at which particles are collided into $d\nu$ from the background plasma, and we should therefore give f_0 its value just prior to the interaction, at the same time preserving the essential irreversible character of $(\partial f/\partial t)_c$. Let irr $\{\ \}$ denote the irreversible part of $\{\ \}$, that is that part that has positive parity under time reversal; then equation (3) is replaced by

$$\left(\frac{\partial f}{\partial t}\right)_c = \text{irr}\,\{f_0(t-\tau_0) - f(t)\}/\tau \tag{4}$$

or

$$\left(\frac{\partial f}{\partial t}\right)_c \approx \tau^{-1}\{f_0(t) - f(t)\} + \frac{\tau_0^2}{2\tau}\mathbb{D}^2 f_0 \tag{5}$$

where the time derivative is evaluated at t, and $\mathbb{D}f_0$ has been omitted in the expansion, since it has the same parity as the left-hand side of equation (1), that is, it is reversible. (Strictly, equation (5) is markovian, since the history has been reduced to a current time derivative, but we shall regard it as non-markovian, to emphasize the distinction between it and equation (3).) With Bogoliubov's hypothesis,

$$\tau_0/\tau \ll 1 \tag{6}$$

equation (5) is reduced to equation (3), and the history of f_0 is thus discarded.

The long range of the Coulomb force throws considerable doubt on equation (6)[2]. A typical charged particle experiences a great number of small-angle collisions, interacting continuously with all of the $n_D = (4\pi/3)\lambda_D^3 n$ particles within a Debye distance, λ_D. The random component of these interactions accumulates over the 'collision' or kinetic time τ to deflect the particle through 90°. In this time the particle typically moves a distance $108\lambda_D n_D$ and hence interacts with $81 n_D^2$ particles. Thus in a typical laboratory plasma, a particle experiences $\sim 10^{13}$ grazing interactions per collision interval τ, which means that a 90° 'collision' is essentially a continuous process, lasting for the whole of the kinetic time τ. If we regard the encounter as occurring between a test particle P and the collection Q of particles within the Debye cylinder described above, or better, as a continuous binary encounter between P and a pseudo-particle Q, that lasts for most of the kinetic time τ, then in place of equation (6) we should write

$$\tau_0 = \alpha\tau \qquad (\alpha = 0(1)) \tag{7}$$

where α is a constant of order unity.

Provided the expansion

$$f_0(t-\tau_0) = -\tau_0\mathbb{D}f_0 + \tfrac{1}{2}\tau_0^2\mathbb{D}^2 f_0 \tag{8}$$

remains a good approximation for the somewhat longer τ_0 given by equation (7), equations (1) and (7) yield

$$\mathbb{D}f = \tau^{-1}(f_0 - f) + \tfrac{1}{2}\alpha^2\tau\mathbb{D}^2 f_0 \tag{9}$$

This is our simple, non-markovian kinetic equation. The principle involved here is simply that the equilibrium 'information' available in $d\nu$ is not the instantaneous value $f_0(t)$, but the value of f_0 about one relaxation time earlier, and the collisional processes tend to drive $f(t)$ irreversibly towards $f_0(t-\alpha\tau)$.

Of course, equation (9) is just a model equation, with a status similar to the relaxation model from which it was derived. But the two alternative non-markovian kinetic theories known to me[3,4] are so complicated that their consequences for mass and energy transport have not been pursued. At least equation (9) offers the chance of checking the importance of time delays in transport in a plasma, even if there remains some uncertainty in the value of the phenomenological constant α.

In a magnetic field, $\mathbf{B} = B\mathbf{b}$, particles with a charge q have a gyro frequency $\omega_c = qB/m$; we shall be concerned with 'strong' fields, that is, those for which $\omega_c\tau \gg 1$, which means that the cloud of particles in $d\nu$ gyrate about the field many times per 90° collision interval.

Transforming equation (9) from the laboratory frame in which the velocity $\mathbf{w}$ is measured, to a frame moving with the fluid velocity $\mathbf{v}$ and rotating with the local fluid angular velocity, $\boldsymbol{\Omega} = \frac{1}{2}\nabla\times\mathbf{v}$, we find (ref. 5)

$$\mathscr{D}f - \omega_c\mathbf{b}\times\mathbf{c}\cdot\frac{\partial f}{\partial\mathbf{c}} = \tau^{-1}(f_0 - f) + \tfrac{1}{2}\alpha^2\tau\mathscr{D}^2 f_0 \tag{10}$$

where

$$\mathscr{D} = D^* + \mathbf{c}\cdot\nabla + (\mathscr{F} - D\mathbf{v} - \mathbf{c}\cdot\boldsymbol{e}^s)\cdot\frac{\partial}{\partial\mathbf{c}} \tag{11}$$

$\boldsymbol{e}^s$ is the symmetrical part of $\nabla\mathbf{v}$, $D\mathbf{v}$ is the fluid acceleration, $\mathscr{F} = (q/m)(\mathbf{E} + \mathbf{v}\times\mathbf{B})$, $\mathbf{E}$ is the electric field and for scalars φ, vectors $\mathbf{a}$ and second-order symmetrical tensors $\boldsymbol{A}$, D^* is defined by

$$D^*\varphi = D\varphi, \qquad D^*\mathbf{a} = D\mathbf{a} - \boldsymbol{\Omega}\times\mathbf{a}, \qquad D^*\boldsymbol{A} = D\boldsymbol{A} - 2\boldsymbol{\Omega}\times\boldsymbol{A} \tag{12}$$

with

$$D = \frac{\partial}{\partial t} + \mathbf{v}\cdot\nabla \tag{13}$$

Thus D^* is a frame-indifferent time derivative.

In reducing $\mathbb{D}^2 f_0$ to $\mathscr{D}^2 f_0$, we have used equation (2), which gives $\mathbf{b}\times\mathbf{c}\cdot\partial f_0/\partial\mathbf{c} = 0$, and the fact that $\mathbb{D}^2 f_0$ must be evaluated with the coefficients of the derivatives held constant. Note that the rapid gyrations about the field lines have been excluded by changing from $\mathbb{D}$ to $\mathscr{D}$, so that now we require that equation (8) holds only for the operator $\mathscr{D}$ and on paths lying roughly parallel to $\mathbf{b}$.

The collision term on the right of equation (10) must conserve the number, momentum and energy of particles, so that

$$\int (1, m\mathbf{c}, \tfrac{1}{2}mc^2)\left(\frac{\partial f}{\partial t}\right)_c d\mathbf{c} = 0 \tag{14}$$

where the integral is over the whole of velocity space. To ensure this we are free to add to $(\partial f/\partial t)_c$ a complementary function $(\alpha_0 + \boldsymbol{\alpha}_1\mathbf{c} + \alpha_2 c^2)f_0$, where α_0, $\boldsymbol{\alpha}_1$, α_2 are functions of $\mathbf{r}$, t and independent of $\mathbf{c}$ (ref. 6). We do this later, using equation (14) to determine these parameters.

Let $\varphi \equiv (f - f_0)/f_0$ and assume $\varphi \ll 1$, then from equations (10) and (2)

$$\underset{(1)}{\varphi} - \underset{(2)}{\omega_c\tau\mathbf{b}\times\mathbf{c}\cdot\frac{\partial\varphi}{\partial\mathbf{c}}} = -\tau\mathscr{D}\ln f_0 + \tfrac{1}{2}\alpha^2\tau^2\{\mathscr{D}^2\ln f_0 + (\mathscr{D}\ln f_0)^2\} \tag{15}$$

where

$$\mathscr{D}\ln f_0 = (u^2 - \tfrac{5}{2})\mathbf{c}\cdot\nabla\ln T + 2\mathbf{uu}:\nabla^0\mathbf{v} \tag{16}$$

$\nabla^0\mathbf{v}$ being the traceless part of $\boldsymbol{e}^s$. We shall assume ∇T to be parallel to unit vector $\hat{\mathbf{r}}$ and perpendicular to $\mathbf{b}$. With $\omega_c\tau$ very large the term (1) in equation (15) yields a heat flux vector parallel to ∇T of the standard type[7],

$$\hat{\mathbf{r}}\cdot\mathbf{q}^{(1)} = -K_\perp\hat{\mathbf{r}}\cdot\nabla T \qquad (K_\perp \sim K_\parallel/(\omega_c\tau)^2) \tag{17}$$

where $K_\parallel$ is the thermal conductivity parallel to $\mathbf{b}$. On the other hand it is easily deduced from equation (16) (details will be published elsewhere) that the non-markovian term (2) in equation (15) gives

$$\hat{\mathbf{r}} \cdot \mathbf{q}^{(2)} = 7\alpha^2 \tau \frac{kp}{qB} H \hat{\mathbf{r}} \cdot \nabla T \qquad (H \equiv \mathbf{b} \times \hat{\mathbf{r}} \cdot \nabla^0 \mathbf{v} \cdot \hat{\mathbf{r}}) \quad (18)$$

The choice $\alpha = (2.5/7)^{1/2} \approx 0.6$ for the electron gas gives

$$\hat{\mathbf{r}} \cdot \mathbf{q}_e^{(2)} = -K_e^* \hat{\mathbf{r}} \cdot \nabla T_e \qquad \left(K_e^* \equiv \frac{5kp_e}{2eB} \tau_e H_e\right) \quad (19)$$

a result previously obtained[8] by quite different arguments. It was shown that in laboratory plasmas K_e^* is several hundred times larger than $K_{\perp e}$. Thus non-markovian effects are of great importance in the transport theory of a magnetoplasma. They may account for the relative failure of tokamaks and similar magnetoplasma machines to confine the plasma energy in the manner originally optimistically predicted by the standard theory. Provided τ_e in equation (19) is replaced by an average bounce time between the tokamak mirrors (good physical arguments can be advanced for this substitution), comparison of equation (19) with observations in tokamaks shows remarkably good agreement. Another interesting feature of equation (19) is that at sufficiently high densities H_e becomes negative and a thermal instability results that must disrupt the discharge; this may explain the well known disruptive instability in tokamaks[9].

To pursue these technical topics here would be inappropriate. The conclusion is that non-markovian effects are of great importance in a magnetoplasma, and that they appear to provide the answers to several unsolved problems in fusion research.

Received 14 September; accepted 11 November 1983.

1. Bogoliubov, N. N. *Studies in Statistical Mechanics* Vol. 1 (eds Boer, J. De & Uhlenbeck, G. E.) (North-Holland, Amsterdam, 1946).
2. Montgomery, D. *Lectures in Theoretical Physics* Vol. 9C (ed. Brittin, W. E.) (Gordon and Breach, New York, 1967).
3. Tschen, C. M. *Phys. Rev.* **114**, 394–411 (1959).
4. Fujita, S. *Lectures in Theoretical Physics* Vol. 9C (ed. Brittin, W. E.) (Gordon and Breach, New York, 1967).
5. Woods, L. C. *J. Fluid Mech.* **136**, 423–433 (1983).
6. Chapman, S. & Cowling, T. G. *The Mathematical Theory of Non-Uniform Gases* (Cambridge University Press, 1970).
7. Bragniskii, S. I. *Reviews of Plasma Physics* Vol. 1 (ed. Leontovich, M. A.) (Consultants Bureau, New York, 1965).
8. Woods, L. C. *J. Plasma Phys.* **29**, 143–154 (1983).
9. Murakami, M., Callen, J. D. & Berry, L. A. *Nucl. Fus.* **16**, 347–354 (1976).

A possible mechanism for epeirogenic uplift

Dan McKenzie

Bullard Laboratories, Madingley Rise, Madingley Road, Cambridge CB3 0EZ, UK

Continental geology is dominated by vertical movements, some of these are the result of crustal shortening and extension associated with large horizontal displacements, involving processes which are now understood, at least in outline. However, both uplift and subsidence can occur without associated shortening or extension of the continental crust. Most investigations of such epeirogenic movements have been concerned with subsidence, partly because of the economic importance of the resulting sedimentary basins, and partly because of the existence of a simple model for the process[1] which can account for the development of several basins in some detail[2–4]. However, uplift has been relatively neglected and cannot be produced by the same mechanism. The purpose of this paper is to examine whether epeirogenic uplift can result from the intrusion of large thicknesses of basic magma into the lower part of continental crust. This suggestion was considered as a possible mechanism for the regional uplift of the Colorado Plateau by Gilbert[5] more than 100 years ago, and also by Holmes[6] (though both rejected the idea). However, a recent discussion of no less than 14 different mechanisms for generating uplift[7] did not include this process, even though it seems able to produce the observed vertical motions and also to account for some of the features of the evolution of sedimentary basins that cannot be explained by the uniform stretching model[3,8,9]. Although the suggestion is not new, the argument used here to support it is, and depends on an understanding of the relationship between ocean island volcanoes and mantle circulation.

In the ocean basins magma is generated in two different environments. Plate separation at ridges leads to adiabatic upwelling and melting. This process produces most of the oceanic crust. The other type of feature which produces magma in the ocean basins is intra-plate volcanism such as that which is occurring in Hawaii, Iceland and the Azores. No detailed estimates of the volume of volcanic material produced by intra-plate volcanism appear to have been made. Ben-Avraham *et al.*[10] suggest that about 10% of the ocean floor is covered by aseismic plateaux, almost all of which are volcanic constructs. The crustal thickness beneath these features is about 20 km, of which perhaps 15 km has been added to the normal oceanic crustal thickness by volcanism. The error in the estimate of the total volume involved may be as much as a factor of two; more accurate estimates are not critical to the present argument. Many of the major ocean island volcanoes which are now active are believed to be at the centre of hot rising regions within the mantle[11–13]. Although the nature of the relationship between the magma and the upwelling is not yet clear, it is generally agreed to exist. The importance of this association is that hot rising regions are generated at the base of the convecting region[11] by instabilities of the lower thermal boundary layer. The distribution of these regions, and hence the rate of magma production from such sources, should therefore be independent of the distribution of continental and oceanic lithosphere, though they cannot yet be mapped beneath the continents by the methods used in the oceans. Many authors have proposed continental volcanoes which they believe to be the surface expression of such rising regions. Certainly some of these volcanoes are likely to have such an origin. There is a major difficulty to be overcome, however, if the rate of magma production in the rising regions is to be the same beneath both continents and oceans. The mean age of oceanic lithosphere is no more than 100 Myr, whereas that of the continental lithosphere is probably at least 1,500 Myr. Hence 15 times more of the continent than of the ocean should be covered by a basaltic layer 15 km thick. Such basalt should therefore make up about 75% of the continental crust of 30 km thickness. Compared with this estimate the volume of flood basalts even in an area such as the Deccan is negligible. Clearly this argument is not compatible with geological observations if the magma is erupted at the surface.

The geological observations are, however, not contradicted if this volume of magma is injected into the lower half of the continental crust. The mean density of the continental crust is about 2.8 Mg m^{-3} (ref. 14), and varies from about 2.6 Mg m^{-3} at the top to 3 Mg m^{-3} at the bottom. The composition of the intruding magma has little effect on its density, which is likely to be between 2.7 and 2.9 Mg m^{-3} (ref. 15) at a depth of 30 km. If much of the primary magma is picritic, as seems likely[16,17], its density will be at the upper end of this range. Therefore, whatever its composition, such a magma could form sills within the plate. This emplacement is likely to be within the crust if the magma density is less than that of the lower crust, or it could form a layer between the crust and the mantle if its density is greater. If the whole of the lower part of the continental crust is produced in this way, the resulting velocity of P waves should be between 6.8 and 7.0 km s^{-1}, in agreement with that observed. Cox[17] has proposed a similar model on petrological grounds, and argued that differentiation within a picritic layer could generate plateau basalts. Large intrusions of basic magma could also provide the source of heat required to generate rhyolites and granites from the continental crust by anataxis[18–20], and in this way satisfy the isotope constraints[21–23]. Such intrusions into

0028–0836/84/070616—03$01.00

© 1984 Macmillan Journals Ltd

the lower crust could account for the horizontal layering seen in many deep reflection profiles of the continental crust (refs 24, 25 and D. H. Matthews, personal communication). Clear reflections from the Moho also suggest that extensive intrusion of dykes is not common. The resulting uplift, followed by erosion, can explain the dominance of high grade metamorphic rocks in the surface outcrop of Archaean terrains[26].

Perhaps the most important consequence of the intrusion of great thicknesses of magma into the lower crust is the associated surface uplift. The addition of 15 km of material of density 2.7 Mg m^{-3} to the crust which overlies mantle of density 3.3 Mg m^{-3} produces 2.7 km of permanent uplift, sufficient to generate features like the Colorado Plateau. The reason Gilbert[5] argued against such a mechanism is that the plateau is bounded by linear monoclines, whereas the smaller intrusions he mapped in the Henry Mountains were circular in plan. However, it would not be surprising if the uplift reactivated old lines of weakness in the upper crust as faults that may underlie the monoclines. Gilbert's objection does not therefore seem to be fundamental. Some uplifted regions, such as Lesotho in southern Africa[27], have probably existed for longer than 60 Myr, the thermal time constant of the lithosphere. This region is also more than 100 km from the continental margin. Such uplift is therefore unlikely to be produced by lithospheric heating[28], which would not extend more than about 100 km from the margin and would decay with a time constant of 60 Myr. There is, however, no difficulty in producing the uplift by large intrusions, and the resulting elevation can only be removed by erosion. Furthermore, many uplifted regions are associated with extensive surface volcanism.

These arguments therefore suggest that large igneous intrusions are a major cause of uplift. Another cause is likely to be the uplift produced by the hot upwelling mantle. In oceanic regions such uplift has a scale of 1,000 km or more, and an amplitude of about 1 km. Similar surface deformation should exist in continental regions, although the subaerial amplitude will be reduced to about 700 m. Such features should be widespread, though their existence has not been clearly established. Unlike uplift produced by crustal thickening, that resulting from mantle upwelling will disappear as the plate moves away from the rising region. Also, the horizontal scale of the uplift produced by mantle upwelling should be greater than that from igneous intrusion. The source of the magma may be the hot upwelling region, especially in regions remote from plate boundaries. This is the only source whose production rate can be estimated. Near plate boundaries basic magma is generated in other ways, by both extension and subduction, and may also be emplaced into continental crust.

The above discussion has been concerned with uplift which is not directly associated with crustal shortening or extension. It is likely that some major igneous intrusions into the lower crust also occur in continental regions undergoing deformation. If such additions occur during the extension associated with the formation of a sedimentary basin, the volume of the crust will not be conserved. This process has been discussed by several authors[9,29], all of whom required that there be a relationship between the extension and the amount of melt produced. No such relationship is, in general, to be expected unless both the temperature and composition of the mantle are constant. The ratio of the final to the initial area, β, cannot then be estimated from the change in crustal thickness. Since the density of the intrusion is similar to the mean crustal density ρ_c, the initial tectonic subsidence, s_i, is approximately related to the initial, t_c, and final, t_f, crustal thicknesses by

$$s_i = \frac{(\rho_m - \rho_c)(t_c - t_f)}{(\rho_m - r_w)} \quad (1)$$

where $t_c > t_f$, ρ_m is the density of the mantle, ρ_w that of seawater and the upper surface of the crust is initially at or below sea level. The final thickness t_f then consists of the sum of the thickness t_c/β of original crust and of that, t_I, of intrusions (Fig. 1). The igneous material will probably not be emplaced as dykes but as sills, because the density of most magma types exceeds that of upper crustal material. After the extension ceases, the thermal subsidence and heat flux will be controlled by the extension of the lithosphere, which will exceed that (β_a) estimated from the change in crustal thickness. The heat flux associated with the intrusion itself will decay quickly, because the thermal time constant of a 30 km thick layer is less than 5 Myr.

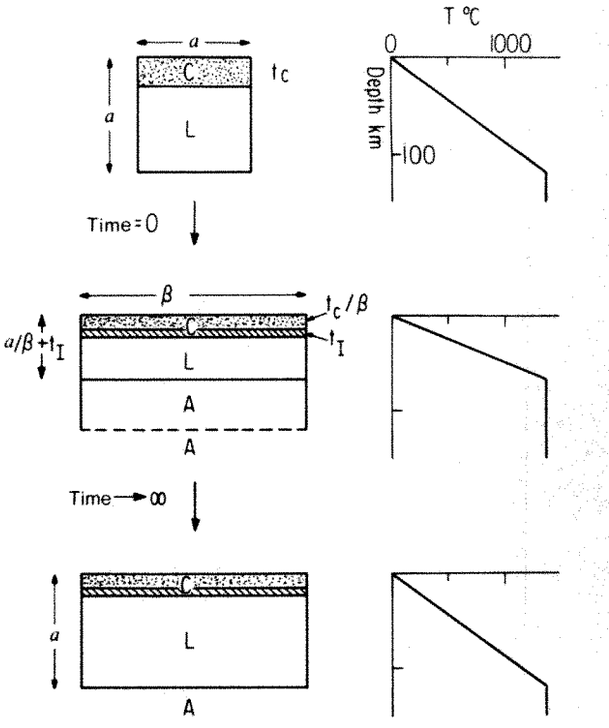


Fig. 1 A piece of thermally equilibrated continental lithosphere of thickness a, consisting of crust C (dotted) and mantle L, underlain by asthenosphere A, is uniformly extended by a factor β at time 0, and a thickness t_I of basic magma (cross-hatched) is added to the crust. The total change in thickness of the continental crust, marked C, is $t_c(1-1/\beta)-t_I$ and controls the tectonic subsidence associated with faulting during the stretching event. In contrast, the variation of temperature with depth is controlled by β, as are also the heat flux and the thermal subsidence. In the example illustrated $\beta = 2$ and $t_I = 7.5$ km. Hence the value of β obtained from the change in crustal thickness would be 1.33.

The effects of extension combined with large additions to the crust therefore closely resemble those which Royden and Keen[8] and Royden *et al.*[3] have argued should be produced by stretching the mantle part of the lithosphere more than the crust. The behaviour of the model proposed above is the same as that of Royden *et al.* if their crustal value of β, β_a, is defined to be

$$\beta_a = t_c/(t_I + t_c/\beta) \quad (2)$$

The value of β_a controls the change in crustal thickness and initial subsidence, whereas the true value of β controls the heat flux and thermal subsidence. In regions such as the Pannonian Basin the magma is more likely to be produced by the subduction zone than by the hot rising jet within the mantle.

Despite the similarity in the behaviour of the model proposed here to that of Royden *et al.*[3], the physical processes involved are rather different. If the crust is everywhere stretched less than the mantle part of the lithosphere, as they suggest, a space problem arises. If, however, the true crustal extension is the same as that of the mantle, but merely appears to be less because crustal volume has not been conserved, there is no such difficulty. In the Pannonian Basin the thermal subsidence requires a value

of β of at least 3, whereas the present crustal thickness and the initial subsidence suggest a value of β of about 2. If the initial crustal thickness was 36 km, these values require the addition of 6 km or more of igneous material. Such a thickness is not unreasonable, as large amounts of volcanic material were being erupted in the surrounding regions during the extensional event, probably generated by a subduction zone. Whether the large horizontal displacements, which must occur if β is to have a value of more than 3, can be accommodated by faulting is uncertain. Though this objection has been raised repeatedly[30,31] against the stretching model even in the absence of additions to the crust, the accuracy of estimates made from fault geometry is still controversial[32]. In contrast to the Pannonian Basin, little volcanism was associated with the extension of the North Sea, and therefore different methods of estimating β yield similar values[4].

Massive intrusions can also occur in regions undergoing shortening, where they will add to the uplift produced by crustal thickening.

I thank K. Cox, L. Royden, K. O'Nions and R. Wood for helpful comments, and NERC and the University of Chicago for support. Department of Earth Sciences contribution 494.

Received 28 June; accepted 7 December 1983.

1. McKenzie, D. P. *Earth planet. Sci. Lett.* **40,** 25–32 (1978).
2. Sclater, J. G. & Christie, P. A. F. *J. geophys. Res.* **85,** 3711–3739 (1980).
3. Royden, L., Horvath, F., Nagymarosy, A. & Stegena, L. *Tectonics* **2,** 91–137 (1983).
4. Wood, R. J. & Barton, P. *Nature* **302,** 134–136 (1983).
5. Gilbert, G. K. *Report on the Geology of the Henry Mountains* (Government Printing Office, Washington DC, 1877).
6. Holmes, A. *Principles of Physical Geology* (Ronald, New York, 1964).
7. McGetchin, T. R., Burke, K. C., Thompson, G. A. & Young, R. A. in *Dynamics of Plate Interiors* (eds Bally, A. W., Bender, P. L., McGetchin, T. R. & Walcott, R. I.) 99–110 (Am. Geophys. Un. & Geol. Soc. Am., Boulder, Colorado, 1980).
8. Royden, L. & Keen, C. E. *Earth planet. Sci. Lett.* **51,** 343–361 (1980).
9. Beaumont, C., Keen, C. E. & Boutilier, R. *Geophys. J. R. astr. Soc.* **70,** 667–715 (1982).
10. Ben-Avraham, A., Nur, A., Jones, D. & Cox, A. *Science* **213,** 47 (1981).
11. McKenzie, D. P., Roberts, J. M. & Weiss, N. O. *J. Fluid Mech.* **62,** 465–538 (1974).
12. Sclater, J. G., Lawver, L. A. & Parsons, B. *J. geophys. Res.* **80,** 1031–1052 (1975).
13. Watts, A. B. *J. geophys. Res.* **81,** 1533–1553 (1976).
14. Cochran, J. R. *Geophys. J. R. astr. Soc.* **68,** 171–201 (1982).
15. Nisbet, E. G. in *Komatiites* (eds Arndt, N. T. & Nisbet, E. G.) Ch. 29 (Allen & Unwin, London, 1982).
16. O'Hara, M. J. *Earth Sci. Rev.* **4,** 69–133 (1968).
17. Cox, K. G. *J. Petrol.* **21,** 629–650 (1980).
18. Pichler, H. & Ziel, W. *Bull. Volcan.* **35,** 424–452 (1972).
19. Wyllie, P. J. *Tectonophysics* **43,** 41–71 (1977).
20. Ewart, A. *J. Petrol.* **23,** 344–382 (1982).
21. Ewart, A. & Stipp, J. J. *Geochim. cosmochim. Acta* **32,** 699–736 (1968).
22. Klerkx, J., Deutsch, S., Pichler, H. & Ziel, W. *J. Volcan. geotherm. Res.* **2,** 48–71 (1977).
23. Mahoney, J. *et al. Earth planet. Sci. Lett.* **60,** 47–60 (1982).
24. Smythe, D. K. *et al. Nature* **299,** 338–340 (1982).
25. Oliver, J., Cook, F. & Brown, L. *J. geophys. Res.* **88,** 3329–3347 (1983).
26. Perkins, D. & Newton, R. C. *Nature* **292,** 144–146 (1981).
27. King, L. *The Morphology of the Earth* (Oliver & Boyd, Edinburgh, 1967).
28. Sleep, N. H. *Geophys. J. R. astr. Soc.* **24,** 325 (1971).
29. Foucher, J.-P., Le Pichon, X. & Sibuet, J.-C. *Phil. Trans. R. Soc.* A**305,** 27 (1982).
30. Ziegler, P. A. *Phil. Trans. R. Soc.* A**305,** 113–143 (1982).
31. Avedik, F. *et al. Phil. Trans. R. Soc.* A**305,** 5 (1982).
32. Bally, A. W. *Phil. Trans. R. Soc.* A**305,** 41 (1982).

Isotopic and geochemical evidence for the evolution of a cyclic unit in the Rhum intrusion, north-west Scotland

Z. A. Palacz*

Department of Earth Sciences, The University, Leeds LS2 9JT, UK

Layered basic intrusions can record processes which operated within basic magma chambers. In particular processes such as magma mixing and fractionation can be identified from the chemistry of cumulus minerals. Isotopes have been used to identify contamination in lavas[1–6], but they can also be applied to cumulates to infer a continuous record of the interactions of crust and magma within the magma chamber. We report here that olivine compositions, rare earth elements (REE) on mineral separates and Sr isotopes on whole rocks and pyroxenes from unit 9 of the Eastern Layered Series of the Tertiary Rhum intrusion, north-west Scotland, suggest that fresh magma entered the chamber and mixed with fractionated and contaminated magmas at the base of the chamber. With prolonged residence in the chamber the fresh magma became fractionated and contaminated, was then erupted off and replaced by fresh magma which produced the basal peridotites of unit 10.

The Eastern Layered Series (ELS) comprises 15 cyclic units[7], each representing the crystallized products of successive injections of magma into the chamber[7,8]. A typical sequence of rocks within a cyclic unit comprises peridotite, grading upwards into troctolite and finally gabbro, corresponding to the first appearance of olivine, plagioclase and the clinopyroxene on the liquidus with decreasing temperature. The basal peridotites are in sharp contact with the gabbros of the preceding unit.

Unit 9 is 21 m thick (Fig. 1), olivine being the only cumulus mineral present throughout the unit. The forsterite content can be directly related to the Mg/Mg+Fe(II)) ratio of the magma (an index of fractionation)[9].

The most Mg-rich olivines would be expected at the base and the most Fe-rich olivines at the top of the unit. In unit 9 the olivines are more Mg rich upsequence through the peridotites (Fig. 2, Table 1). Within the troctolites the compositions vary, while the olivines within the gabbros become more Fe-rich, particularly towards the top of the unit. The base of unit 10 has more Mg-rich olivines than the top of unit 9. This would, perhaps, be consistent with the eruption of fractionated magma and replacement by fresh magma.

In contrast, the olivines in the gabbros at the top of unit 8 have almost identical compositions with those in the basal peridotite of unit 9, while olivines 2 m below the unit 8/9 contact (41597) are the most evolved yet found in the ELS. The basal peridotite (42142) of unit 9 contains a unique occurrence of cumulus clinopyroxene, restricted to the basal metre of unit 9. They form up to 15% of the mode and are small subhedral crystals occasionally enclosed within cumulus olivine, but more typically within intercumulus pyroxene and plagioclase. They are unzoned with compositions comparable with the cumulus pyroxenes in the gabbros at the top of unit 8.

The approximate equivalence of olivine and pyroxene compositions across unit contacts (Table 1) and the progressively more primitive nature of olivines upsequence through the peridotites has been interpreted as being due to magma mixing[10] between a fractionated magma and a fresh magma batch. The presence of cumulus pyroxenes at the base of unit 9 may support this.

Sr isotopes measured on whole rocks and pyroxene separates are presented in Fig. 2 and Table 1. Rb/Sr measurements on several samples indicate that the age correction (60 Myr) is insignificant and within experimental error, therefore ratios where no Rb data are available are assumed to be initial ratios. The variations in $^{87}Sr/^{86}Sr$ through unit 9 are large.

Clinopyroxenes through unit 9 are generally in isotopic equilibrium with the whole rocks. The only exceptions are at the tops of units 8 and 9. Disequilibrium between pyroxene and magma can be caused by magma mixing between two or more isotopically distinct magmas with the pyroxenes being derived from one magma[11]. Because the rate of Sr diffusion in pyroxene is slow compared with that in the magma[12], it is possible for the pyroxene to retain the $^{87}Sr/^{86}Sr$ of the magmas it originally crystallized from and not reequilibrate with the magma it settled in. Pyroxenes are also resistant to hydrothermal alteration[13]. Because the pyroxenes were leached[14], any hydrothermal Sr will be removed, therefore because pyroxenes and whole rock $^{87}Sr/^{86}Sr$ are equivalent these variations are considered primary and not the result of hydrothermal alteration.

* Present address: Department of Earth Sciences, The Open University, Walton Hall, Milton Keynes MK7 6AA, Buckinghamshire, UK.

0028–0836/84/070618—03$01.00

© 1984 Macmillan Journals Ltd

Table 1 Composition analyses

Sample		Unit (height above base, m)	Rb‡ (p.p.m.)	Sr‡ (p.p.m.)	Rb/Sr	$^{87}Sr/^{86}Sr$†	$^{87}Sr/^{86}Sr_{60}$‡	Fe mole % olivine	Mgr CPX
42162 Peridotite	WR	10(1)	ND	69	—	0.70411±5		85.2±0.2	
42158 Peridotite	WR	10(0)	0.25	56.1	0.004	0.70382±7	0.70381	86.3±0.4	
42157 Gabbro	WR	9(21)	0.92	265.8	0.003	0.70538±6	0.70537	76.6±0.1	
	CPX		ND	24.9	—	0.70495±12			
42156 Gabbro	WR	9(19)	ND	307.9	—	0.70425±5		79.2±0.1	
42155 Gabbro	WR	9(17)	1.55	311.6	0.005	0.70395±4	0.70394	79.9±0.7	
42154 Gabbro	WR	9(14)	1.35	238.7	0.005	0.70405±3	0.70404	78.9±0.3	
	CPX		ND	ND	—	0.70397±7			
42149 Gabbro	WR	9(10)	0.87	286.1	0.003	0.70413±3	0.70412	80.1	
	CPX		ND	28.36	—	0.70417±4			
42148 Troctolite	WR	9(9)	ND	314.7	—	0.70550±7		84.2±0.2	
42147 Troctolite	WR	9(7)	0.43	204.6	0.002	0.70496±5	0.70496	82.6±0.3	
42144 Peridotite	WR	9(6)	0.55	125.9	0.004	0.70544±6	0.70543	84.4±0.04	
	CPX		ND	30.29	—	0.70543±4			
42143 Peridotite	WR	9(2)	0.55	141.7	0.004	0.70532±5	0.70531	83.6±0.2	
42142 Peridotite	WR	9(0)	0.44	138.5	0.003	0.70525±2	0.70525	81.5±0.5	83±0.6
	CPX		ND	28.3	—	0.70530±			
41596 Gabbro	WR	8 (top)	ND	275.6	—	0.70622±2		81.1±0.2	83.8±0.17
	CPX		0.14	50.4	0.0027	0.70633±5	0.70633		
41597 Gabbro	WR	8 (2m below top)	ND	289.5	—	0.70543±2	—	73.8±0.4	

WR, whole rock; CPX, clinopyroxene.
* Isotope dilution (p.p.m.).
† Errors 2 s.e. SRM 987 0.710254±12 (47 analyses); VG.MM 30. Mgr, Mg/(Mg+Fe(II)), 10 analyses errors 1 s.e. ND, not determined.
‡ 60 Myr; $\lambda 1.42\times10^{-11}$.
§ Mean at least five analyses by WDS. Errors l.s.e.

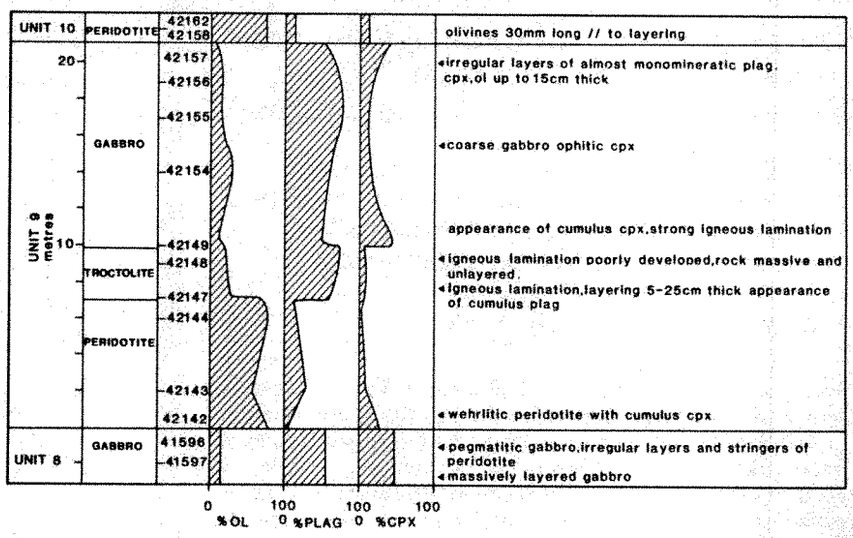


Fig. 1 Lithological log through unit 9.

The unit can be divided isotopically into two. In the peridotites $^{87}Sr/^{86}Sr$ is ~0.705, while the gabbros are mainly ~0.704. The troctolites appear to have transitional values. Within the gabbros an increase in $^{87}Sr/^{86}Sr$ at the top of unit 9 coincides with Fe enrichment in olivine and may represent the process of combined assimilation of high $^{87}Sr/^{86}Sr$ crust and fractional crystallization (AFC)[3]. A sharp reduction in $^{87}Sr/^{86}Sr$ at the base of unit 10 coincides with more Mg olivines which may suggest that contaminated and fractionated magmas were erupted off and replaced by relatively uncontaminated and unfractionated magmas.

In the peridotites of unit 9 a more complex situation is present. The olivine compositions indicate that magma mixing has operated. The Sr isotopes may support this because the $^{87}Sr/^{86}Sr$ of the peridotites is substantially higher than the basal peridotites of unit 10 and may represent a mixture of high $^{87}Sr/^{86}Sr$ magma which crystallized the top gabbro of unit 8 and low $^{87}Sr/^{86}Sr$ fresh magma.

The REE contents of separated pyroxenes are presented in

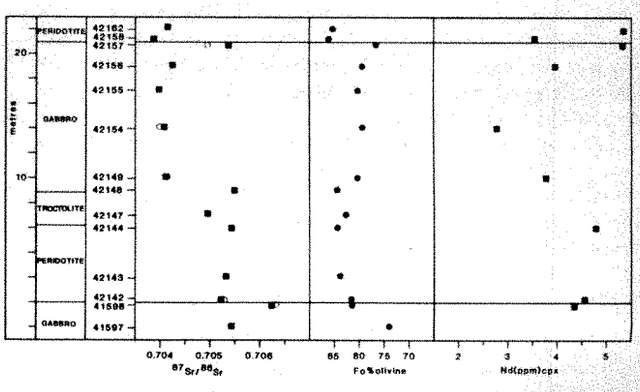


Fig. 2 Variation in whole rock (squares) and clinopyroxene (circles) $^{87}Sr/^{86}Sr$ through unit 9, compared with Fe content in olivine and Nd concentration in clinopyroxene.

Table 2 REE by isotope dilution

Sample	La	Ce	Nd	Sm	Eu	Gd	Dy	Er	Yb
42162 CPX*	0.90	3.60	5.33	2.00	0.69	2.60	2.75	1.37	1.00
42158 CPX	0.59	2.66	3.57	1.36	0.47	1.79	1.78	0.90	0.68
42157 CPX	0.83	3.73	5.34	2.15	0.75	2.92	2.99	1.54	1.20
42156 CPX	—	2.94	3.99	1.54	0.48	—	—	—	0.81
42154 CPX	0.44	2.06	2.77	1.08	0.38	1.42	1.45	0.71	0.55
42149 CPX	0.79	2.77	3.790	1.48	0.49	1.95	—	0.94	0.83
42144 CPX	0.79	3.63	4.79	1.83	0.58	2.42	—	1.29	1.000
42142 CPX	—	3.78	4.57	1.75	0.56	2.29	—	1.29	0.97
41596 CPX	0.77	3.26	4.360	1.65	0.52	2.03	—	1.19	0.94

From ref. 15. Errors 2 s.e. are 1%. CPX, clinopyroxene.

Table 2. The REE patterns are parallel, with variable, small, negative Eu anomalies. Because the patterns are parallel the total REE content ($\sum$ REE) of the pyroxenes can be expressed in terms of one element, Nd, for example. The variation of Nd through unit 9 (Fig. 2) is remarkably similar to $^{87}Sr/^{86}Sr$. Although Nd can increase in magma during fractionation, $^{87}Sr/^{86}Sr$ cannot, consequently, these variations represent the addition of a high Nd, high $^{87}Sr/^{86}Sr$ contaminant. This addition is seen in the gabbros at the top of unit 9 and coincides with fractionation. The peridotites of unit 9 also seem to be contaminated. However, this contamination could be the result of magma mixing between the fresh magma influx and any contaminated magma left in the chamber.

I thank Drs M. F. Thirlwall, R. A. Cliff and B. M. Wilson for helpful discussion and criticism and Dr E. Condliffe for advice on the microprobe. Isotope work at Leeds is funded by the Royal Society and NERC, and this work was performed during the tenure of a NERC studentship.

Received 25 May; accepted 18 November 1983.

1. DePaolo, D. & Wasserburg, G. *Geochim. cosmochim. Acta* **43,** 615–627 (1979).
2. James, D. E. *Earth planet. Sci. Lett.* **57,** 47–62 (1982).
3. DePaolo, D. *Earth planet. Sci. Lett.* **53,** 189–202 (1982).
4. Moorbath, S. & Welke, H. *Earth planet. Sci. Lett.* **5,** 217–230 (1969).
5. Moorbath, S. & Thompson, R. N. *J. Petrol.* **21,** 295–321 (1980).
6. Dickin, A. P. *J. Petrol.* **22,** 155–189 (1980).
7. Brown, G. M. *Phil. Trans. R. Soc.* B**240,** 1–53 (1956).
8. Huppert, H. E. & Sparks, R. S. J. *Contr. Miner. Petrol.* **75,** 279–289 (1980).
9. Roeder, P. L. & Emslie, R. F. *Contr. Miner. Petrol.* **29,** 275–289 (1970).
10. Dunham, A. C. & Wadsworth, W. J. *Min. Mag.* **42,** 347–356 (1979).
11. Vollmer, R., *et al. Geotherm. Res.* **11,** 317–327 (1981).
12. Sneeringer, M. & Hart, S. *EOS* **59,** 402 (1978).
13. Taylor, H. P. & Forester, R. W. *J. Petrol.* **20,** 355–419 (1979).
14. Stosch, H. G., Carlson, R. W. & Lugmair, G. W. *Earth. planet. Sci. Lett.* **47,** 263–271 (1980).
15. Thirlwall, M. F., *Chem. Geol.* **35,** 155–166 (1982).

Oxygen isotope calibration of the onset of ice-rafting and history of glaciation in the North Atlantic region

N. J. Shackleton*, J. Backman, H. Zimmerman, D. V. Kent, M. A. Hall, D. G. Roberts, D. Schnitker, J. G. Baldauf, A. Desprairies, R. Homrighausen, P. Huddlestun, J. B. Keene, A. J. Kaltenback, K. A. O. Krumsiek, A. C. Morton, J. W. Murray & J. Westberg-Smith

We report here that DSDP Site 552A, cored with the hydraulic piston corer on the west flank of Rockall Bank, recovered an undisturbed sequence of alternating white deep-sea carbonate oozes and dark-coloured layers that are rich in glacial debris. Oxygen isotope analysis of the sequence together with detailed nannofossil and palaeomagnetic stratigraphy shows that the first major horizon of ice-rafting occurred at about 2.4 Myr, and was preceded by a minor pulse of ice-rafting at about 2.5 Myr. The carbon isotope record shows that the site has been bathed by a water mass of similar characteristics to present-day North Atlantic deep water at least since 3.5 Myr.

Despite the concentration of members of the deep-sea drilling community around the North Atlantic, surprisingly few Deep Sea Drilling Project sites have been drilled that are suitable for even a cursory study of North Atlantic palaeoenvironments. Until recently the best sites in which to study the onset of glaciation around the North Atlantic were from DSDP Leg 12, sites 111 and 116, in which Berggren[1] studied the stratigraphic position of the earliest ice-rafted debris in the region and estimated its age at 3.0 Myr (Backman[2] re-examined this material and obtained a younger estimate of 2.5 Myr). Sediments from the Leg 12 sites were extensively disturbed by rotary drilling, and not suitable for detailed analysis of the palaeoenvironmental record. During Leg 81, the *Glomar Challenger* used the newly developed hydraulic piston corer (HPC)[3] with considerable success, and in particular recovered a section from the west flank of Rockall Bank (Fig. 1) that is largely undisturbed.

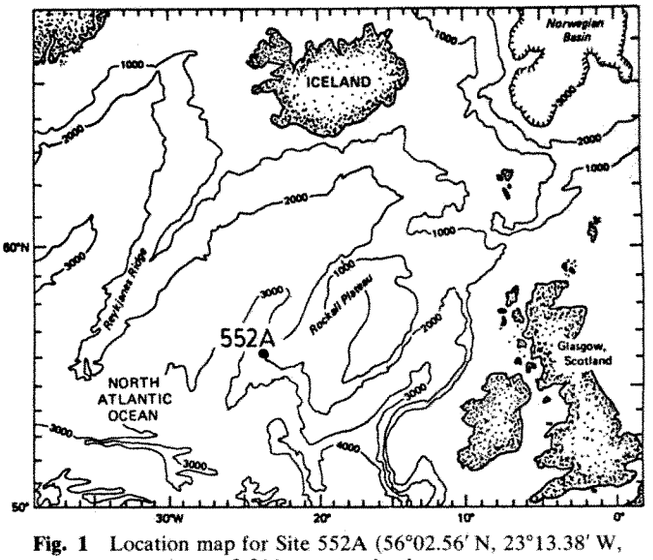


Fig. 1 Location map for Site 552A (56°02.56′ N, 23°13.38′ W, 2,311 m water depth.

* Authors' addresses: Godwin Laboratory for Quaternary Research, Free School Lane, Cambridge CB2 3RS (N.J.S.); Department of Geology, University of Stockholm, S-10690 Stockholm, Sweden (J.B.); Department of Civil Engineering, Union College, Schenectady, New York 12308, USA (H.Z.); Lamond Doherty Geological Observatory of Columbia University, Palisades, New York 10964, USA (N.J.S., D.V.K.); British Petroleum (Overseas) Development Ltd, Britannic House, Moorgate, London EC1, UK (D.G.R.); Department of Oceanography, University of Maine, Walpole, Maine 04573, USA (D.S.); United States Geological Survey, 345 Middlefield Road, Menlo Park, California 94025, USA (J.G.B.); Laboratoire de Géochimie des Roches Sédimentaires, University of Paris XI, 91405 Orsay, France (A.D.); Laboratorium für Erdölgewinnung, Deutsche Texaco AG, 3101 Wietze, FRG (R.H.); Georgia Geological Survey, 19 Martin Luther King Jr Drive, Atlanta, Georgia 30334, USA (P.H.); Department of Geology, University of Melbourne, Parkville, Victoria 3052, Australia (J.B.); Denver Research Center, Marathon Oil Co., 7400 South Broadway, Littleton, Colorado 80160, USA (A.J.K.); Geologisches Institut, Nussallee 8, D-53 Bonn 1, FRG (K.A.O.K.); Institute of Geological Sciences, Ring Road, Halton, Leeds LS15 8TQ, UK (A.C.M.); Department of Geology, University of Exeter, Exeter, UK (J.W.M.); Scripps Institution of Oceanography, La Jolla, California 92092, USA (J.W.-S.).

0028–0836/84/070620—04$01.00

© 1984 Macmillan Journals Ltd

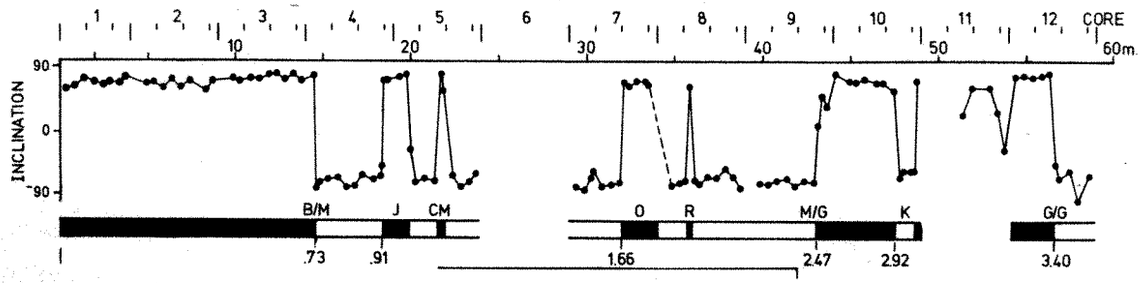


Fig. 2 Magnetic record for Site 552A. Demagnetized inclinations are shown only for apparently undisturbed parts of the cores (the data from core 11 suggest that some unrecognized disturbance may be present).

The upper 40 m of the section comprises alternating units of white and dark grey colour. In the white intervals, the >150-μm fraction consists almost exclusively of foraminifera; in the dark intervals angular sand-sized rock fragments predominate although sufficient benthic foraminifera for isotopic analysis are present at almost every horizon examined. The dark-coloured intervals containing ice-rafted material are visually clear and also show unmistakably in a record of carbonate content (typically between 80 and 90% in the white layers and 10–40% in the dark layers).

We have made isotope analyses at 10-cm intervals over the interval from well below the first ice-rafting layer, through to the recent sediment. Only core 6 was excluded from analysis because it was very severely disturbed by coring. Three species were used: *Globocassidulina subglobosa*, *Uvigerina peregrina* and *Planulina wuellerstorfi*. In Figs 3 and 4 data are presented using the previously established 'correction factors' to relate the oxygen isotope values obtained for *G. subglobosa* and *P. wuellerstorfi* to the equilibrium value which is thought to be given by *Uvigerina* spp.[4,5]. Carbon isotope values are related to *P. wuellerstorfi* rather than to *Uvigerina* because this procedure provides a good estimate of the ^{13}C content of ocean dissolved CO_2 (refs 6, 7).

Samples for palaeomagnetic analysis were taken approximately every 25 cm, avoiding sediment that showed any sign of disturbance. Only orientation with respect to the vertical axis was preserved so that the inclination component is used to infer polarity at this high-latitude site. The natural remanent magnetization (NRM) was measured on a two-axis cryogenic magnetometer[8] both before and after alternating-field demagnetization at 10–30 mT. NRM intensities are typically of the order 10^{-2} A m^{-1} in the upper 45 m of the section, decreasing to about 10^{-3} A m^{-1} in the lower part. Measured inclination data are shown in Fig. 2. Inclinations are generally well-grouped near to the expected dipole value (71°, positive for normal and negative for reversed) for the latitude of the site, indicating that a reliable record of the geomagnetic field has been obtained to the base of the Gauss normal magnetochron. The lower boundary of the Olduvai subchron is unfortunately obscured by disturbance at the break between successive cores, while the Mammoth subchron is unclear, probably lying in a slightly disturbed section of core 11.

Analyses of the carbonate content of the sediment have also been made at 10-cm intervals through the upper 59 m of the section. In this part of the North Atlantic, carbonate content is controlled chiefly by variations in the influx of non-carbonate material transported by ice[9]. Changing carbonate production also has a major role, while carbonate dissolution has little impact at such shallow water depths in the North Atlantic.

The samples taken for stable isotope analysis were also used for quantitative nannofossil studies; here we show (Fig. 4) only those nannofossil data which have a bearing on the biostratigraphical position of the early ice-rafting horizon.

Figure 3 shows the oxygen isotope and carbonate data for the upper five cores, together with the oxygen isotope record of core V28-238 (ref. 4) for comparison. Both records are plotted linearly with respect to depth in sediment between magnetic reversals. Since it has been established that the sediment in V28-238 accumulated at a fairly regular rate[10], the axis

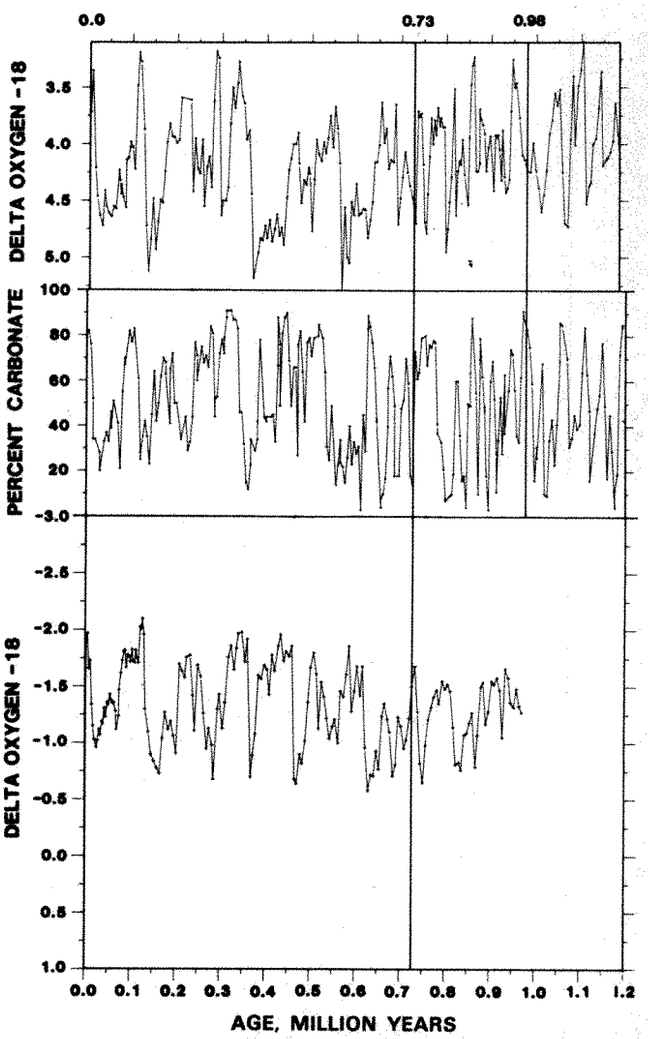


Fig. 3 Top: Oxygen isotope record of Site 552A cores 1–5. The plotting scale is linear between magnetic reversal horizons. Middle: carbonate content in the same interval; low carbonate content implies dilution by ice-rafted material. Bottom: oxygen isotope record of Pacific core V28-238 (ref. 4) for comparison. Vertical lines indicate the horizons used for time control: top (zero age), base Bruhnes normal chron (0.73 Myr), base Jaramillo subchron (0.98 Myr).

is shown as an approximate age scale calibrated on the basis of the single magnetic reversal in this core. Thus the comparison shows that there have been quite marked accumulation rate variations in DSDP 552A. This is hardly surprising in view of the lithological variations that are evident. Nonetheless the oxygen isotope record of Site 552A is complete to the extent of preserving every isotope stage.

In Figure 4, the oxygen isotope data for the lower part of the sequence are shown and compared with the oxygen isotope data for piston core V28-179. (Figure 4 shows additional measurements that we have made in core V28-179. Note that the position

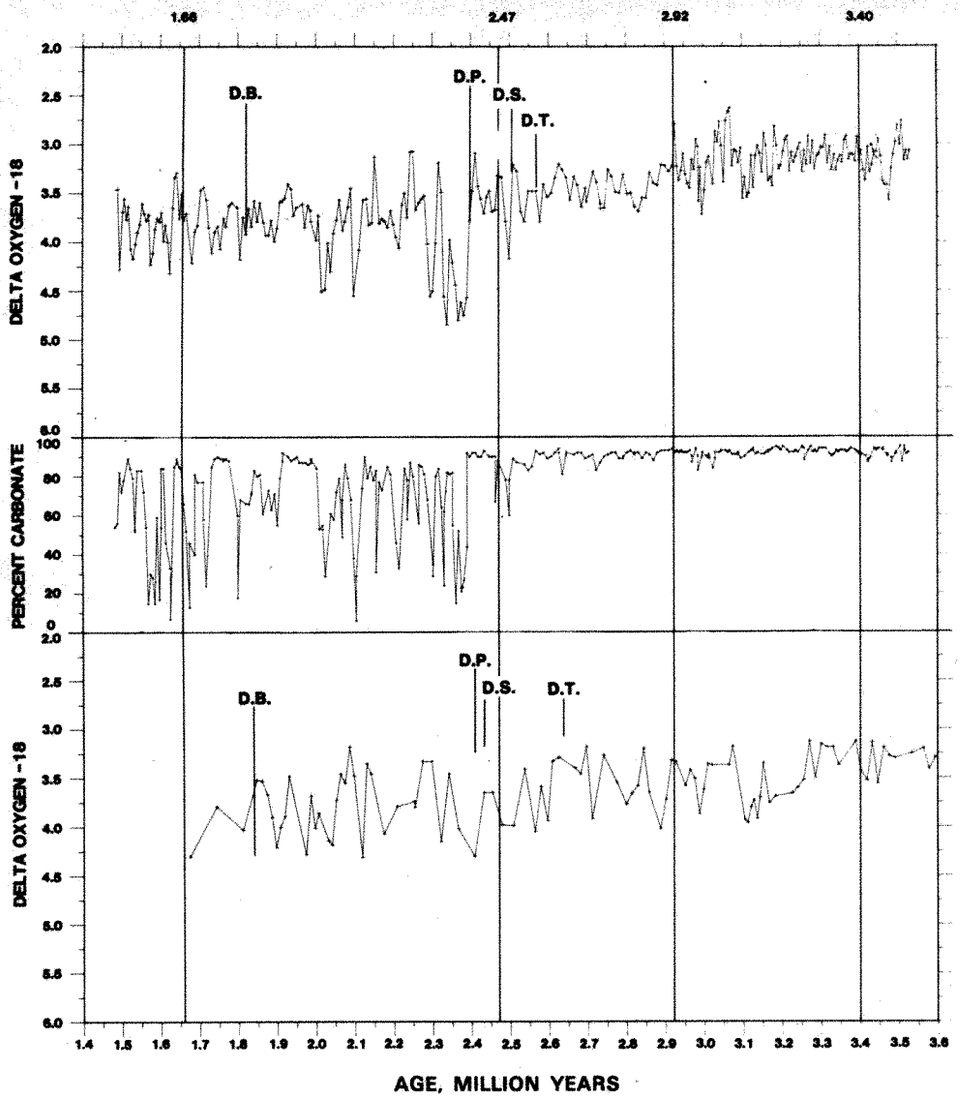


Fig. 4 Top: Oxygen isotope record of Site 552A cores 7–12. The plotting scale is linear between magnetic reversal horizons. Middle: carbonate content in same interval. Bottom: oxygen isotope record in Pacific core V28-179 (ref. 11) for comparison. Vertical lines show horizons used for time control: top Olduvai normal subchron (1.66 Myr), base Matuyama reversed chron (2.47 Myr), top Kaena reversed subchron (2.92 Myr), base Gauss normal chron (3.40 Myr). Nannofossil extinction horizons determined in both cores indicated by: DT, *Discoaster tamalis*; DS, *D. surculus*; DP, *D. pentaradiatus*; DB, *D. brouweri.*

of the Gauss–Matuyama boundary was incorrectly printed in ref. 11; it was in fact located at 1,338 cm, not 1,358 cm.) Again, to facilitate comparison, the plotting scale for both records has been adjusted at magnetostratigraphic boundaries as indicated, so as to approximate an age scale using a palaeomagnetic time scale[12]. Obviously the application of this age scale becomes less accurate far from the magnetic reversals. However, comparison between the two records is good considering the low accumulation rate, and relatively coarse sampling interval in V28-179, and many small-scale features can be correlated between the two. The amplitude of variation is substantially greater in Site 552A than in V28-179. This is probably due to the effect of bioturbation in the Pacific core; since the accumulation rate of core V28-179 was only about 0.55 cm kyr^{-1}, one would expect climatic extremes lasting only a few thousand years to be severely degraded by bioturbation.

The series of nannofossil extinction data shown in Fig. 4 have also been determined quantitatively in core V28-179 (ref. 13) and their ages estimated. Using these estimates, the average accumulation rate over the pre-ice-rafting interval in Site 552A may be estimated at approximately 1.7 cm kyr^{-1}, surprisingly close to the average for the upper part, 1.8 cm kyr^{-1} (although the rate has certainly varied over short intervals during the intense climatic fluctuations of the past few million years). Palaeomagnetic stratigraphy and nannofossil stratigraphy in this site give identical estimates for the age of the first major northern hemisphere glacial event about 2.37 Myr.

It is clear from an examination of Figs 3 and 4 that there is a remarkable correspondence between the records of oxygen isotopic composition in the foraminifera, and carbonate content, back to about 2.4 Myr. This is not particularly surprising since both are clearly causally related to glaciation. However, it is worth noting that if the late Pliocene isotopic fluctuations were attributed to glaciation in Antarctica, one would not expect the influx of ice-rafted debris during the glaciation at 2.4 Myr to be as dramatic as that observed in the last glacial, whereas this is what we observe in Site 552A. A brief ice-rafting episode, coinciding with positive ^{18}O values, is observed at about 2.5 Myr. Prior to 2.5 Myr, the influx of ice-rafted debris is minute and has little impact on the record of carbonate content. Presumably such glaciation as may have occurred before that time did not give rise to extensive calving of icebergs into the North Atlantic.

If we compare the isotopic composition of benthonic foraminifera at the level of the first glacial maximum at 2.37 Myr with values in glacial levels in the upper part of the sequence, it becomes apparent that the early event represents a truly glacial interval, with an ice volume similar to maxima during the middle Pleistocene. This must, for instance, be correlative with a fully glacial environment in Britain although obviously the actual ice-margin positions cannot be determined from these data.

Other data relating to a major climatic threshold being crossed at this time comes from the Netherlands and New Zealand. In the Netherlands, Zagwijn[14] has shown that there was a complete

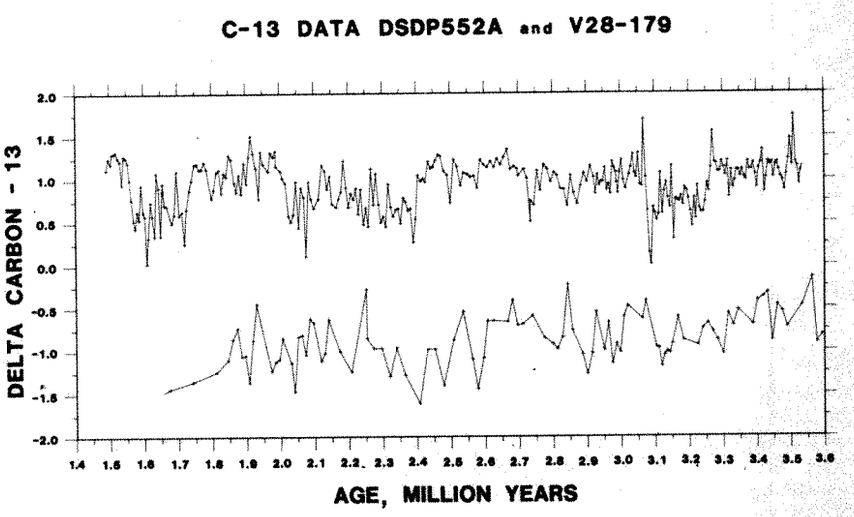


Fig. 5 Carbon isotope records for Site 552A cores 7–12 (top) and Pacific core V28-179 (bottom). Both are plotted on the same ^{13}C scale and represent estimates of the ^{13}C contents of intermediate depth (2,311 m) water in the North Atlantic (above) and of deep (4,509 m) water in the equatorial Pacific (below). Today there is an observed difference of a little over 1‰ which reflects the fact that ocean deep waters are ventilated in the North Atlantic.

overturn in vegetational type just after the Gauss–Matuyama boundary; indeed he regards this as representing the Pliocene–Pleistocene boundary. In New Zealand, Stipp and others[15] showed that there was a major drop in sea level between about 2.4 and 2.6 Myr; again, they correlated this event, which they interpreted as the first glacio-eustatic fall in sea level, with the Pliocene–Pleistocene boundary. We disagree with these opinions, only because we consider it well established that the Pliocene–Pleistocene boundary at the newly proposed stratotype at Vrica[16], as well as at previous Calabrian sections, has a much younger age of about 1.6 Myr (ref. 17). If the boundary were selected on the basis of a significant climatic event rather than on historical precedent, it might appropriately be re-positioned so as to coincide with the climatic change about 2.4 Myr, but we do not recommend such an approach to the definition of geological boundaries.

The data shown in Fig. 4 also indicate that there was considerable climatic variability, somewhere on the globe, even before 2.5 Myr. It is well established that glaciation in Iceland occurred as early as 3.1 Myr (ref. 18) although recent work suggests that glaciation did not reach sea level in Iceland until about 2.0 Myr (ref. 19) and there is also evidence for glaciation in the Sierra Nevada, California, at about the same time[20]. However, palaeooceanographic conditions on Rockall Bank do not suggest severe climates in North-west Europe. Occasional grains of ice-rafted debris do occur, and the nannofossil floras certainly show marked variation, but the scale of variation was much less before 2.4 Myr than it was after the event at that time.

Figure 5 shows the carbon isotope records for DSDP 552A and V28-179. The data are plotted on the same isotope scale and the same species-dependent correction factors have been made to both. The persistent isotopic difference between the two records of over 1‰ indicates a continuing situation in which the water bathing the sea floor on Rockall was isotopically heavier for ^{13}C than deep water in the Pacific, as is the case today. This difference reflects the difference between newly-formed, oxygen rich North Atlantic deep water (NADW) and older, oxygen-depleted deep Pacific water[21,22]. We (and also Blanc *et al.*[23]) disagree with Keigwin's deduction[24] that the formation of NADW only began with the closing of the Panama Straits at 3.0 Myr, although this event may well have affected the contribution that this water mass made to the deep water of the Caribbean deep waters that were the topic of Keigwin's study[24].

In the upper part of the section, it is clear that DSDP Site 552A preserves the whole of the standard oxygen isotope record of the past million years. Although the fine structure of many stages is obscure, every single stage is present. By comparison, we note that the stratigraphically longest piston core from the North Atlantic, core Kane 708-7 (ref. 9), extends only to Stage 17 at about 0.6 Myr. For the earlier record, only DSDP Site 116, recently re-investigated from the palaeoclimatic point of view[25], was previously available. Site 116 was recovered by conventional rotary drilling and the sediment was so disturbed that glacial and non-glacial materials are intermixed, precluding any high-resolution studies being made. It is to be hoped that the new technique of ^{13}C analysis in the organic carbon portion of the sediment, pioneered in Site 116 (ref. 25), will be applied to Site 552A. We believe that the material recovered in Site 552A is ideal for detailed studies of climatic evolution in the ocean adjacent to the north-west European region where the Plio–Pleistocene continental record is best known.

N.J.S. thanks NERC for support and Mike Tabecki for laboratory assistance. D.V.K. tanks NSF for support, Lamont-Doherty Geological Observatory contribution no. 3552.

Received 22 August; accepted 31 October 1983.

1. Berggren, A. A. *Init. Rep. DSDP* **12,** 953–963 (1972).
2. Backman, J. *Stockh. Contr. Geol.* **32,** 113–137 (1979).
3. Prell, W. L. *et al. Init. Rep. DSDP* **68,** 5–6 (1982).
4. Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* **3,** 39–55 (1973).
5. Shackleton, N. J. *Coll. int. Cent. natn. Rech. Scient.* **219,** 203–210 (1974).
6. Graham, D. W., Corliss, B. H., Bender, M. L. & Keigwin, L. D. Jr *Mar. Micropalaeont.* **6,** 483–497 (1981).
7. Duplessy, J.-C. *et al. Quat. Res.* (in the press).
8. Goree, W. S. & Fuller, M. *Rev. Geophys. Space Phys.* **14,** 591–608 (1976).
9. Ruddiman, W. F. & McIntyre, A. *Mem. Geol. Soc. Am.* **145,** 111–146 (1976).
10. Imbrie, J. *et al.* in *Milankovich and Climate* (eds Berger, A. L. *et al.*) (Reidel, Dordrecht, in the press).
11. Shackleton, N. J. & Opdyke, N. D. *Nature* **270,** 216–219 (1977).
12. Berggren, W. A., Kent, D. V. & van Couvering, J. *Geol. Soc. Lond. Spec. Publ.* (ed. Snelling, N. L.) (in the press).
13. Backman, J. A. & Shackleton, N. J. *Mar. Micropalaeont.* **8,** 141–170 (1983).
14. Zagwijn, W. *Boreas* **3,** 75–97 (1974).
15. Stipp, J. J., Chappell, J. H. A. & McDougall, I. *Am. J. Sci.* **265,** 462–474 (1967).
16. Selli, R. *et al. Giorn. Geol.* **42,** 181–204 (1977).
17. Backman, J., Shackleton, N. J. & Tauxe, L. *Nature* **304,** 156–158 (1983).
18. McDougall, I. & Wensink, H. *Earth planet. Sci. Lett.* **1,** 232–236 (1966).
19. Albertsson, K. J. *Náttúrufraedingurinn* **48,** 1–8 (1978).
20. Curry, R. R. *Science* **154,** 1121–1142 (1966).
21. Duplessy, J.-C. thesis, Univ. Paris VI (1972).
22. Kroopnick, P., Deuser, W. G. & Craig, H. *J. geophys. Res.* **75,** 7668–7671 (1970).
23. Blanc, P. L., Rabussier, D., Vergnaud-Grazzini, C. & Duplessy, J.-C. *Nature* **283,** 553–555 (1980).
24. Keigwin, L. D. *Science* **217,** 350–353 (1982).
25. Blanc, P. L., Fontugne, M. R. & Duplessy, J.-C. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **42,** 211–224 (1983).

Seasonal fluctuations in deep-sea sediment community oxygen consumption: central and eastern North Pacific

K. L. Smith Jr & R. J. Baldwin

Marine Biology Research Division, A-002,
Scripps Institution of Oceanography,
La Jolla, California 92093, USA

Historically the deep sea was viewed as a homogeneous environment in time and space characterized by high hydrostatic pressure, low temperature and low food supply[1,2]. The highly diverse but low-density communities of organisms[3,4] occupying this environment were dependent on a slow, constant rain of small particulate organic matter from the overlying surface water for food. This material slowly settled through thousands of metres of the water column, dampening any pulses in primary production at the surface. The rate at which this 'constant' food source was used was found to be slow[5–8]. However, there was early evidence[9–12], with much recent support[13–17], that some deep-sea animals exhibited seasonal reproduction. In addition, the flux of small particulate organic matter to the deep-sea floor has been shown to be seasonal and in phase with rates of surface primary productivity[18–20]. We report here the first evidence of seasonality in biological rates in the deep sea: *in situ* rates of sediment community oxygen consumption at two abyssal stations in the central and eastern North Pacific are highest in early summer, decreasing to the lowest rates in late autumn and winter.

We have measured sediment community oxygen consumption (SCOC) seasonally at two stations over the past 6 yr: Station CNP in the oligotrophic central North Pacific north of Hawaii (31° N, 159° W; water depth 5,900 m), and Station C in the eutropic eastern North Pacific, west of the southern California Continental Borderlands (32°33′ N, 120°31′ W; water depth 3,815 m).

Sediment community oxygen consumption was measured *in situ* using a free vehicle grab respirometer (FVGR)[21,22]. This instrument consists of an integrated flotation and instrument tripod which is capable of continuously measuring the dissolved oxygen consumed by the undisturbed sediment and overlying water in four replicate grabs (413 cm^2 each). Polarographic oxygen sensors measure the dissolved oxygen tension in each grab with amplified outputs continuously recorded in the instrument package. Each sensor is calibrated using chilled saturated and nitrogen-purged seawater as limits and corrected for hydrostatic pressure effects and electrode oxygen consumption. Initial and final control bottom-water samples were incubated on the FVGR for each deployment (≤2.7 days at Station C, ≤4.9 days at Station CNP). On recovery of the FVGR, the undisturbed sediment with overlying water was analysed for macrofauna abundance and biomass, and organic carbon and total nitrogen. Total oxygen consumption rates, corrected for the overlying water rates in each grab, yielded SCOC rates[23]. Sediments in two or three grabs from each FVGR deployment were sieved through a 297-μm mesh screen on board ship and the abundance and wet weight biomass of the retained macrofauna determined in the laboratory. Small aliquots of surface sediment (0–1 cm) from each grab were frozen for CHN analysis in the laboratory where these samples were acidified with 0.1 M HCl, dried, and three replicate samples analysed using a Perkin-Elmer 240C CNH analyser.

Characterization of each bottom station is presented in Table 1. Variations of the sediment biological and chemical parameters measured are large and may represent intra-station heterogeneity.

Rates of SCOC at both stations were significantly higher ($P < 0.05$) in June than in November (Station CNP) and February (Station C) (Fig. 1). At Station CNP, June rates of SCOC were almost four times higher than in November. The high and low measured rates of SCOC in June and November bracketed intermediate values measured in August over two consecutive years. The non-significant difference ($P > 0.05$) between the SCOC rates measured in August of 1978 and 1979 suggests a repeatable pattern.

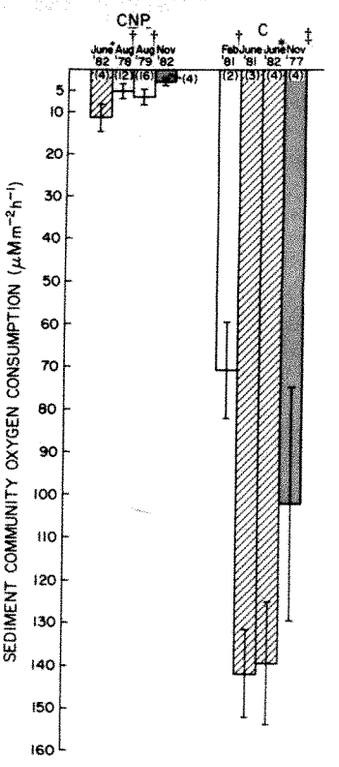


Fig. 1 Sediment community oxygen consumption measured *in situ* on a seasonal basis at Stations CNP and C. Mean rates with standard deviations are presented; the number of replicates is given in parentheses. * † ‡ Values reported previously (refs 23, 24 and 21, respectively).

Although substantially higher rates of SCOC were measured at Station C, a pattern similar to Station CNP is exhibited. Mean rates of SCOC are twice as high in June as in February (Fig. 1) with intermediate rates measured in November. These seasonal measurements of SCOC at both stations fall within the range of values measured sporadically at various depths and temperatures in the world oceans[24–28].

We propose that the fluctuations in SCOC measured at three different times of the year at both stations reflect seasonal variations in biological rates in the deep sea. The logical question is what causes such fluctuations in a seemingly constant environment. On the hypothesis[29] that the environment is food-limited, the most obvious answer is food supply; food energy used by these soft-bottom abyssal communities is ultimately derived from primary production in the euphotic zone. On a variety of temporal scales, a fraction of this production must reach bottom waters directly by sinking, diffusion and advection, indirectly by trophic transfers, or by a combination of both. This food can exist in three forms: (1) small particulate organic matter, (2) large organic falls and (3) dissolved organic matter.

(1) The flux of small particulate organic matter has not been measured seasonally in our study areas. However, a correlation has been shown between the annual cycle of surface primary productivity and the flux of small particulate organic matter reaching abyssal depths in the western North Atlantic[18] and eastern tropical Pacific[19]. The close synchrony between the annual cycles of surface primary productivity and particulate organic matter collected by sediment traps at 3,200 m (ref. 18) and 3,560 m (ref. 19) suggests rapid settling rates (>65 m per

0028–0836/84/070624—03$01.00

© 1984 Macmillan Journals Ltd

Table 1 Characterization of two stations in the North Pacific, C and CNP

Station parameters	Station CNP June 1982	Station CNP August 1978*	Station CNP August 1979*	Station CNP November 1982	Station C February 1981*	Station C June 1981	Station C June 1982	Station C November 1977†
Bottom water								
Dissolved O_2 (μmol)	167‡	167*	168*	171	138*	162	138‡	172†
Temperature (°C)	1.6‡	1.5*	1.5*	1.6	1.6*	1.6	1.6‡	2§
Sediments								
Sediment type	Clay‡	Clay*	Clay*	Clay	Silty clay*	Silty clay	Silty clay‡	Silty clay†
Surface organic carbon (mg C g^{-1})	3.50‡	4.20 ± 2.08 (3)*	5.40 ± 3.24 (3)*	3.40	13.7*	11.70	15.70‡	12.24 ± 0.11 (2)†
Surface total nitrogen (mg N g^{-1})	0.20‡	0.50 ± 0.44 (3)*	0.47 ± 0.25 (3)*	1.10	1.4*	1.30	1.00‡	1.74 ± 0.14 (2)†
Macrofaunal abundance (individuals m^{-2})	752 ± 103 (2)	201 ± 189 (6)*	584 ± 272 (8)*	497 ± 223 (2)	8,697 ± 1,179 (3)*	7,202 ± 868 (3)	4,695 ± 1,115 (2)	5,274 ± 3,115 (2)†
Macrofaunal biomass (mg wet wt m^{-2})	30.94 ± 6.71 (2)	61.5 ± 74.9 (6)*	20.6 ± 39.1 (8)*	50.7 ± 61.8 (2)	664.8 ± 423.0 (3)*	1,713.4 ± 1,060.6 (3)	1,936.0 ± 526.5 (2)	1,539.0 ± 531.1 (2)†

Means ± s.d. are presented where more than one grab was sampled (number of replicates in parentheses). Macrofaunal biomass for Station C in November excludes two large animals per grab—inclusion of these animals would increase biomass to 24,190 ± 13,407.0 mg wet wt m^{-2}.
* † ‡ Values previously reported (refs 24, 21 and 23, respectively).
§ Value rounded to nearest degree (ref. 21).

day) in both areas[19,30]. More precise estimates of sedimentation rates may be determined using time-lapse photography. Billet *et al.* report rates of 100–150 m per day in the north-east Atlantic[20]. Although complete seasonal measurements of primary productivity are lacking for our two stations, data are available for areas in close proximity.

Seasonal rates of surface primary productivity are well documented for the Southern California Bight, an area 17 miles inshore of Station C. Our high rates of SCOC in June and low rates in February correlate well with the seasonal productivity maxima between March and July[31], allowing for sinking rates of ≤38 days[20] over the 3,800-m water column.

A less clear picture of primary productivity exists for the central North Pacific. Early estimates of productivity in the central gyre area recorded winter peaks in primary productivity corresponding to increased mixing of subsurface nutrient-rich water with surface water[32]. Although Hayward and associates[33], on analysing recent ^{14}C measurements at a station south of Station CNP, found no obvious trends in primary productivity, seasonal variation may be masked by the large intraseasonal variability. One indication of seasonal variation is the existence of a high subsurface oxygen maximum in this same area in summer, which, if photosynthetically produced, would yield substantially higher estimates of primary productivity than indicated by the ^{14}C method[34]. Of the three times of the year measured, SCOC at Station CNP was highest in June and lowest in November (Fig. 1). If we assume that the photosynthetically produced particulate organic matter which escapes the euphotic zone sinks at a rate of 100–150 m per day (ref. 20), then one would expect this food supply to reach the bottom community within 2 months. Therefore, our data predict that peaks in primary productivity would occur in spring. However, faster sinking rates up to 1,028 m per day have been reported for zooplankton and midwater fish faecal pellets[35,36] yielding bottom arrival times of less than 10 days at 5,900 m.

(2) The contribution of large organic falls (for example, carcasses of pelagic fishes and squid, shallow benthic and terrestrial plant remains) to the food supply of the deep sea is unknown[37]. Large pelagic fishes such as tuna, *Thunnus alalunga*, undergo seasonal migrations in the North Pacific with high numbers occurring in winter in the vicinity of Station CNP, and in the spring and early summer in the vicinity of Station C[38,39]. Although seasonal mortality rates are unknown for these and other large pelagic animals, the potential importance of this food source would increase during periods of maximum abundance. In addition, Station C may be influenced by seasonal inputs of drift kelp; peak kelp abundances appear on beaches and probably offshore during the stormy winter months[40].

(3) Although dissolved organic matter (DOM) represents a large pool of potential food in the deep sea, measurements of DOM in the central and eastern North Pacific near the sediment–water interface reveal this source to be 'old' and refractory[41,42]. However, seasonal measurements of combined dissolved and particulate organic carbon at 300–1,500 m depth in the central Pacific do show significantly higher concentrations in the winter than in summer[43].

The seasonality of any of these potential food sources may be influenced more directly by abyssal advection than by surface and atmospheric conditions. Abyssal currents have been shown to have seasonal signals in the eastern North Atlantic, generated by high surface wind stress in winter with temporal lag and decay in magnitude with depth[44]. This, coupled with observations of temporal variability in the nepheloid layer at abyssal depths in the western North Atlantic[45], suggests the potential impact of seasonal pulses on abyssal advection of food.

Our results suggest a seasonal pattern in SCOC which is related to the seasonal productivity regime of the overlying waters in the eutrophic eastern boundary current region (Station C) and possibly in the oligotrophic central gyre region (Station CNP). Deep-sea areas can no longer be considered to be in static equilibrium exhibiting constant biological rates, but must be examined as components in dynamic equilibrium with the overall open ocean ecosystem. Mass balance calculations of organic matter remineralization which are based on a single measurement in time must be re-evaluated considering fluctuations which in the case of SCOC can vary by as much as a factor of four between seasons.

This study was supported by NSF grant OCE 81-17661. We thank M. B. Laver and N. O. Brown for technical assistance and A. Carlucci, R. Eppley, R. Hessler, M. Laver, C. Reimers and P. Williams for commenting on the manuscript.

Received 22 August; accepted 25 November 1983.

1. Bruun, A. F. *Mem. geol. Soc. Am.* **67**, 641–672 (1957).
2. Menzies, R. J. *Oceanogr. mar. Biol. A. Rev.* **3**, 195–210 (1965).
3. Sanders, H. L., Hessler, R. R. & Hampson, G. R. *Deep Sea Res.* **12**, 845–867 (1965).
4. Hessler, R. R. & Sanders, H. L. *Deep Sea Res.* **14**, 65–78 (1967).
5. Jannasch, H. W., Eimhjellen, K., Wirsen, C. O. & Farmanfarmian, A. *Science* **171**, 672–675 (1971).
6. Jannasch, H. W. & Wirsen, C. O. *Science* **180**, 641–643 (1973).
7. Smith, K. L. Jr & Teal, J. M. *Science* **179**, 853–858 (1973).
8. Turekian, K. K. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 2829–2833 (1975).
9. Mead, G. W., Bertelsen, E. & Cohen, D. *Deep Sea Res.* **11**, 569–596 (1964).
10. George, R. Y. & Menzies, R. J. *Nature* **215**, 878 (1967).
11. George, R. Y. & Menzies, R. J. *Nature* **220**, 80–81 (1968).
12. Schoener, A. *Ecology* **49**, 81–87 (1968).
13. Rokop, F. J. *Science* **186**, 743–745 (1974).
14. Rokop, F. J. *Mar. Biol.* **43**, 237–246 (1977).
15. Lightfoot, R. H., Tyler, P. A. & Gage, J. D. *Deep Sea Res.* **26A**, 967–973 (1979).
16. Tyler, P. A., Grant, A., Pain, S. L. & Gage, J. D. *Nature* **300**, 747–750 (1982).
17. Gage, J. D. *Deep Sea Res.* **29**, 1565–1586 (1982).
18. Deuser, W. G. & Ross, E. H. *Nature* **283**, 364–365 (1980).
19. Honjo, S. *Science* **218**, 883–884 (1982).
20. Billett, D. S. M., Lampitt, R. S., Rice, A. L. & Mantoura, R. F. C. *Nature* **302**, 520–522 (1983).
21. Smith, K. L. Jr, White, G. A. & Laver, M. B. *Deep Sea Res.* **26A**, 337–346 (1979).
22. Smith, K. L. Jr & Baldwin, R. J. in *Handbook on Polarographic Oxygen Sensors* (eds Gnaiger, E. & Forstner, H.) 298–319 (Springer, Berlin, 1983).
23. Smith, K. L. Jr & Laver, M. B. *Limnol. Oceanogr.* (submitted).
24. Smith, K. L. Jr, Laver, M. B. & Brown, N. O. *Limnol. Oceanogr.* **28**, 882–898 (1983).

25. Smith, K. L. Jr *Limnol. Oceanogr.* **19,** 939–944 (1974).
26. Smith, K. L. Jr *Mar. Biol.* **47,** 337–347 (1978).
27. Hinga, K. R., Sieburth, J. McN. & Heath, G. R. *J. mar. Res.* **37,** 557–579 (1979).
28. Murray, J. M. & Grundmanis, V. *Science* **209,** 1527–1530 (1980).
29. Dayton, P. K. & Hessler, R. R. *Deep Sea Res.* **19,** 199–208 (1972).
30. Deuser, W. G., Ross, E. H. & Anderson, R. F. *Deep Sea Res.* **28A,** 495–505 (1981).
31. Smith, P. E. & Eppley, R. W. *Limnol. Oceanogr.* **27,** 1–17 (1982).
32. McGowan, J. A. in *Biology of the Oceanic Pacific* (ed. Miller, C. B.) 9–28 (Oregon State University, Corvallis, 1974).
33. Hayward, T. L., Venrick, E. L. & McGowan, J. A. *J. mar. Res.* **41,** 711–729 (1983).
34. Schulenberger, E. & Reid, J. L. *Deep Sea Res.* **28A,** 901–919 (1981).
35. Komar, P. D., Morse, A. P., Small, L. F. & Fowler, S. W. *Limnol. Oceanogr.* **26,** 172–180 (1981).
36. Robison, B. H. & Bailey, T. G. *Mar. Biol.* **65,** 135–142 (1981).
37. Stockton, W. L. & DeLaca, T. E. *Deep Sea Res.* **29A,** 157–169 (1982).
38. McGary, J. W., Graham, J. J. & Otsu, T. *Calif. Coop., Oceanic Fish. Invest. Rep.* **8,** 45–53 (1961).
39. Laurs, R. M. & Lynn, R. J. *Fish. Bull.* **75,** 795–822 (1977).
40. Zobell, C. E. *Nova Hedwigia* **32,** 269–318 (1971).
41. Williams, P. M., Carlucci, A. F. & Olson, R. *Oceanol. Acta* **3,** 471–476 (1980).
42. Williams, P. M., Stenhouse, M. C., Druffel, E. M. & Koide, M. *Nature* **276,** 698–701 (1978).
43. McGowan, J. A. & Williams, P. M. *J. exp. Mar. Biol. Ecol.* **12,** 187–217 (1973).
44. Dickson, R. R., Gould, W. J., Gurbutt, P. D. & Killworth, P. D. *Nature* **295,** 193–198 (1982).
45. Gardner, W. D. & Sullivan, L. G. *Science* **213,** 329–331 (1981).

Phosphorus limitation of net production in a confined aquatic ecosystem

S. V. Smith

Hawaii Institute of Marine Biology, University of Hawaii, PO Box 1346, Kaneohe, Hawaii 96744, USA

M. J. Atkinson

Department of Zoology, University of Western Australia, Nedlands, Western Australia 6009

The sediment composition of confined aquatic ecosystems with little terrigenous input reflects net ecosystem production. Here we present nutrient budgets for one such system, Shark Bay in Western Australia, which suggest that net ecosystem production is limited by the oceanographic delivery of P, while the system meets its N requirements by nitrogen fixation. We suggest that N versus P limitation of the net production of aquatic ecosystems reflects the degree of confinement of those systems.

An aquatic ecosystem isolated from exogenous inputs of organic material must have a long-term net organic production rate which equals or exceeds zero. Were such an isolated system not biased towards net autotrophy, the biomass and sedimentary organic materials would be consumed and the system would eventually collapse. If the system also does not export organic material, then the sediments must reflect the long-term net production of organic material. While these relationships must be true in principle, they rarely have application: organic additions or losses usually obscure the sedimentary record of net organic metabolism.

Shark Bay, Western Australia (Fig. 1), is a large (13,000 km^2), seagrass-dominated coastal lagoon[1] which might be expected to preserve this theoretical relationship between net organic production and sediment composition. The bay is surrounded by desert; there is virtually no input of fresh water via runoff, rainfall or groundwater. Exchange of materials with the ocean is slow; average water residence time in the bay, estimated from water and salt budgets[1] exceeds 1 yr.

Net uptake of dissolved inorganic materials from incoming water, although very slow, nevertheless drastically modifies the water composition. This modification represents the net signal of biogeochemical fluxes, integrated over the long water residence time[1]. For example, ocean water entering the bay has a reactive P concentration of ~0.2 mmol m^{-3}; reactive P in the bay decreases to ~0.03 mmol m^{-3}.

We refer to budgets of net C and P uptake derived from a box model of water and salt balance as the 'oceanographic budgets'. The rate term for these budgets is derived from net evaporation rates[1]. Because of the virtual isolation of the bay from particulate inputs and outputs, we hypothesized that the sediments should reflect the net production. We refer to the sedimentary record of net production as the 'sediment budgets'. Because the absolute sedimentation rate of Shark Bay is unknown, we do not have a normalizing parameter for the sediment budgets. We therefore compare data for the oceanographic and sediment budgets by means of material ratios, thus removing the absolute rate terms from both sets of budgets.

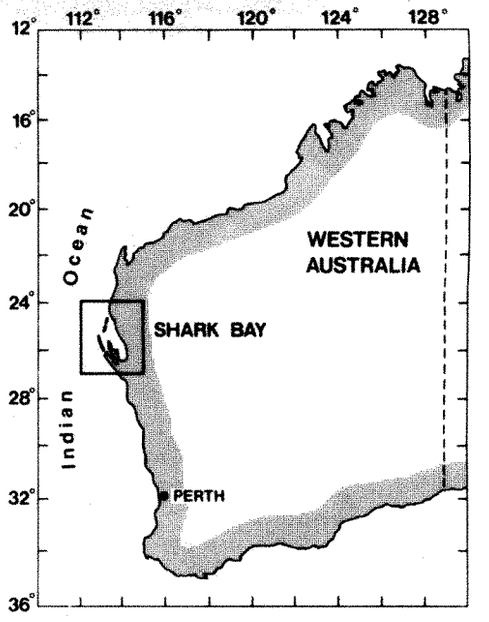


Fig. 1 Location map of Shark Bay, Western Australia.

Water loss from net evaporation is compensated by net advective (tidal) inflow. The salt balance is maintained by advective inflow and loss by horizontal eddy diffusion. Deviation of the concentrations of other chemical constituents from proportionality with salinity is attributed to net uptake.

Table 1 is a summary of net uptake of C into $CaCO_3$ and the net uptake of both C and reactive P into organic materials, as derived from the oceanographic budgets. Note that 27% of the carbon taken up enters the organic fraction and the remainder enters $CaCO_3$. Moreover, the molar ratio of C to P taken up by the net production of organic materials is ~320:1.

Sediment grab samples were collected along the same transect through the eastern gulf of Shark Bay as was used for the oceanographic budgets. Samples were dried at 110 °C. Weighed aliquots were muffled at 550 °C for 2 h, re-weighed, remuffled at 1,000 °C, and weighed again. Combustion at 550 °C oxidizes organic materials[2]. Organic carbon was calculated as 40% of the weight loss at 550 °C. Combustion of $CaCO_3$ at 1,000 °C drives off CO_2. Inorganic carbon was calculated to be 27% of this latter weight loss.

This procedure gives results more reproducible for the C composition of organic-poor calcareous sediments than do CHN analyses according to the technique of Hirota and Szyper[3]. We found no evidence of significant carbonate loss at 550 °C, although some Mg calcite loss could have escaped our notice[2].

Total sediment N was determined by standard colorimetric techniques after sulphuric acid Kjeldahl digestion of samples, and total P was determined colorimetrically after sample digestion with nitric and perchloric acids.

Table 2 summarizes sediment composition. The inorganic C content of the sediments averages 8.2 mmol g^{-1} (82% $CaCO_3$). The average organic C content of the sediments is 2.1 mmol g^{-1}

0028–0836/84/070626—02$01.00
© 1984 Macmillan Journals Ltd

Table 1 Net rates of carbon and phosphorus uptake by biogeochemical processes in the eastern gulf of Shark Bay

Process	Rate (mmol per m^2 per day)
$CaCO_3$ production	3.2
Organic carbon production	1.2
Phosphorus uptake	0.0037

Rates derived from Smith and Atkinson[1].

Table 2 Sediment composition in the eastern gulf of Shark Bay

Component	Amount (mmol g^{-1})
Inorganic C	8.2 ± 0.18 (38)
Organic C	2.1 ± 0.10 (39)
Total N	0.05 ± 0.06 (39)
Total P	0.007 ± 0.005 (39)

Values are the mean ± s.d. (n).

(6% organic material). Thus, no more than 12% of the sediments come from non-carbonate, inorganic sources.

Organic C represents 20% of the C in the sediments, about one-quarter lower than the organic fraction of C uptake derived from the oceanographic budgets (Table 1). Broecker and Takahashi[4] were unable to reconcile an oceanographically determined C budget for the Bahama Banks with the organic C content of sediments there. In most settings where the biomass in the system studied exceeds that of the surrounding waters, there may be significant net export of organic materials by advective and mixing losses and by the migration of organisms. Extremely slow water exchange and apparently small migration fluxes obviate such a problem in Shark Bay. Even so, the slight discrepancy between the organic C fraction of the oceanographically determined C uptake and the organic C fraction of the sediments may represent loss of organic materials.

The average P content of the sediments is 0.007 mmol g^{-1}, and the sediment molar ratio of organic C to P is 300:1 (Table 2), not significantly different from the uptake ratio derived from the oceanographic budget (Table 1). From the correspondence between the oceanographic and sediment budgets for C and P, we are confident that the sediments adequately reflect net ecosystem uptake. Sediment N composition should therefore reflect net N uptake. The sediment N content is 0.05 mmol g^{-1} (Table 2), and the molar C:N:P ratio of the sediments is ~300:7:1.

We now use the oceanographically derived rates of C and P uptake and the sediment C:N:P ratio to scale N uptake, and we derive a rate of 0.03 mmol per m^2 per day. If an error exists in this estimate, it should be of the same order as the discrepancy between the oceanographic and sediment C budgets (<25%). The net N uptake by this system is <0.04 mmol per m^2 per day.

Nitrate plus ammonia concentration throughout the bay is less than 0.5 mmol m^{-3}, and there is no coherent pattern of spatial variation (Smith and Atkinson, unpublished data). Applying this average concentration and a nitrogen/salinity gradient of 0 to our box model[1] yields an oceanographic supply of N of 0.002 mmol per m^2 per day; less than 10% of the N being taken up into the sediments can be supplied by oceanographic input. The remainder must enter by other pathways, with atmospheric N fixation being the most likely one.

We did not measure N fixation, nor do we believe that such measurements are directly relevant. It would be impossible to estimate the N fixation rate for such a large, heterogeneous area from a few spot measurements of local N fixation, and the phenomenon of N fixation is adequately documented. Capone and Carpenter[5] report N fixation rates of 1–0.02 mmol per m^2 per day for environments ranging from seagrass beds to bare estuaries and shallow benthic environments. Our initial estimate of N fixation in Shark Bay falls near the low end of this range.

The sedimentary end product of Shark Bay organic materials appears greatly depleted with respect to N compared with the N/P ratios of materials being produced. The low sediment N/P ratio (7:1) compared with either plankton (16:1 (ref. 6); confirmed with our own data for Shark Bay) or benthic plants (30:1 (ref. 7); confirmed for Shark Bay) suggests that N is more readily released from particulate organic materials in the sediments than are P and C. This release may represent denitrification[8]. In that event, our estimate of N fixation might be too low by 2–4-fold. The upper limit of possible N fixation in Shark Bay (<0.16 mmol per m^2 per day) is still well within the range for comparable environments elsewhere[5].

We offer the following simple model to explain our observations. The two primary components to the model are the flushing rate of water through the system and the presence of N fixation as a supplement to the oceanographic (and, in other systems, land-derived) N sources.

As summarized above, marine plants ordinarily contain 16 to 30 times as much N as P, yet surface seawater typically has an inorganic N/P ratio of ≤10. Surface seawater is thus deficient in N, relative to the nutritional requirements of plants[9]. A highly productive environment subjected to such N-deficient water may fix N (for example, coral reef flats[10]); however such open environments export the locally fixed N (ref. 11) before the supply of P has been lowered measurably[12]. By contrast, confined systems such as Shark Bay will accumulate fixed N. They supplement their N deficiency until P is exhausted. The system-wide rates required to exhaust available P are small relative to documented community or organismic N fixation rates[5]. Therefore the systems clearly have the capacity to 'fine-tune' N fixation rates to use available P. The supply of N is effectively limitless; the supply of P is not.

The apparent conflict between N and P limitation of net community production in aquatic systems can be resolved into extremes of a continuum. Many open systems receiving water which is deficient in N fail to accumulate N and are examples of open, N-limited systems[9]. The net community production of progressively confined systems will tend towards P limitation, because of accumulation and recycling of internally fixed N until P is exhausted. Isolated hypersaline coastal lagoons such as Shark Bay, some relatively enclosed coral atoll lagoons[13], many lakes with long water-residence times[14], and the whole of the world's oceans[15] are examples of confined, P-limited ecosystems.

We thank C. Crossland, J. Hansen, A. Hatcher, B. Hatcher, R. Johannes, F. Mackenzie and D. Smith for critical comments on the manuscript.

Received 20 April; accepted 29 November 1983.

1. Smith, S. V. & Atkinson, M. J. *Limnol. Oceanogr.* **28,** 625–639 (1983).
2. Krom, M. D. & Berner, R. A. *J. sedim. Petrol.* **53,** 660–663 (1983).
3. Hirota, J. & Szyper, J. P. *Limnol. Oceanogr.* **20,** 896–900 (1975).
4. Broecker, W. S. & Takahashi, T. *J. geophys. Res.* **71,** 1575–1602 (1966).
5. Capone, D. G. & Carpenter, E. J. *Science* **217,** 1140–1142 (1982).
6. Redfield, A. C., Ketchum, B. H. & Richards, F. A. in *The Sea* Vol. 2 (ed. Hill, M. N.) (Wiley, New York, 1963).
7. Atkinson, M. J. & Smith, S. V. *Limnol. Oceanogr.* **28,** 568–574 (1983).
8. Nixon, S. W. in *Estuaries and Nutrients* (eds Neilson, B. J. & Cronin, L. E.) 111–138 (Humana, Clifton, New Jersey, 1981).
9. Ryther, J. H. & Dunstan, W. M. *Science* **171,** 1008–1013 (1971).
10. Wiebe, W. J., Johannes, R. E. & Webb, K. L. *Science* **188,** 257–259 (1975).
11. Webb, K. L. *et al. Limnol. Oceanogr.* **20,** 198–210 (1975).
12. Pilson, M. E. Q. & Betzer, S. B. *Ecology* **54,** 581–588 (1973).
13. Smith, S. V. *et al. Univ. Hawaii/Univ. South Pacific Int. Seagrant Prog. Tech. Rep.* (in the press).
14. Schindler, D. W. *Science* **195,** 260–262 (1977).
15. Broecker, W. S. & Peng, T.-H. *Tracers in the Sea* 690 (Eldigio, Palisades, New York, 1982).

Mallomonadacean microfossils provide evidence of recent lake acidification

John P. Smol

Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6

Donald F. Charles & Donald R. Whitehead

Department of Biology, Indiana University, Bloomington, Indiana 47405, USA

The Mallomonadaceae (Chrysophyceae), a group of flagellated algae commonly found in freshwater systems, are characterized by an armour of overlapping siliceous scales that are taxonomically diagnostic[1]. Mallomonadacean scales are usually abundant and well preserved in lake sediments[2]. Previous palaeolimnological studies have used the stratigraphic distribution of mallomonadacean assemblages to document patterns of lake eutrophication[2–5]. Our data demonstrate that the distribution of certain mallomonadacean taxa is also closely related to lakewater *p*H, that stratigraphic analyses of fossil scales can be used to indicate past changes in lake acidity, and that such stratigraphic studies provide important insights concerning the responses of sensitive lake systems to acid deposition.

Subfossil mallomonadacean assemblages from the surface sediments of 38 lakes in the Adirondack Mountains (New York, USA) have been studied with respect to the limnological factors that appear to influence the distribution of individual species and the characteristics of assemblages[6]. The Adirondack area is a largely forested and mountainous region that contains about 3,000 lakes and ponds, and is one of the most important lake regions in North America. The lakes are generally dilute and unproductive. The area receives intense acidic precipitation and, since many lakes have been affected[7,8], it is important that we be able to determine the recent acidification histories of sensitive lake systems. Detailed information on lake locations, morphometry, and chemistry has been presented elsewhere[9].

Surface sediment samples (0–1 cm) were taken from the deepest areas of the study lakes using a modified Cushing and Wright piston corer[10]. Because mallomonadacean scales are siliceous, sediment digestions and microscopic techniques were the same as those used for fossil diatoms[2]. Although electron microscopy is required to confirm taxonomic identifications, scales can usually be distinguished to the species level with light microscopy. An average of 600 scales was counted from each sediment sample. A total of 30 species from five genera was identified[6].

Reciprocal averaging and cluster analyses indicated that *p*H and factors correlated with it exerted the greatest influence on mallomonadacean assemblages[6]. The distributions of individual taxa were plotted against each of the 21 limnological factors studied (as in Table 1). Scales of *Mallomonas hindonii* Nicholls and/or *M. hamata* Asmund (Fig. 1) were especially common in acidic lakes (Fig. 2). Notable exceptions were the three bog lakes (pH < 5.4) in which only trace populations were noted, and in the two highest elevation lakes (pH < 4.8) where scales of *M. hamata* were never observed. The distribution of these two taxa did not correlate closely with any of the other limnological factors studied. These data suggest that *M. hindonii* and *M. hamata* are reliable indicators of acidic non-bog conditions. In the few acidic lakes where neither of these two species were common, scales of *Synura echinulata* Korsh. were usually abundant.

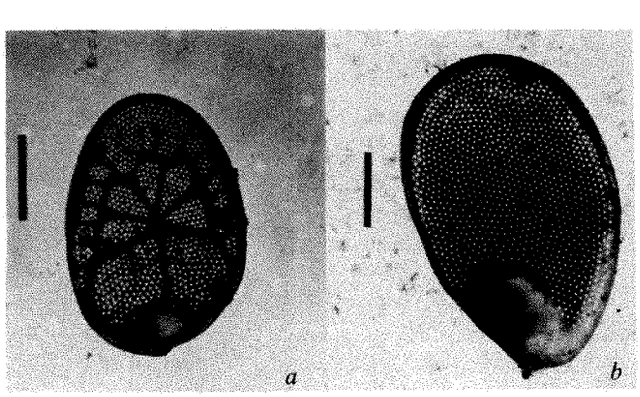


Fig. 1 Transmission electron micrographs of scales of *Mallomonas hindonii* (*a*) and *Mallomonas hamata* (*b*). Scale bar, 1 μm.

Table 1 Morphometric, physical and chemical characteristics of Deep Lake

Lake characteristic	
Surface area	11 hectare
Maximum depth	24 m
Elevation	789 m
Watershed area	72 hectare
*p*H	4.7
Alkalinity	−18 μequiv. l^{-1}
Conductivity	29 μS cm^{-1} at 25 °C
Colour, true	7.5 Pt. Co units
Colour, Forel-Ule, median	7 units
Secchi disk transparency	8.4 m
P, total	3.1 μg l^{-1}
Chlorophyll *a*	1.7 μg l^{-1}
Ca	64 μequiv. l^{-1}
Mg	26 μequiv. l^{-1}
Na	13 μequiv. l^{-1}
K	6 μequiv. l^{-1}
SO_4	150 μequiv. l^{-1}
Cl	7 μequiv. l^{-1}
NO_3	12 μequiv. l^{-1}
Al, total	611 μg l^{-1}
SiO_2	2.7 mg l^{-1}

Alkalinity, *p*H, conductivity, and true colour data are for surface (0.5 m) samples. Total P, chlorophyll *a*, cations, anions, Al, and SiO_2 data are for composite lake-average samples. All data are 1978–1979 summer (May–August) averages except for cation, anion, Al, and SiO_2 which are for samples taken 1 September, 1979.

Because many lakes in the Adirondacks have been affected by anthropogenic acidic precipitation[7], a stratigraphic analysis of fossil Mallomonadaceae was initiated to establish whether these microfossils could be used to trace past changes in lakewater *p*H. Deep Lake (43°36′58″ N, 74°39′52″ W; Table 1) was chosen for this study because of its suitability for such a palaeological study. It is a headwater lake with a deep cup-shaped basin, it is at a relatively high elevation (789 m), is acidic (pH = 4.7), and is similar to other lakes in the area that have recently become more acidic[8]. The drainage basin is underlain by granitic gneiss bedrock that is covered by thin tills and soils. Watershed vegetation is a mixed deciduous–coniferous forest consisting largely of sugar maple (*Acer saccharum* Marsh.), yellow birch (*Betula alleghaniensis* Britton.) and American beech (*Fagus grandifolia* Ehr.), with lesser amounts of red maple (*Acer rubrum* L.) and red spruce (*Picea rubens* Sarg.). The area was logged in the late 1800s, and then again in the early 1950s (F. Lamphear, personal communication). Our unpublished palynological data do not indicate any marked differences in vegetation succession between these two episodes of disturbance. The watershed is in the West Canada Creek Wilderness Area and contains no cultural development other than hiking trails. Historical data indicate that Deep Lake probably became more acidic sometime between the 1930s and the mid-1950s. Professional guides reported good fishing for brook trout (*Salvelinus fontinalis*) in Deep Lake during the 1930s. However, although Deep Lake was stocked annually with brook trout fingerlings between 1951 and 1968, only three trout were

0028–0836/84/070628—03$01.00

© 1984 Macmillan Journals Ltd

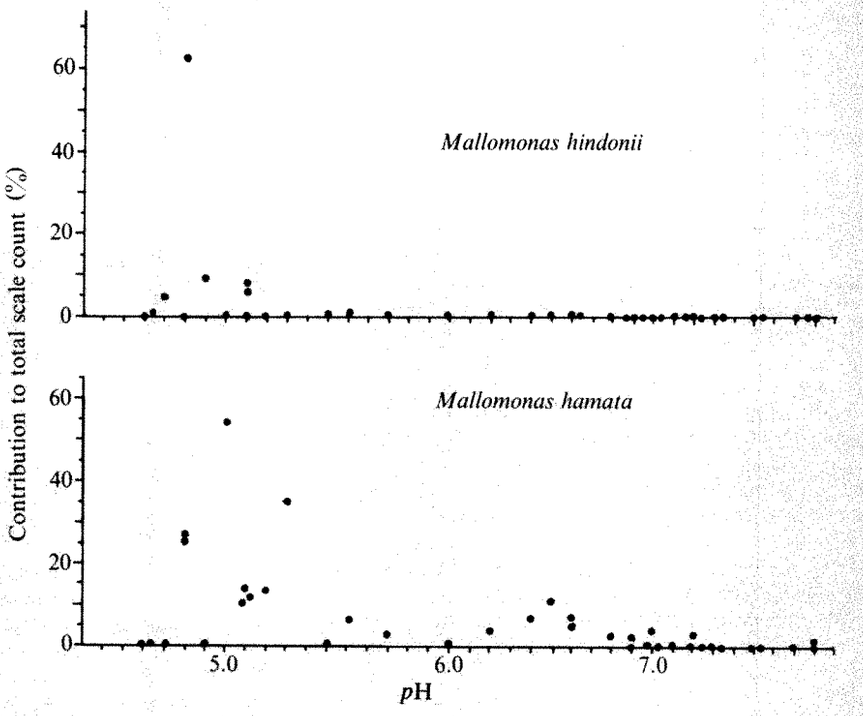


Fig. 2 The percentage contribution of *Mallomonas hindonii* and *M. hamata* scales plotted relative to the *p*H of each of the 38 study lakes.

captured during fish surveys in 1954, 1964 and 1965. No fish were caught during 1966, 1967 and 1968 surveys[11]. Because brook trout usually cannot survive in lakes with a *p*H < 5.0 and high Al concentrations[12], conditions that currently exist in Deep Lake (Table 1), lake acidification was probably responsible for the disappearance of fish populations.

A 60-cm-long core was removed from the centre of Deep Lake and sectioned in the field at 1 cm intervals. The rise in non-arboreal pollen [ragweed (*Ambrosia*) and other weedy taxa] was recorded at the 5–6 cm level (Fig. 3). This horizon can be dated circa AD 1850, since an examination of historical records indicates that land clearance in the region southwest of the Adirondacks intensified at this time. Although the scales of 13 mallomonadacean taxa were identified in the Deep Lake stratigraphy, only six species ever represented more than 2% of the total scale sum (Fig. 3). Throughout the lower part of the core, assemblages are dominated by *Mallomonas crassisquama* (Asmund) Fott with lesser amounts of *M. punctifera* Korsh. and *M. acaroides* Perty em. Iwanoff. However, a significant change occurs near the 3 cm level. There is a marked increase in *M. hindonii* and *M. hamata*, and, to a lesser extent, *Synura echinulata*. No changes in Mallomonadaceae coincided with the rise in non-arboreal pollen.

We believe that this shift in species composition was in response to lake acidification. As previously noted, *M. hindonii* and *M. hamata* are indicative of acidic lakes. Among the 38 Adirondack study lakes, *Synura echinulata* scales were also most abundant in the surface sediments of acidic lakes (for example, 81% of the total scale sum in Lake Tear of the Clouds, *p*H = 4.6; 74% in Mountain Pond, *p*H = 4.7; 59% in Lake Colden, *p*H = 5.0). In contrast, *M. crassisquama*, which is the most widespread *Mallomonas* sp. in the world[13–15], occurred only rarely in

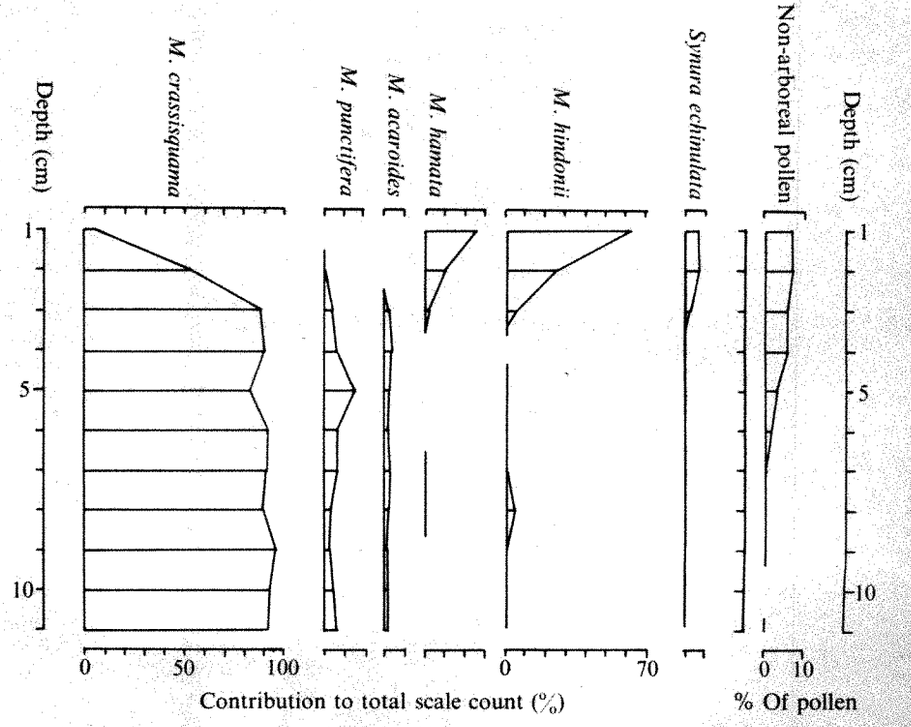


Fig. 3 Percentage diagram of the stratigraphic distribution of Mallomonadaceae in the Deep Lake sediments. The 15-cm and 20-cm levels were also analysed, but they were similar to the 11-cm level and are not presented here. An average of 600 scales was identified and enumerated from each level. *Mallomonas punctifera* Korsh. is synonymous with *M. pulchella* (Kisselev) Cronberg & Kristiansen and *M. reginae* Teil[15].

assemblages from lakes with a $pH < 5.0$. A similar distribution for this taxon has been reported in other areas[13]. Similarly, *M. punctifera* was more abundant in circumneutral to slightly alkaline waters, and reached its highest abundance in the surface sediments of Clear Pond ($pH = 7.2$).

The earliest date at which the shift in Mallomonadaceae could have occurred was approximately 60–70 years ago. This estimate is made by assuming a constant sedimentation rate (~25 years cm^{-1}) between the rise in non-arboreal pollen at the 6 cm level (~1850) and 1979, the year the core was taken. However, the sediment was less dense at the top of the core, so that accumulation rates must have been greater near the sediment surface, and the shift in Mallomonadaceae undoubtedly occurred more recently. Nevertheless, the acidification began at least 30 years ago, as the earliest limnological data available for Deep Lake are from 1954 when the *p*H was already recorded as low as 4.7[11]. This is consistent with fisheries data cited previously, since it is likely that the decline in lake *p*H preceded the decline in fish populations. The inferred acidification probably resulted in large part from the increased atmospheric deposition of strong acids.

Our interpretation of Deep Lake's recent history is further substantiated by the stratigraphic distribution of diatoms. Before the proposed period of acidification, the diatom flora consisted of a diverse assemblage of indifferent to acidophilous taxa [for example *Frustulia rhomboides* (Ehr.) De T., *Anomoeoneis serians* var. *brachysira* (Bréb. *ex* Kütz.) Hust.]. However, in the surface sediment, the assemblage is dominated (56%) by an as yet undescribed taxon [*Fragilaria* cf. *virescens* var. 1,]. Based on Charles' diatom survey of the Adirondack lakes[9], this new *Fragilaria* is an acidobiontic form, reaching its highest abundance in lakes with a $pH < 5.0$.

Our data indicate that the Mallomonadaceae are sensitive to changes in *p*H, and can therefore provide important palaeoecological data on lake acidification. Because sediment preparations for mallomonadacean scales and diatoms are identical, scales can easily be incorporated into palaeolimnological studies where diatoms are being analysed. In contrast to euplanktonic diatoms, which appear to be excluded in waters with $pH < 5.8$–6.0[9], several mallomonadacean taxa thrive in acidic lakes. Hence, mallomonadacean scales probably represent the only algal microfossils that will reflect the euplankton in acidic lakes. Palaeolimnological studies that utilize both diatom frustules and mallomonadacean scales should provide a more accurate reconstruction of lake history.

This study was supported in part by NSF grants DEB 77-03907 (to D.R.W. and P. M. Vitousek) and DEB 79-12210 (to D.R.W.), and by the Natural Sciences and Engineering Research Council of Canada (operating grant A-808 to Dr S. R. Brown and as fellowships to J.P.S.). We thank Drs S. R. Brown, M. M. Boucherle, T. Oliver and G. Cronberg for helpful comments, and Ms F. Murrin for assistance with electron microscopy.

Received 10 August; accepted 17 October 1983.

1. Takahashi, E. *Electron Microscopical Studies of the Synuraceae (Chrysophyceae) in Japan* (Tokai Univeristy Press, 1978).
2. Smol, J. P. *Can. J. Bot.* **58**, 458–465 (1980).
3. Munch, C. S. *Freshwat. Biol.* **10**, 61–66 (1980).
4. Battarbee, R. W., Cronberg, G. & Lowry, S. *Hydrobiologia* **71**, 225–232 (1980).
5. Smol, J. P., Brown, S. R., & McNeely, R. N. *Hydrobiologia* **103**, 125–130 (1983).
6. Smol, J. P., Charles, D. F., & Whitehead, D. R. *Can. J. Bot.* (in the press).
7. Schofield, C. L. *Acidification of Adirondack Lakes by Atmospheric Precipitation: Final Report, Project F-28-R* (New York State Department of Environmental Conservation, Albany, 1976.)
8. Pfeiffer, M. H. & Festa, P. J. *Acidity Status of Lakes in the Adirondack Region of New York in Relation to Fish Resources* (New York State Department of Environmental Conservation, Albany, 1980.)
9. Charles, D. F. thesis, Indiana Univ. (1982).
10. Cushing, E. J. & Wright, H. E. Jr *Ecology* **46**, 380–384 (1965).
11. New York State Department of Environmental Conservation Lake and Pond Survey records, Region 5, Ray Brook, New York, USA.
12. Driscoll, C. T., Jr, Baker, J. P., Bisogni, J. J., Jr, & Schofield, C. L. *Nature* **284**, 161–164 (1980).
13. Cronberg, G. & Kristiansen, J. *Bot. Notiser* **133**, 595–618 (1980).
14. Nicholls, K. *Nova Hedw.* **34**, 88–124 (1982).
15. Kling, H. & Kristiansen, J. *Nord. J. Bot.* **3**, 269–290 (1983).

Photodriven charge separation in a carotenoporphyrin–quinone triad

Thomas A. Moore*†§, Devens Gust*‡§, Paul Mathis†, Jean-Claude Mialocq‡, Claude Chachaty‡, René V. Bensasson§, Edward J. Land‖, Denis Doizi‡, Paul A. Liddell*, William R. Lehman*, Gregory A. Nemeth* & Ana L. Moore*§

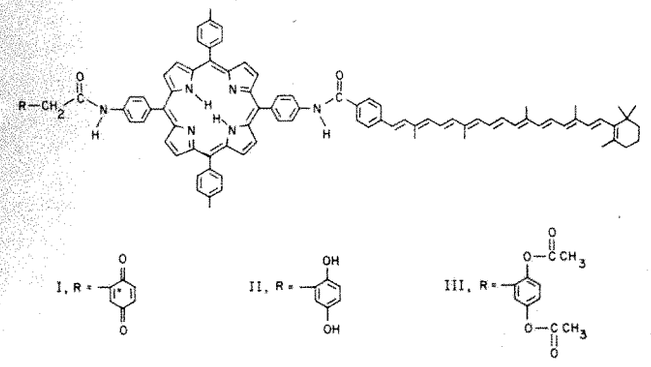
* Department of Chemistry, Arizona State University, Tempe, Arizona 85287, USA
† Département de Biologie and ‡ Département de Physico-Chimie CNRS (LA 331), CEN Saclay, 91191 Gif-sur-Yvette Cedex, France
§ Laboratoire de Biophysique, INSERM U.201, ERA 951 du CNRS, Musée National d'Histoire Naturelle, 61 Rue Buffon 75005, Paris, France
‖ Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, UK

The key steps in the photosynthetic conversion of light to chemical potential energy include not only photodriven charge separation, but also prevention of the back-reaction (charge recombination). Although the first of these steps has been achieved in several biomimetic solar energy conversion systems, retarding the back-reaction has proved more difficult. This may be accomplished by rapidly moving the electron, the hole, or both away from the site of excitation to more stabilizing environments. In photosynthetic membranes, the electron is transferred sequentially over several closely coupled molecules, including tetrapyrroles and quinones[1–3]. In semiconductor/liquid interfacial systems both the electron and the hole migrate following excitation[4,5]. We now report that substantial slowing of the back-reaction has been achieved with a tripartite molecule in which a long-lived photodriven charge-separated state of relatively high potential is formed from an excited singlet state in accordance with the above principles. This molecular triad (compound I) consists of a tetraarylporphyrin covalently linked to both a carotenoid and a quinone. In solution, excitation of the porphyrin moiety by visible light results in the rapid (<100 ps) formation of a transient species $C^{+\cdot}$–P–Q–$^{\cdot-}$, with a lifetime on the μs time scale and an energy more than 1 eV above the ground state.

Compound I was synthesized using methods previously developed for carotenoporphyrins[6–10]. The 7′-apo-7′-(4-carboxyphenyl)-β-carotene was prepared by the Wittig reaction of the ylide of 4-carbomethoxybenzyltriphenylphosphonium bromide with β-apo-8′-carotenal, followed by saponification of the resulting methyl ester. The 5,15-bis(4-aminophenyl)-10,20-bis(4-methylphenyl)porphyrin was prepared by reduction of the corresponding dinitro porphyrin, and the carotenoid was linked to this porphyrin using dicyclohexylcarbodiimide and 4-dimethylaminopyridine. Coupling of this carotenoporphyrin to 2,5-diacetoxyphenylacetic acid yielded compound III, which was deacetylated with potassium borohydride to generate hydroquinone II. The oxidation of II to I was achieved with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone or lead oxide. The fast atom bombardment (FAB) mass spectrum of III yielded a monoisotopic mass ($M+1$) of 1,423.5 (calculated for $C_{96}H_{90}N_6O_6$; 1,423.7).

The 500 MHz ^{1}H-NMR spectra of I, II and III in $CDCl_3$ solution support the assigned structures and also yield conformational information. The aromatic, 7′ and 8′ proton resonances of the carotenoid portions of the molecules are shifted downfield from their positions in the corresponding carotenoid aniline amide, whereas the chemical shifts of the remaining carotenoid protons are virtually unchanged. These downfield shifts (≤0.16 p.p.m.) indicate that the carotenoid is in the deshielding region of the porphyrin ring current field. Quantitative calculations[11] for III show that the carotenoid chain lies approximately in the plane of the porphyrin and is extended away from the

0028-0836/84/070630—03$01.00
© 1984 Macmillan Journals Ltd



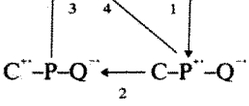
porphyrin π-electron system (Fig. 1). The protected hydroquinone side chain is also in an extended, rather than folded, conformation. Ring current calculations yield the conformation shown in Fig. 1, and are consistent with rapid rotational jumps of the $-CH_2$-diacetoxyphenyl residue among the various equilibrium positions about the CH_2–CO bond, only one of which is shown. Conversion of III to II or I results in small changes in chemical shifts for the protons of the quinone side chain, as expected, and has very little effect ($\Delta\delta \leq 0.05$ p.p.m.) on the porphyrin or carotenoid proton resonances. Thus, it is reasonable to conclude that the time average solution conformation of each of these triads is also extended with the carotenoid and quinone moieties rather widely separated.

Nanosecond flash excitation[12] at 600 nm of a solution of compound I in CH_2Cl_2 resulted in the transient absorption spectrum shown in Fig. 2 with $\lambda_{max} = 970$ nm and $t_{1/2} \sim 170$ ns. This signal was not observed with II or III. Figure 2 also shows the transient absorption after pulse radiolysis of III and of a model for its carotenoid component. The assignment of the pulse radiolysis[13] spectrum, and therefore the flash photolysis spectrum, to a carotenoid cation radical is straightforward[14–17]. A carotenoid ground state bleaching signal ($\lambda_{max} \sim 460$ nm, $t_{1/2} \sim 170$ ns) appeared concomitantly with the cation radical absorption. (A small, long-lived bleaching component ascribed to a carotenoid triplet arising from trace amounts of II was also observed.) Saturation of the CH_2Cl_2 solution with tetra-*n*-butylammonium tetrafluoroborate increased the yield of carotenoid cation radical by a factor of at least six, increased $t_{1/2}$ to ~ 2.5 μs, and blueshifted the absorption maximum to ~940 nm. In acetonitrile solution $\lambda_{max} = 910$ nm and $t_{1/2}$ was ~3 μs.

Picosecond techniques were used to measure the rise of the cation radical (Fig. 3). An absorbance increase was observed at 950 nm and assigned to the carotenoid cation radical, as confirmed by a spectrum taken at 500 ps. The rise of the cation radical occurs within ~100 ps, and is delayed by ~30 ps with respect to the response time of the apparatus as determined by the photobleaching of pinacyanol chloride[18]. At 600 nm, an absorbance increase appears with the response time of the instrument and decays within 100 ps to a small residual absorption. This absorbance increase may be assigned to the porphyrin first excited singlet state (^{1}P) and/or an intermediate porphyrin cation radical (see below)[19,20].

The quantum yield of carotenoid cation radical was ~0.25 in CH_2Cl_2 solution saturated with tetra-*n*-butylammonium tetrafluoroborate. This measurement was made by a comparative method[21] using free base *meso*-tetra-*p*-tolylporphyrin in benzene as the standard ($\varepsilon_{441}(^3\text{porphyrin}) = 6.8 \times 10^4\ M^{-1}\ cm^{-1}$ (ref. 8) and $\phi(^3\text{porphyrin}) = 0.9$ (ref. 22)). An ε_{970} (carotenoid cation radical) $\sim 1.6 \times 10^5\ M^{-1}\ cm^{-1}$ was determined from the ratio of δA_{460} (the bleaching component having $t_{1/2} \sim 170$ ns) to $\delta A_{970} = 1:2.7$ and assuming[17] ε_{460} (carotenoid cation radical) $= 0.5\ \varepsilon_{460}$ (carotenoid S_0)[15–17]. In pure CH_2Cl_2, the quantum yield was ~0.04; similar values were found in acetonitrile and acetone.

The simplest scheme that is consistent with the spectroscopic observations is shown below



(minor pathways, triplet states and spin multiplicities of the ionic states are ignored). By analogy with simple porphyrin–quinone systems[19,20,23–25], it is reasonable to assume that the dominant relaxation pathway (1) from C–^{1}P–Q is quenching of ^{1}P via electron transfer to yield C–P$^{+\cdot}$–Q$^{-\cdot}$. The immediate rise of the 600-nm absorption on excitation (Fig. 3) is consistent with the formation of C–^{1}P–Q and its rapid conversion to C–P$^{+\cdot}$–Q$^{-\cdot}$. (The relative contributions of ^{1}P and P$^{+\cdot}$ to this

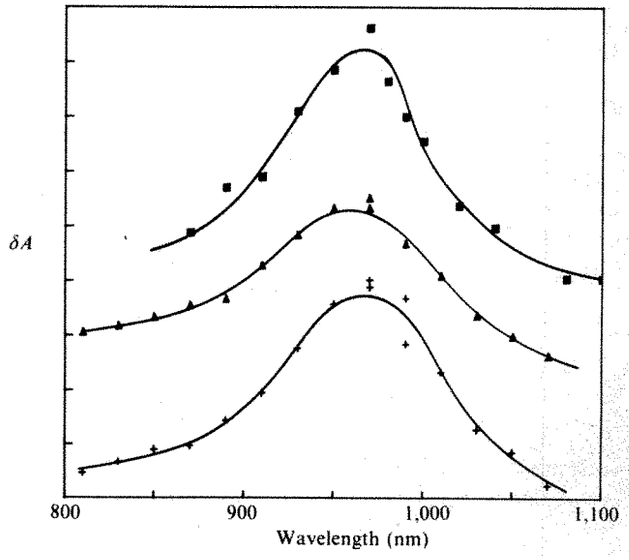


Fig. 2 Transient absorption spectra of carotenoid cation radicals. ■, Absorption spectrum immediately after 10-ns 600-nm flash excitation[12] of 10^{-5} M compound I in CH_2Cl_2. ▲, Absorption spectrum 2 μs after pulse radiolysis of argon-flushed $\sim 5 \times 10^{-5}$ M compound III in CH_2Cl_2. +, Absorption spectrum 4 μs after pulse radiolysis of argon-flushed 10^{-5} M aniline amide of 7′-apo-7′(4-carboxyphenyl)-β-carotene in CH_2Cl_2. For the pulse radiolysis experiments, radiation doses (~20 Gy per 50 ns pulse) were provided by a 12-MeV Vickers electron linear accelerator[13]. Quartz capillary cells of 2 cm optical path were used. The δA scales for the three spectra are different, and baselines have been offset for clarity.

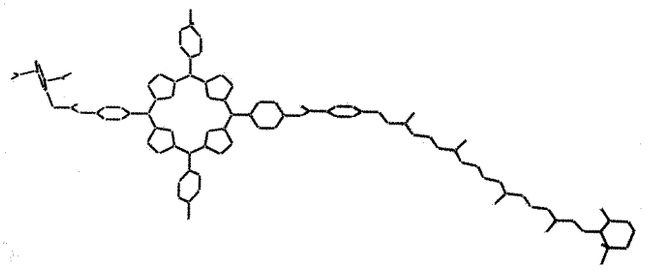


Fig. 1 Time averaged solution conformation of triad III as calculated from porphyrin ring current-induced ^{1}H-NMR chemical shift changes. The hydrogen atoms are not shown. The acetylated hydroquinone moiety is seen edge-on at the left, whereas the carotenoid extends to the right. In this perspective, the porphyrin is not coplanar with the plane of the figure. The chemical shifts of the acetyl methyl protons are consistent with rapid reorientation by π radians about the bonds linking the acetoxy oxygens to the aromatic ring.

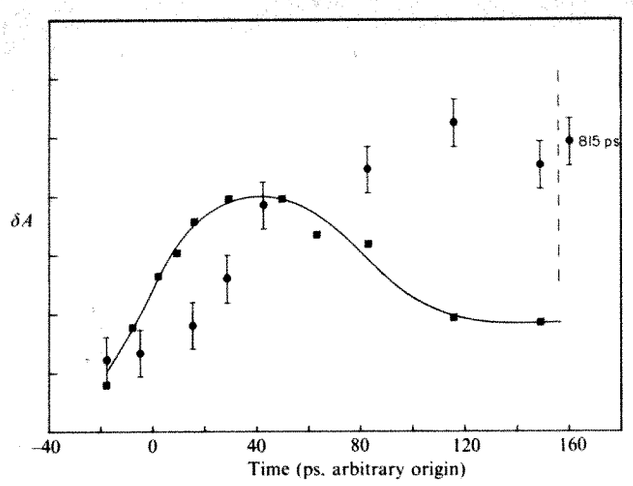


Fig. 3 Transient absorption kinetics for compound I (10^{-4} M) in CH_2Cl_2 solution saturated with tetra-*n*-butylammonium tetrafluoroborate after 532 nm pulse excitation (27 ps FWHM)[32]. The probe light was the ps continuum of light, analysed after the sample cell (1 mm path length) using Pomfret interference filters (3 nm bandwidth) centred at 600 nm and at 950 nm ($\delta A = 0.08$ per division). Each point is an average of four laser shots. The circles with error bars represent the response at 950 nm (carotenoid cation radical), whereas the squares give δA at 600 nm. The error bars at 600 nm are comparable with those at 950 nm, but have been omitted for clarity.

absorption are not resolved.) The decay of this absorption and concurrent rise of the carotenoid cation radical absorption reflect the formation of $C^{\cdot+}$–P–$Q^{\cdot-}$ (step 2). The dramatic shortening of the porphyrin first excited singlet lifetime ($\tau \sim 10$ ns for a typical porphyrin) suggests that the species C–$P^{\cdot+}$–$Q^{\cdot-}$ is formed with a quantum yield approaching unity. Furthermore, the rapid decay of ^{1}P and rise of carotenoid cation radical rule out the participation of the porphyrin triplet in the primary electron transfer process. In addition, there is no evidence for direct excitation of Mulliken-type[26] porphyrin–quinone charge transfer states, because the absorption spectra at 400–800 nm for I, II and III are identical to within 1%.

The next step in the formation of the final charge-separated state is the electron transfer process C–$P^{\cdot+}$–$Q^{\cdot-}$ → $C^{\cdot+}$–P–$Q^{\cdot-}$ (step 2) which competes with the recombination reaction (step 4). In pure CH_2Cl_2 the yield of (2) is ~4%, whereas at high salt concentration it increases markedly. This observation suggests that the outcome of the competition between (2) and (4) is strongly dependent on the ionic environment. It seems certain that the redox potential of the final state $C^{\cdot+}$–P–$Q^{\cdot-}$ is >1 V, based on $E_{1/2}$ values for related unlinked molecules[27–29]. Thus, a substantial fraction of the 1.8-eV porphyrin lowest excited singlet state has been conserved as chemical potential.

As was found with certain other carotenoporphyrins[7,8], the triads mimic antenna and photoprotective processes observed in photosynthetic membranes. In compounds II and III, singlet energy transfer (^{1}C–P–Q → C–^{1}P–Q, antenna function) is ~10% efficient. As is the case in the natural system, triplet states are formed in much greater yield when the primary acceptor is reduced (II or III) before excitation. Quenching of the porphyrin triplet state in III ($C-{}^3P-Q \rightarrow {}^3C-P-Q$) occurs in less than 20 ns, ensuring essentially complete photoprotection from singlet oxygen (a detailed report of the singlet and triplet energy transfer processes in the triads will be presented elsewhere).

The observation of a carotenoid cation radical in I has biological relevance in that the formation of a carotenoid cation radical has been observed in photosystem II in certain conditions[27]. Note also that the formation of a carotenoid radical cation in I in organic solvents suggests that charge conduction by a carotenoid through media of low dielectric constant[30] is possible. Furthermore, the observation that an ionic environment promotes the formation of $C^{\cdot+}$–P–$Q^{\cdot-}$ is consistent with the suggestion that specific charged sites on proteins at the reaction centre in photosynthetic membranes may be important in mediating charge separation[31].

The formation of a long-lived charge-separated state from the first excited singlet state of this molecular triad with a reasonable quantum yield has significance for the design of artificial photosynthetic systems. The key to obtaining long lifetimes of the charge-separated state appears to be the interposition of a neutral porphyrin between the widely separated ions, coupled with energetics which are unfavourable for recombination via species such as C–$P^{\cdot+}$–$Q^{\cdot-}$ or $C^{\cdot+}$–$P^{\cdot+}$–Q. Moreover, note that I is relatively stable; there is little change in the sample even after hundreds of laser flashes over several days.

This material is based on work supported in part by the NSF under grants CHE-8209348 and INT-8212583 (CNRS-NSF Exchange Program), CNRS grant 3064, the North Atlantic Treaty Organization under grant RG.083.81 and the Cancer Research Campaign (UK). We thank Hoffmann-La Roche, Inc. for a gift of β-apo-8′-carotenal, and David N. Heller, Catherine Fenselau and the Middle Atlantic Mass Spectrometry Laboratory, a NSF Shared Instrumentation Facility, for the FAB mass spectral determinations.

Received 22 August; accepted 1 December 1983.

1. Dutton, P. L., Prince, R. C. & Tiede, D. M. *Photochem. Photobiol.* **28,** 939–949 (1978).
2. Sauer, K. *Acc. chem. Res.* **11,** 257–264 (1978).
3. Mathis, P. & Paillotin, G. in *The Biochemistry of Plants* Vol. 8 (eds Hatch, M. D. & Boardman, N. K.) 97–161 (Academic, New York, 1981).
4. Gerischer, H. in *Topics appl. Phys.* **31,** 115–172 (1979).
5. Wrighton, M. S. *Acc. chem. Res.* **12,** 303–310 (1979).
6. Dirks, G., Moore, A. L., Moore, T. A. & Gust, D. *Photochem. Photobiol.* **32,** 277–280 (1980).
7. Moore, A. L., Dirks, G., Gust, D. & Moore, T. A. *Photochem. Photobiol.* **32,** 691–695 (1980).
8. Bensasson, R. V. *et al. Nature* **290,** 329–332 (1981).
9. Moore, A. L. *et al. Science* **216,** 982–984 (1982).
10. Liddell, P. A. *et al. Photochem. Photobiol.* **36,** 641–645 (1982).
11. Chachaty, C. *et al. Org. magn. Reson.* (in the press).
12. van Best, J. A. & Mathis, P. *Rev. scient. Instrum.* **49,** 1332–1335 (1978).
13. Keene, J. P. *J. scient. Instrum.* **41,** 493–496 (1964).
14. Dorfman, L. M., Sujdak, R. J. & Bockrath, B. *Acc. chem. Res.* **9,** 352–357 (1976).
15. Mathis, P. & Vermeglio, A. *Photochem. Photobiol.* **15,** 157–164 (1972).
16. Dawe, E. A. & Land, E. J. *JCS Faraday Trans. I* **71,** 2162–2169 (1975).
17. Chauvet, J.-P., Viovy, R., Land, E. J., Santus, R. & Truscott, T. G. *J. phys. Chem.* **87,** 592–601 (1983).
18. Mialocq, J. C. *Chem. Phys.* **73,** 107–115 (1982).
19. Migita, M. *et al. Chem. Phys. Lett.* **84,** 263–266 (1981).
20. Bergkamp, M. A., Dalton, J. & Netzel, T. L. *J. Am. chem. Soc.* **104,** 253–259 (1982).
21. Bensasson, R. & Land, E. J. *Photochem. photobiol. Rev.* **3,** 163–191 (1978).
22. Moore, T. A., Benin, D. & Tom, R. *J. Am. chem. Soc.* **104,** 7356–7357 (1982).
23. Kong, J. L. Y., Spears, K. G. & Loach, P. A. *Photochem. Photobiol.* **35,** 545–553 (1982).
24. Tabushi, I., Koga, N. & Yanagita, M. *Tetrahedron Lett.* 257–260 (1979).
25. Ho, T. F., McIntosh, A. R. & Bolton, J. R. *Nature* **286,** 254–256 (1980).
26. Mulliken, R. S. *J. Am. chem. Soc.* **72,** 600–608 (1950); **74,** 811–824 (1952).
27. Schenck, C. C., Diner, B., Mathis, P. & Satoh, K. *Biochim. biophys. Acta* **680,** 216–227 (1982).
28. Paramonova, L. I., Naush, Ya., Kreslavskii, V. D. & Stolovitskii, Yu. M. *Biophysics* **27,** 199–203 (1982).
29. Prince, R. C., Gunner, M. R. & Dutton, P. L. in *Function of Quinones in Energy Conserving Systems* (ed. Trumpower, B. L.) 29–33 (Academic, New York, 1982).
30. Platt, J. R. *Science* **129,** 372–374 (1959).
31. Mauzerall, D. *Israel J. Chem.* **21,** 321–324 (1981).
32. Mialocq, J. C., Amouyal, E., Bernas, A. & Grand, D. *J. phys. Chem.* **86,** 3173–3177 (1982).

Sensitivity for vertical retinal image differences

Gerald Westheimer

Department of Physiology–Anatomy, University of California, Berkeley, California 94720, USA

The detection of horizontal disparities between the right and left retinal images is accepted as an important component in the visual perception of depth. It has occasionally been pointed out[1–3] that a disparity will also occur in the vertical when a target is nearer to one eye than the other and could be used as a second component[3]. Here it is shown that while it is possible to detect size differences between the retinal images in the vertical direc-

0028–0836/84/070632—03$01.00

© 1984 Macmillan Journals Ltd

tion, the sensitivity is at least one order of magnitude less than the horizontal differences. It had been generally accepted that vertical disparities cannot be detected (literature summarized by Tschermak[4]), but there are clear reports of the ability of subjects to respond to relative differences in the vertical magnification of the images of the two eyes, the so-called induced size effect[2]. It manifests itself in the appearance of a visual scene as if there had been an opposite horizontal magnification and is, therefore, in the correct direction if it is to be utilized in asymmetrical convergence.

Two reasons make it opportune to examine this matter experimentally. Mayhew and Longuet-Higgins[3] have questioned the applicability of recent findings[5,6] which have again failed to show any significant sensitivity of normal observers to imposed vertical disparities in their retinal images. They point out that in actual viewing situations where a target is nearer to one eye than the other, as indeed also in the more artificial conditions for observing the induced size effect, the vertical disparities are appropriately distributed throughout the visual field in a plausible manner. Tests for detection of vertical disparity in a simple unitary target may, therefore, not be successful. The second reason for looking into this matter with some care is the extraordinary sensitivity of most subjects to horizontal disparities. Thresholds of a few seconds of arc indicate that a complex apparatus has to be present to extract this information from the retinal images. Neurophysiological studies of early cortical stages in the mammal indicate disparity coding with at least one and possibly two orders of magnitude less sensitivity[7] and, significantly, the scatter of disparities is about the same in the horizontal and the vertical direction[8,9]. One would thus like to know whether the mechanism for distilling the cortical cells' coarse disparity coding into the fine disparity signals demonstrated in stereoacuity is needed not only in the horizontal direction, but also vertically as would be the case if subjects indeed had good ability to detect vertical disparities, no matter how this ability actually manifested itself.

In the experiments a compromise was struck between the abstract, single feature patterns used in previous disparity detection research and the more variegated visual configurations that seem to be needed to elicit the induced size effect[2]. Five small squares with sides of 5 arc min were seen by both eyes at a distance of 3 m. They were laid out in an inverted V, the middle two squares being 30 arc min apart horizontally and the lower pair 1° apart (Fig. 1). This pattern was chosen because any implicit horizontal disparity induced by vertical magnification[6] would not manifest itself in a tilt of the fronto-parallel plane around a vertical axis, which is the essence of the induced size effect. The rest of the visual field was entirely dark. Because the induced size effect is said to take some time to develop, exposure duration was 1 s, several times longer than needed for good horizotal disparity discrimination.

During an experimental session, the pattern was presented every 3 s and good fixation was maintained in the intervals by showing a fronto-parallel set of four brackets with a central cross, all dimensions being equal in the two eyes. By means of polaroids and a half-silvered mirror it was arranged that one eye saw the display on one oscilloscope and the other eye on another. During each stimulus presentation, the vertical gain of one scope relative to that of the other was set to a value chosen at random from an ensemble consisting of seven gains, for example, 0.94, 0.96, 0.98, 1.00, 1.02, 1.04 and 1.06. That is to say, the whole pattern seen by one eye was 6%, 4%, 2% or 0% larger or smaller vertically than that of the other, randomly chosen for a given presentation. The subject's response was restricted to the decision whether the little squares in the right or left limb of the inverted V configuration were nearer to him. Results (Fig. 1, left), accumulated in several sessions of 300 responses, were fitted by the methods of probits, which enables the identification of a threshold, that is, that image magnification at which the subject on 75% of occasions saw the pattern rotated out of the fronto-parallel plane around a vertical axis in the indicated direction. Thresholds are summarized in Table I for three experienced stereoscopic subjects. Fragmentary results in a fourth subject gave concordant findings. Also shown are the results for equivalent runs in which the magnification changes were given in the horizontal direction, where the situation accurately mimics an actual rotation of the fronto-parallel plane around a vertical axis. Each pair of conditions (horizontal and vertical) is based on a total of 2,000 responses, obtained over several days under strictly comparable conditions.

The findings summarized in Table 1 allow conclusions about both of the questions raised. First, the sensitivity for discrimination of vertical magnification differences in the two eyes is 14, 12 and 3.5 times lower in the three subjects, respectively, than that for horizontal magnification differences. Second, it seems indeed possible to show a small induced effect with appropriate kinds of abstract stimuli: complex patterns, long exposure durations and a relatively conflict-free visual environment seem necessary. Vertical magnification of one eye then produces a restructuring of visual space as if the other eye had been subjected to horizontal magnification. The experiment reported here is, however, not an exact replication of the standard demonstration of the induced size effect[2], where an ink-splattered glass pane is set to appear fronto-parallel by a subject wearing a vertically magnifying lens over one eye.

Succinctly stated, our three subjects viewing a fronto-parallel pattern with a 3% horizontal magnification would call it tilted towards their left on 98%, 99% and 78% of all presentations, respectively; of the presentations with 3% vertical magnification, 58%, 60% and 61% respectively would be called tilted towards their right. The percentages each have a standard error of no more than 3% and even in the second set they are clearly

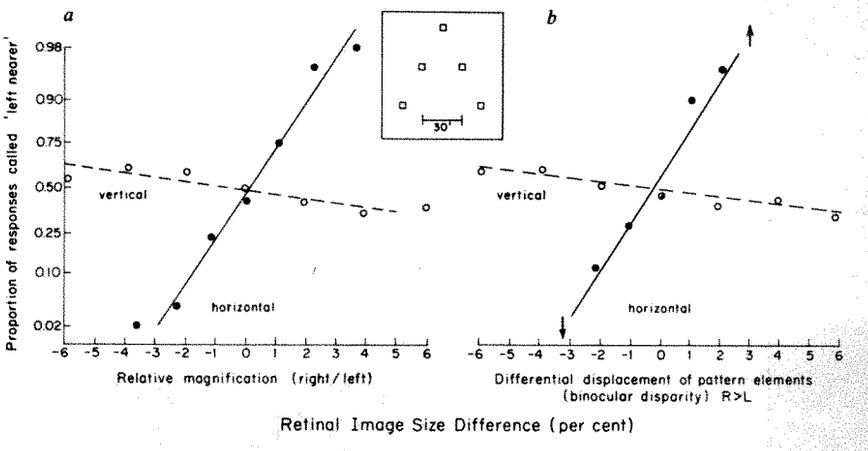


Fig. 1 *a*, Results of 2,000 forced-choice responses from subject G.W., without error feedback, in which right and left retinal images of the pattern seen binocularly (shown in inset) were presented at random with one of the indicated magnification ratios, either horizontally or vertically. *b*, Results of a similar experiment where the pattern elements remained of constant size throughout, but were given differential horizontal or vertical displacements in the two eyes to produce binocular disparities. Lines are best fit probits and allow accurate threshold quantitation: half the image differences for which 75% of the responses are at criterion. Sensitivity for vertical image size differences, whether generated by magnification (*a*) or disparity (*b*), is about an order of magnitude less than for horizontal ones and the responses are of opposite polarity.

Table 1 Threshold difference between subject's right and left retinal images at which he could detect an apparent rotation of a fronto-parallel plane around a vertical axis

Direction	Subject		
	GW	MG	RY
		Magnification	
Horizontal	1.1+0.06	0.9+0.05	2.9+0.2
Vertical*	−12.9+2.3	−12.7+2.7	−10.1+1.8
		Disparity	
Horizontal	0.9+0.06	0.9+0.06	1.5+0.11
Vertical*	−15.5+2.6	−16.9+3.0	−29.3+10.2

Magnification: whole pattern was magnified in the indicated direction-threshold is expressed as per cent magnification (right/left) retinal images. Disparity: pattern elements remained of same size but were differentially displaced in the two eyes to give binocular disparity in the indicated direction, threshold is expressed as per cent difference in target separations in the two eyes.

* The minus sign signifies that the apparent fronto-parallel plane appeared nearer at the subject's right when the retinal image of the right eye was larger vertically than that of the left.

statistically different from random, which would give a 50% response. In a related experiment using gratings with spatial frequency differing in the two eyes, Riguadiere[10] observed much less tilt of the apparent fronto-parallel plane for horizontal than for vertical patterns.

A large difference between horizontal and vertical also showed up when the experiments were repeated with the same geometrical target and exposure duration, but with the depth clue given by pure disparity and not magnification. In practice this meant that the squares making up the target were of identical size in the two eyes under all stimulus conditions, but their relative placements on the two retinas was dictated solely by disparity considerations. This could, however, still be expressed in percentage terms, although now referring to the relative separation of identically sized target elements on the two retinas. The results (Fig. 1, right, and Table 4) confirm the earlier observations of massive differences in sensitivity for horizontal versus vertical disparities[5]. The ratio is about 20 for all three observers.

An induced size effect, therefore, exists not only in the situation used by Ogle, but even here where the experimental situation is governed by the kind of stimulus abstraction commonly used in the psychophysical analysis of the substrates of space perception. The particular patterns in the experiment described here preclude the direct use of implicit horizontal disparity information contained in stimuli with uniocular vertical magnification. This cannot, therefore, be the sole clue for the induced size effect, as has sometimes been thought[5,6].

To see whether there are simple ways of enhancing vertical disparity detection beyond the levels shown in the experiments reported above, two additional factors have been cursorily examined, longer exposure durations and increased pattern size. There was no important change in the threshold when the target was presented for 5 s; and a target consisting of a 7×7 sheet of dots extending over the central 3° of the visual field still did not suffice to lower the disparity threshold materially, so it would appear necessary to postulate spatial integration over a much larger portion of the visual field. If this is the case, we have yet another variable that sets off vertical from horizontal disparity detection, because stereoacuity can be a few seconds of arc for just two small squares separated by 10–20 arc min.

This research was supported by the National Eye Institute, U.S. Public Health Service under grant EY-00220.

Received 25 April; accepted 3 November 1983.

1. Householder, A. S. Bull. *Math. Biophys.* **5**, 155–160 (1943).
2. Ogle, K. N. *Binocular Vision* (Saunders, Philadelphia, 1950).
3. Mayhew, J. E. W. & Longuet-Higgins, H. C. *Nature* **297**, 376–378 (1982).
4. Tschermak, A. *Handbuch der normalen und pathologischen Physiologie* Vol. XII/2 (Springer, Berlin, 1932).
5. Westheimer, G. *Invest. ophthal. vis. Sci.* **17**, 545–551 (1978).
6. Arditi, A., Kaufman, L. & Movshon, J. A. *Vision Res.* **21**, 755–764 (1981).
7. Poggio, G. F. and Talbot, W. H. *J. Physiol., Lond.* **315**, 469–492 (1981).
8. Joshua, D. E. and Bishop, P. O. *Expl Brain Res.* **10**, 389–416 (1970).
9. Ferster, D. *J. Physiol., Lond.* **311**, 623–655 (1981).
10. Rigaudiere, F. *Vision Res.* **15**, 931–938 (1975).

Interspecific chimaerism between sheep and goat

Carole B. Fehilly, S. M. Willadsen & Elizabeth M. Tucker

ARC Institute of Animal Physiology, Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ, UK

In rodents, chimaeric blastocysts produced by combining embryonic cells of two different species have been used in investigations of cell lineage and interaction during development (*Mus musculus–Rattus norvegicus*[1,2], *M. musculus–Clethrionomys glareolus*[3], *M. musculus–Mus caroli*[4]). However, interspecific chimaerism also offers new approaches to the study of reproductive incompatibilities between species and may even allow such incompatibilities to be neutralized, thus improving the chances of successful hybridization and interspecific embryo transplantation[5]. We report here the production of sheep–goat chimaeras by embryo manipulation and the use of interspecific chimaerism to allow successful interspecific embryo transplantation in sheep and goats.

So far, the *M. musculus–M. caroli* chimaeras described by Rossant *et al.*[4–6] seem to be the only viable interspecific mammalian chimaeras to have been produced by embryo manipulation. The two species concerned are closely related and, although they do not normally interbreed, they can be hybridized[7]. The domestic sheep (*Ovis aries*, $2n = 54$) and the domestic goat (*Capra hircus*, $2n = 60$) are less closely related[9,10] but neither a sheep nor a goat has carried a live hybrid fetus to term in a controlled experiment except when special procedures were used to reduce interspecific incompatibility[11]. The rationale of the latter procedures as well as the reproducibility of the results have been questioned[12]. Experimental hybrid pregnancies in both sheep and goat females have often proceeded beyond the time of implantation but, invariably, by the end of the second month the fetus has died[12–14]. The same applies to pregnancies resulting from transfer of sheep embryos to goat recipients, while goat embryos transferred to sheep recipients have not survived beyond the first month[13,15]. The transfer of an embryo belonging to the same species as the recipient together with the foreign embryo does not seem to alter the survival prospects of the latter[15]. In all the above categories of interspecific pregnancies, placental abnormalities, possibly due to maternal immunological reaction against foreign antigens of the conceptus, have been the immediate cause of abortion[16–19].

The present study comprises three series of experiments. In the first series, single blastomeres from 4-cell goat embryos were combined with single blastomeres from 4-cell sheep embryos (Table 1, expt 1*a*), or with single blastomeres from 8-cell sheep embryos (expt 1*b*) in evacuated zonae pellucidae as described by Willadsen[20]. Up to four interspecific embryos of identical composition were made from the same two parent embryos. In the second series of experiments, interspecific 'giant' embryos were produced either by surrounding an 8-cell goat embryo from which the zona pellucida had been removed with the dissociated blastomeres of three 8-cell sheep embryos (expt 2*a*) or by surrounding a similarly denuded 8-cell sheep embryo with the separated blastomeres of three 8-cell goat embryos.

0028–0836/84/070634—03$01.00

© 1984 Macmillan Journals Ltd

Table 1 The development of chimaeric embryos composed of sheep and goat cells

Experiment	1		2		3	
	a	*b*	*a*	*b*	*a*	*b*
Composition of embryos*	$\frac{1}{4}$S+$\frac{1}{4}$G	$\frac{1}{8}$S+$\frac{1}{4}$G	$\frac{8}{8}$G+3×$\frac{8}{8}$S	$\frac{8}{8}$S+3×$\frac{8}{8}$G	ICMG+BLS	ICMS+BLG
No. cultured in temporary recipients	31	4	4	3		
No. forming interspecific blastocysts	17	2	4	3		
Species of definitive recipients	S	G	S	G	S	G
No. transferred to definitive recipients	17	2	4	3	11	3
No. of full-term young	7	2	3	2	9	3
No. of lambs	4	2(?)	1†	0	6	1
No. of kids	0	0	0	1†	1	2
No. of interspecific chimaeras	3	0	2	1	2	0

* As regards embryo composition, $\frac{1}{4}$ represents one blastomere from a 4-cell embryo; $\frac{1}{8}$ represents one blastomere from an 8-cell embryo; and $\frac{8}{8}$ represents all eight blastomeres of an 8-cell embryo. BL, day 8 blastocyst; ICM, inner cell mass and polar trophectoderm from day 8 blastocyst (S, sheep and G, goat).

† Intraspecific chimaera.

The composite embryos in expts 1 and 2 were embedded in agar and cultured in ligated sheep oviducts for 5 (expt 1) or 4 days (expt 2)[20]. They were then examined as fresh specimens (Table 1) and those which had developed into normally organized chimaeric blastocysts were transferred to recipient ewes (expt 1*a* and 2*a*) or recipient goats (expt 1*b* and 2*b*), on day 7 of the oestrous cycle (day of onset of oestrus = day 0).

In a third series of experiments, the inner cell mass and polar trophectoderm from day 8 goat blastocysts were inserted into day 8 sheep blastocysts by the technique described by Gardner[21] (expt 3*a*). In the same way, sheep inner cell masses and polar trophectoderm were combined with goat blastocysts (expt 3*b*). The blastocysts were transferred to sheep (expt 3*a*) or goat recipients (expt 3*b*) on day 8 of their oestrous cycle.

The main results of the experiments are presented in Table 1. The proportion of composite embryos which formed normally organized blastocysts during culture *in vivo* was somewhat lower than that observed in parallel experiments on intraspecific chimaerism in sheep, but judged by the initial pregnancy rate obtained after transplantation of interspecific blastocysts, they were as viable as intraspecific embryos of the same cell number, whether chimaeric or non-chimaeric. However, one pregnancy failed at about day 60, and two fetuses were aborted near term in expt 1*a*.

The seven full-term young (and the two aborted fetuses) in expt 1*a* all had the general appearance of lambs, but in three of these animals the fleece had transverse bands and patches of hair contrasting sharply with the surrounding densely curled wool (Figs 1, 2). Two of these animals each had a twin, produced from the same two parent embryos. Both of the respective twins were lambs with ordinary fleeces. The hairy bands were therefore thought to represent goat tissue, and the three animals were judged to be overt sheep–goat chimaeras (Fig. 3). The two young in expt 1*b* both looked like ordinary lambs; both were stillborn. The three young produced in expt 2*a* also had the general appearance of lambs, but two of these had hairy bands and patches in their fleeces similar to those observed in three of the lambs in expt 1*a*. These two lambs, which were dead at delivery, were therefore also judged to be overt sheep–goat chimaeras. They had developed from a single 'giant' blastocyst. Both of the young produced in expt 2*b* were of the same general appearance as ordinary kids. However, in one of them (Fig. 4) the normal straight goat hair was replaced by densely curled wool in symmetrically situated areas on the neck, shoulders and thighs. The wool was thought to be of sheep origin, and the animal was therefore judged to be an overt sheep–goat chimaera.



Fig. 1 Sheep–goat chimaera (standing) and its sheep twin (see expt 1*a* of Table 1). The two animals, both phenotypically male, developed from chimaeric embryos which shared both their sheep (Suffolk × Welsh Mountain) and their goat (Nubian × Saanen) parent embryo, and were, therefore, monozygotic twins with respect to their sheep component. Note the hairy bands and patches on the neck and trunk of the chimaera. Apart from the white patches on the legs (and ears) the pattern of pigmentation in the chimaera did not differ from that seen in its sheep twin, although the colours were brighter in the hairy areas than in the woolly areas.



Fig. 2 Dorsal view of another sheep–goat chimaera from expt 1*a*. Note the essentially asymmetric distribution of the relatively narrow hairy bands and patches along the midline. The type of fibre (wool or hair) produced in the epidermis is determined by the origin of the underlying dermis (sheep or goat) thus chimaerism, as observed at this stage, had its basis in mesodermally rather than ectodermally derived tissues, despite the absence of blood chimaerism. The pattern of distribution of the hairy bands is of somatic origin.

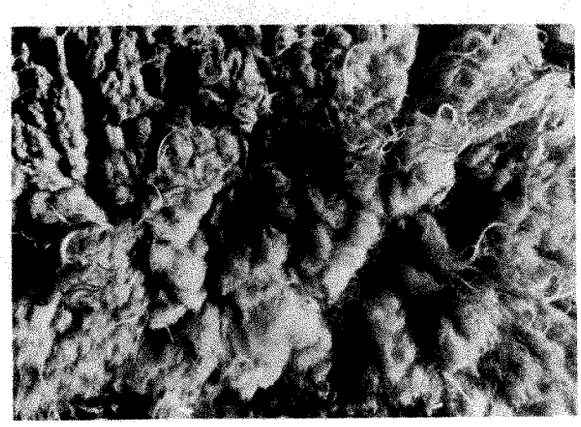


Fig. 3 Close-up of the back of a $1\frac{1}{2}$ yr-old sheep–goat chimaera. By now the wool fibres have outgrown the hair fibres in the fleece so that the hairy bands and patches, although still clearly demarcated, are less conspicuous. Note that two types of fibres can be distinguished within the woolly areas: densely curled fibres of the type normally seen in Suffolk × Welsh Mountain sheep constitute the bulk of the fleece, but in addition there are tufts of longer, wavy (goat wool) fibres, which form a smaller proportion of the fleece but occur throughout. The presence of two types of wool fibres indicated chimaerism in ectodermally derived tissues in addition to the chimaerism in mesodermally derived tissues reflected in the presence of the hairy bands. The latter probably consist mainly of sheep hair but with tufts of goat hair interspersed.



Fig. 4 Sheep–goat chimaera from expt 2*b* at 1 yr old. In this animal many chimaeric features are evident, of which the strongly curved goat horns and the large patches of long, wavy (goat) wool are the most obvious. The distribution of wool, interesting by being essentially symmetric, coincides with areas of the epidermis overlying dermis derived from the lateral mesoderm. The animal, which contains both a male sheep and a male goat component, behaves like a male goat but has proved infertile in natural matings with female goats. The semen is of normal density, but almost all the spermatozoa have a characteristic tail defect.

Six of the nine young produced in expt 3*a* looked in every respect like ordinary lambs. Of the remaining three, one was similar to the chimaeric lambs in expt 1*a*, and another was similar to the chimaeric kid in expt 2*b*, except that its skin had transverse bands and patches of wool along the back; both of these animals were judged to be overt sheep–goat chimaeras. The last of the three animals produced in expt 3*a* looked in every respect like an ordinary kid. Two of the three young produced in expt 3*b* resembled ordinary kids, but the third, which was born as a twin to one of the kids, looked like a lamb.

Blood samples from those lambs and kids which were born alive were tested with respect to the following proteins which differ electrophoretically between sheep and goat[22]: haemoglobin (adult and fetal), carbonic anhydrase, nucleoside phosphorylase, X-protein, malic enzyme, NADH diaphorase and transferrin. The only animal in which interspecific blood chimaerism was demonstrated was the overtly chimaeric kid in expt 2*b*, in which both parent species were represented in all blood proteins tested. The other overt chimaeras all had sheep-type blood proteins, while the remaining animals, including the kid in expt 3*a* and the lamb in expt 3*b*, had blood proteins entirely of the type corresponding to their species of physical appearance.

Thus, the present experiments show that sheep and goat blastomeres can form composite blastocysts and that such interspecific blastocysts are, in principle, viable and may give rise to animals which are sheep–goat chimaeras. In addition, the experiments have shown that a goat fetus can develop to term in a sheep and, similarly, that a sheep fetus can develop to term in a goat. In expt 1*a* the high abortion rate, the relatively low incidence of chimaeras among the offspring, and the disproportionately large contribution made by the sheep blastomeres even in those animals which were overt sheep–goat chimaeras, probably all reflect a reaction on the part of the recipient ewes against the goat component of the conceptus. Although intraembryonic incompatibility or competition between the two species may also have influenced the results, this is less likely to have been the principal cause of the predominance of the sheep component since extensive interspecific chimaerism was observed in one animal in expt 2 and in another in expt 3. The results of the latter experiment indicate that in sheep and goats it is possible to completely neutralize incompatibility between the conceptus and the recipient female by constructing the chimaeric embryo in such a way that the trophectoderm and hence the chorionic epithelium consists entirely of cells belonging to the same species as the recipient. It is probable that the low rate at which this was achieved reflects inadequacy of the particular approach used in that experiment. The results of expt 1*b*, although incomplete, suggest that a more economical and practical way of achieving the same objective is the use of asynchronous parent embryos and reduction of the total cell number to near the minimum required to form a normally organized blastocyst. This is in agreement with the observation made in experiments on intraspecific chimaerism in sheep (C.B.F. and S.M.W., unpublished observations).

We thank Miss S. Clarke for help with the bloodtyping and Dr A. Dain for karyotyping blood lymphocytes of the animal in Fig. 4. C.B.F. was supported by Research Studentships from the MRC and Trinity College, Cambridge.

Received 7 September; accepted 2 December 1983.

1. Stern, M. S. *Nature* **243,** 472–473 (1973).
2. Gardner, R. L. & Johnson, M. H. *Ciba Fdn Symp.* **29,** 183–200 (1975).
3. Mystkowska, E. T. *J. Embryol. exp. Morph.* **33,** 731–744 (1975).
4. Rossant, J. & Frels, W. I. *Science* **208,** 419–420 (1980).
5. Rossant, J., Croy, B. A., Chapman, V. M., Siracusa, L. & Clark, D. A. *J. Anim. Sci.* **55,** 1241–1248 (1982).
6. Rossant, J., Mauro, V. M. & Croy, B. A. *J. Embryol. exp. Morph.* **69,** 141–149 (1982).
7. West, J. D., Frels, W. I., Papaioannou, V. E., Karr, J. P. & Chapman, V. M. *J. Embryol. exp. Morph.* **41,** 233–243 (1977).
8. Gray, A. P. *Mammalian Hybrids*, 130–132 (Commonwealth Agricultural Bureaux, Slough, England, 1972).
9. Roca, R. & Rodero, A. *Arch. Zootec.* **20,** 235–247 (1971).
10. Bunch, T. D., Foote, W. C. & Spillet, J. J. *Theriogenology* **6,** 379–385 (1976).
11. Bratanov, C. & Dikov, V. *Proc. 4th int. Congr. Anim. Reprod.*, The Hague, 4, 744–749 (1962).
12. Hancock, J. L. *Proc. 5th int. Congr. Anim. Reprod.*, Trento, **3,** 445–450 (1964).
13. Warwick, B. L. & Berry, R. O. *J. Hered.* **40,** 297–303 (1949).
14. Hancock, J. L., McGovern, P. T. & Stamp, J. T. *J. Reprod. Fert., Suppl.* **3,** 29–36 (1968).
15. Hancock, J. L. & McGovern, P. T. *Res. Vet. Sci.* **9,** 411 (1968).
16. Dent, J., McGovern, P. T. & Hancock, J. L. *Nature* **231,** 116–117 (1971).
17. Tucker, E. M., McGovern, P. T. & Hancock, J. L. *J. Reprod. Fert.* **27,** 417–425 (1971).
18. McGovern, P. T. *J. Reprod. Fert.* **34,** 215–220 (1973).
19. McGovern, P. T. *Br. vet. J.* **132,** 691–706 (1976).
20. Willadsen, S. M. *Nature* **277,** 298–300 (1979).
21. Gardner, R. L. *Nature* **220,** 596–597 (1968).
22. Tucker, E. M. & Clark, S. W. *Anim. Blood Grps biochem. Genet.* **11,** 163–183 (1980).

Experimental chimaeras—removal of reproductive barrier between sheep and goat

Sabine Meinecke-Tillmann

Institut für Tierzucht und Haustiergenetik der Justus-Liebig-Universität Giessen, Bismarckstrasse 16, 6300 Giessen, FRG

B. Meinecke

Ambulatorische und Geburtshilfliche Veterinärklinik der Justus-Liebig-Universität Giessen, Frankfurter Strasse 106, 6300 Giessen, FRG

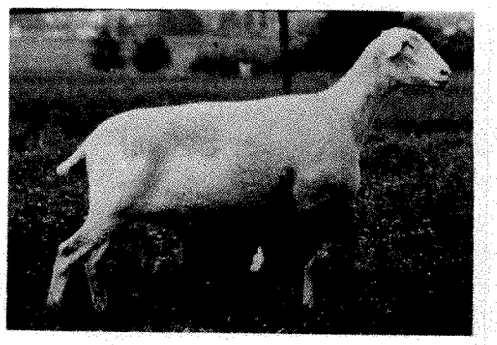


Fig. 1 Male goat–lamb by a Merino mother.

Following reciprocal embryo transfer between sheep and goats, the embryo of the foreign species is able to induce early pregnancy but the embryos do not survive beyond the first weeks of gestation[1,2]. Similar results have been obtained from hybridization experiments on sheep and goats. While the causes for early embryonic death of hybrid eggs from donor sheep are unknown, the reciprocal event has been associated with immunological implications[2–7]. As a means of overcoming the reproductive barrier between sheep and goats, we have transferred interspecific chimaeric embryos. We report here the birth of a goat kid from a sheep mother.

Following oestrus synchronization and natural breeding, embryos were collected from super-ovulated donors on day 2 of pregnancy in sheep (onset of oestrus = day 0) and on day 3 of pregnancy in goats (second day of oestrus = day 0). The chronological difference between the embryos of the two species was about 24 h.

To produce interspecific chimaeric embryos we combined one sheep blastomere of the 4-cell stage with two goat blastomeres of the 8-cell stage, or two sheep blastomeres of the 'early' 8-cell stage with two goat blastomeres of the 'late' 8-cell stage in a common pig zona pellucida. To prevent wastage of cells the slit in the first zona pellucida was completely covered by a second one which surrounded the aggregated embryo with its first host zona. Micromanipulation was done as described previously[8].

The embryos were then transferred to the oviducts of an intermediate recipient and were recovered again when blastulation was expected. Embryos which reached the blastocyst stage were transferred to the uterine horns of the final sheep recipients. Diagnosis of pregnancy was by radioimmunological measurement of plasma progesterone in the recipients on day 18 and 50 of pregnancy.

The results of the experiments are summarized in Table 1. Of 15 interspecific chimaeric embryos analysed after recovery from the intermediate recipients, 9 had developed into a common blastocyst. Of four further embryos which reached the blastocyst stage, one blastomere of the aggregate did not continue to cleave, and in two cases two small blastocysts instead of one developed in their common zona pellucida.

Of 15 final recipients 8 did not return to oestrus and early pregnancy was confirmed by radioimmunoassay. The remaining seven animals returned to oestrus between day 21 and 36 but in all of the cases except one the plasma progesterone values were well above 2 ng ml^{-1} on day 18. On day 50 plasma progesterone was above 2 ng ml^{-1} in six animals.

Three recipients went to term and gave birth to two sheep– and one goat–lamb (Fig. 1). One of the sheep lambs was stillborn because of delayed parturition. The stillborn male sheep–lamb was derived from a combination of two sheep and two goat blastomeres, but in the common blastocyst one goat blastomere was not integrated. The female sheep–lamb was born after transfer of two blastocysts in one common zona pellucida. The goat–lamb developed after aggregation of one blastomere of a 4-cell sheep embryo and two blastomeres of an 8-cell goat embryo. All blastomeres had participated in the development of the common blastocyst.

Table 1 Development of interspecific chimaeric embryos

		Development after transfer to intermediate recipients			Development after transfer to the final recipients			
	Type of chimaeric embryo*	Common blastocyst	Blastocyst (one original blastomere did not cleave)	Two separate blastocysts	Plasma progesterone (ng ml^{-1}) day 18	day 50	Return to oestrus	Delivery
1	●●○○	+			7.6	3.2		
2	●○○	+			7.2	3.9		
3	●○○	+			6.6	5.0		♂ Goat–lamb
4	●○○	+			3.4	3.7		
5	●○○	+			0.1		+	
6	●○○	+			3.3		+	
7	●●○○	+			6.9		+	
8	●○○	+			8.2		+	
9	●●○○	+			3.1		+	
10	●○○		+○		3.4		+	
11	●○○		+●		4.2		+	
12	●○○		+○		2.8	1.4		
13	●●○○		+○		2.2	1.5		♂ Sheep–lamb
14	●○○			+	4.5	5.5		
15	●○○			+	4.1	2.3		♀ Sheep–lamb

* ●, Sheep; ○, Goat.

0028-0836/84/070637–02$01.00

© 1984 Macmillan Journals Ltd

Cytogenetic analysis, haemoglobin and transferrin typing, blood group serology, polyacrylamide gel electrophoretic analysis of blood and muscle proteins and breeding experiments gave no indications of chimaerism.

Thus, by micromanipulation it is possible to overcome the reproductive barrier between sheep and goats, but only when there is a protective mechanism which prevents recognition of the foreign fetus by the mother of the other species. It is assumed that at least the important parts of the placenta, particularly the chorion epithelium, which developed from the trophoblast of the chimaeric blastocyst, were derived from the sheep blastomere, and that the sheep trophoblast components were able to protect the goat embryonic cells from maternal immune rejection. This has been accomplished by combining chronologically different blastomeres. In the embryo which is formed, the younger component tends to give rise to the extra-embryonic membranes and the older component to the fetus. Recent reports on developmental potential of sheep blastomeres[9] and interspecific mouse chimaeras[10] confirm these concepts. Equally, the experiments of S. M. Willadsen and C. B. Fehilly (personal communication) which resulted in the birth of sheep–goat chimaeras revealed the importance of constructing embryos so that the outside cells belong to the species into which the embryos have been transferred. Also, the endocrinological status indicates the sheep origin of the trophoblast. It is known that the goat placenta is not a source of progesterone as the sheep placenta is. After day 55 of pregnancy in sheep the survival of the fetus depends on proper progesterone production by the placenta[11].

The technique described here could be useful for rescuing endangered species.

We thank Dr Glahn-Luft, Dr Heinert, Dr Höhn and Professor Schmid for their technical assistance. This study was supported by a grant from the Deutsche Forschungsgemeinschaft.

Received 11 August; accepted 14 December 1983.

1. Warwick, B. L. & Berry, R. O. *J. Hered.* **40**, 297–303 (1949).
2. Hancock, J. L. & McGovern, P. T. *Res. vet. Sci.* **9**, 411–415 (1968).
3. Sokolovskaja, I. I. *Anim. Breeding. Abstr.* **10**, 204 (1940).
4. Alexander, G., Williams, D. & Bailey, L. *Aust. J. biol. Sci.* **20**, 1217–1220 (1967).
5. Hancock, J. L., McGovern, P. T. & Stamp, J. T. *J. Reprod. Fert.* Suppl. **3**, 29–36 (1968).
6. Dent, J., McGovern, P. T. & Hancock, J. L. *Nature* **231**, 116–117 (1971).
7. McGovern, P. T. *J. Reprod. Fert.* **34**, 215–220 (1973).
8. Meinecke-Tillmann, S. & Meinecke, B. *Zentbl. VetMed.* A**30**, 146–153 (1983).
9. Willadsen, S. M. & Fehilly, C. B. in *Fertilization of the Human Egg* in Vitro (eds Beier, H. M. & Lindner, H. R.) 353–357 (Springer, Berlin, 1983).
10. Rossant, J., Maurer, V. M. & Croy, B. A. *J. Embryol. exp. Morph.* **69**, 141–149 (1982).
11. Amoroso, E. C. & Perry, J. S. in *The Ovary* Vol. 2, 2nd edn (eds Lord Zuckerman & Weir, B. J.) 315–398 (Academic, New York, 1977).

An antigenic difference between cells forming early and late haematopoietic spleen colonies (CFU-S)

R. A. Harris*, P. M. Hogarth†, L. J. Wadeson*, P. Collins†, I. F. C. McKenzie† & D. G. Penington*

* University of Melbourne Department of Medicine, St Vincent's Hospital, Fitzroy, Victoria, Australia 3065
† Research Centre for Cancer and Transplantation, University of Melbourne, Parkville, Victoria, Australia 3052

Murine pluripotent haematopoietic stem cells have generally been assayed by their ability to form macroscopic colonies of haematopoietic cells in the spleens of heavily irradiated recipient mice (colony-forming unit-spleen or CFU-S assay)[1]. However, recent evidence suggests that there are distinct subpopulations of CFU-S[2]. Most spleen colonies present 7–8 days after injection consist of differentiated erythroid cells, contain no primitive myeloid or erythroid precursor cells and disappear from the spleen within 3 days, whereas the majority of colonies present at 10–12 days contain primitive precursors, are multilineal and cannot be detected at 7–8 days[2]. Furthermore, many 10-day-old spleen colonies do not contain cells capable of forming spleen colonies in secondary irradiated recipients (an index of the self-renewal capacity of stem cells) whereas at day 14 almost all colonies contain these CFU-S[3]. These studies suggest that only when colonies are scored at or later than 11–12 days is the spleen colony technique adequate for the assay of multipotential stem cells. We now report an antigenic difference between subpopulations of CFU-S forming early (day 8) and late (day 14) spleen colonies which has been used to purify multipotential haematopoietic stem cells from murine bone marrow.

A cytotoxic IgG2b monoclonal antibody to a 'Qa-2 like' alloantigen, Qa-m2 (ref. 4), reacts with a minority (~10–15%) of bone marrow cells[4] (mostly lymphocytes[5]) and has been shown to kill, in the presence of complement, megakaryocyte colony-forming cells (MEG-CFC) but not granulocyte-macrophage (GM-CFC) or 7-day erythroid colony-forming cells (E-CFC)[5]. These experiments were extended to investigate whether more primitive haematopoietic cells (that is, CFU-S) express Qa-m2. Treatment with anti-Qa-m2 and complement had no effect on the capacity of C57BL/6 bone marrow cells to form spleen colonies 8 days after injection into irradiated hosts (Table 1). However, spleen colony-forming ability was progressively inhibited at later time points. By day 14, spleen colony numbers were reduced by 82% compared with the number formed by cells treated with a negative control antibody, anti-Ly-2.1 (ref. 6), and complement. In contrast, treatment with monoclonal anti-H-2.2 and complement (positive control) abolished colony formation at all times. These results suggest progressive loss of Qa-m2 expression with differentiation from more primitive multipotential cells (day 14 CFU-S) to those with restricted potential (day 8 CFU-S).

Since the CFU-S assay may not adequately predict the capacity of haematopoietic tissue to repopulate the bone marrow of lethally irradiated recipients[7], the effect of anti-Qa-m2+C on this property was studied (Table 2). Anti-Qa-m2+C

Table 1 Effect of anti-Qa-m2 and complement (C) on spleen colony formation at various times after injection of bone marrow cells

Days after injection of cells	CFU-S/1×10^5 marrow cells*			% Reduction by anti-Qa-m2 +C (no. of experiments)†
	Anti-H-2.2 +C	Anti-Ly 2.1 +C	Anti-Qa-m2 +C	
8	0	22.4±3.0	21.1±2.9	7 (5)
10	0	19.6±2.0	15.5±2.3	20 (3)
11	0.2±0.4	19.3±1.2	10.8±1.0	44 (2)
12	0.3±0.5	15.3±2.1	4.2±2.6	71 (3)
14	0	18.6±2.8	3.1±2.0	82 (4)

C57BL/6 bone marrow cells (2×10^6) in 0.1 ml HEPES-buffered alpha medium with 10% heat-inactivated fetal calf serum (FCS) were incubated with monoclonal anti-Qa-m2 (serum from hybridoma-bearing mice, final dilution 1:100), monoclonal anti-H-2.2 serum (1:100 dilution, positive control) or monoclonal anti-Ly-2.1 serum[6] (1:100 dilution, negative control) for 30 min on ice. The concentration of anti-Qa-m2 serum used was >1,000-fold greater than that required for maximal lysis of MEG-CFC[5]. The cells were washed, resuspended in 0.4 ml absorbed rabbit serum (diluted 1:8) as a source of complement (C), and incubated at 37 °C for 45 min. The cells were washed in phosphate-buffered saline (PBS) and injected into groups of 7–10 syngeneic irradiated (825R) specified-pathogen free recipients. From 3×10^4 (for day-14 colonies) to 1×10^5 cells (day-8 colonies) were given to each mouse. At the indicated times after injection, mice were killed by cervical dislocation. Their spleens were removed, fixed in Bouin's fixative, and surface macroscopic colonies were counted.

* Results from a typical experiment (mean ± 1 s.d.).

† Comparison is with anti-Ly-2.1+C-treated cells (negative control). Mean per cent reduction from indicated number of experiments (in parentheses) is shown. The spleens of uninjected irradiated control mice contained <1 colony per 10 spleens at each time point. Treatment of C57BL/6 bone marrow cells as described above produced 9.8±3.2% (mean±s.d.) lysis of anti-Qa-m2+C treated cells compared with 7.3±2.1% lysis after treatment with anti-Ly-2.1+C (three experiments)[5].

0028-0836/84/070638—04$01.00

© 1984 Macmillan Journals Ltd

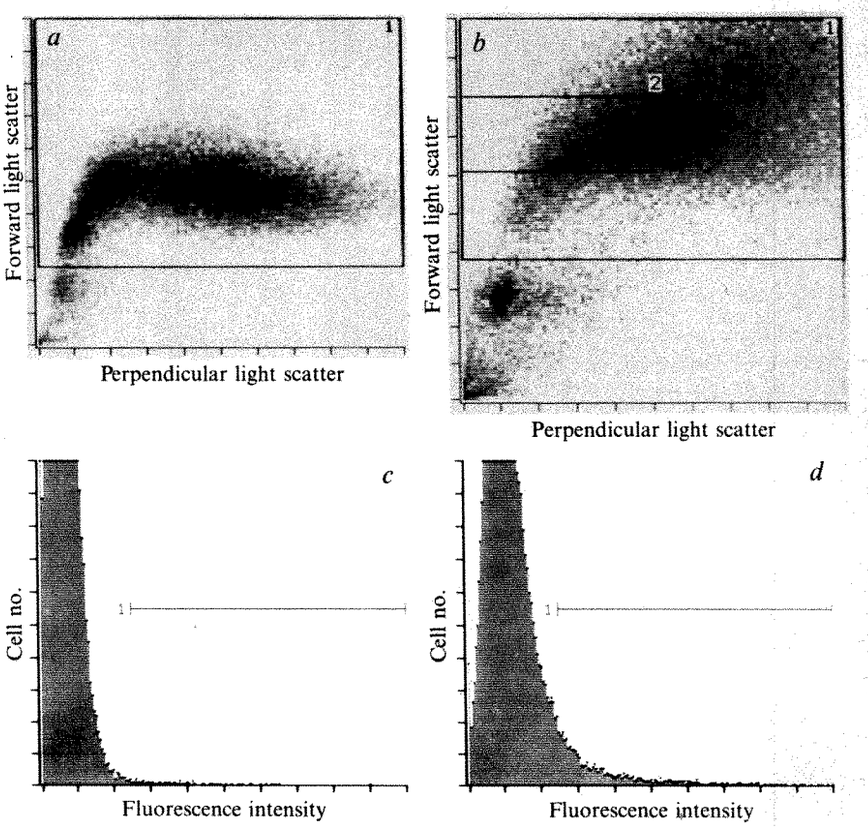


Fig. 1 *a*, Cytogram of lymphocyte-depleted normal marrow cells according to forward and 90° light scatter characteristics. *b*, Cytogram of 5FU8BM cells similarly analysed. *c*, *d*, Examples of fluorescence-intensity profiles for cells in region 2 of 5FU8BM: *c*, treated with anti-Ly-2.1 (negative control) and *d*, treated with anti-Qa-m2. The horizontal bar indicates the region in which cells were sorted as Qa-m2$^+$.

Methods: Normal C57BL/6 bone marrow cells (NBM) or marrow cells from mice treated 8 days previously with 5-fluorouracil (150 mg kg^{-1} intravenously) and endotoxin (*Escherichia coli* 0111:B4 (Difco), 6 μg per mouse intraperitoneally) (5FU8BM) were depleted of lymphocytes, red cells and dead cells as described[5]. Briefly, marrow cells were treated with monoclonal anti-Thy-1.2 serum (1:100 dilution), washed, rosetted with sheep red blood cells coupled with sheep anti-mouse Ig, and depleted of rosetted lymphocytes (T and B), red cells, dead cells and some neutrophils by centrifugation through an Isopaque–Ficoll gradient (ρ 1.090 g cm^{-3})[5]. The lymphocyte-depleted marrow interface cells were treated with anti-Qa-m2 serum (1:100 dilution) for 30 min on ice, washed three times and developed for indirect immunofluorescence by incubating with fluorosceinated F(ab^1)$_2$ goat anti-mouse IgG (Cappel Laboratories) (1:20 dilution) for 30 min on ice. Cells were washed and suspended in phosphate-buffered saline containing 1% bovine serum albumin and 0.02% sodium azide before analysis and sorting on an Ortho Cytofluorograph System 50L cell sorter. Unsorted and sorted fractions were assayed for their content of day-8 and day-14 CFU-S in lethally irradiated syngeneic recipients (Table 1). Cells were sorted on the basis of fluorescence only in large rectangular windows (region 1) in *a* and *b* (that is, gating out only dead cells which fluoresce non-specifically) or by three-parameter sorting by selecting fluorescent cells in a region (region 2 in *b*) enriched for CFU-S in 5FU8BM by light scatter (forward and 90°) characteristics, based on preliminary experiments (unpublished). Background fluorescence (that is <0.5% of cells) in each region was set using an aliquot of lymphocyte-depleted marrow cells (NBM and 5FU8BM) treated with anti-Ly-2.1 serum (negative control, 1:100 dilution) but otherwise treated as above. Anti-Qa-m2-treated cells with fluorescence intensity greater than background in each region were sorted as Qa-m2$^+$ and the remainder of cells in each region sorted as Qa-m2$^-$.

treatment significantly reduced the ability of bone marrow cells to reconstitute haematopoiesis, as measured by platelet counts, marrow cellularity and the femoral content of MEG-CFC and GM-CFC in recipients 14 days after injection of cells. Since anti-Qa-m2 with complement has no significant direct cytotoxic effect on GM-CFC or E-CFC (ref. 5), the reduction in marrow reconstituting ability probably reflects complement-mediated lysis of more primitive multipotential cells (such as day 14 CFU-S or 'pre-CFU-S'[7]) which generate these committed progenitor cells. The smaller reduction in repopulation indices (~50%) compared with reduction of day 14 CFU-S (~80%, Table 1) produced by anti-Qa-m2 and complement probably reflects incomplete regeneration from residual undamaged primitive precursors over the 14-day assay period.

As most C57BL/6 bone marrow cells are Qa-m2$^-$ (refs 4, 5) the anti-Qa-m2 antibody was used to separate subpopulations of CFU-S and to purify day 14 CFU-S from bone marrow by indirect immunofluorescence using a cell sorter (Fig. 1, Table 3). All T-lymphocytes and ~25% of B-lymphocytes in C57BL/6 mice are Qa-m2$^+$ (ref. 4). Therefore normal bone marrow was first depleted of lymphocytes (plus erythrocytes, dead cells and some neutrophils) using a rosetting procedure (ref. 5 and Fig. 1). The lymphocyte-depleted bone marrow cells contained >80% of day 8 and day 14 CFU-S (Table 3). The cells in this fraction were treated with anti-Qa-m2 and stained by indirect immunofluorescence. Viable cells were sorted into Qa-m2$^+$ and Qa-m2$^-$ fractions using lymphocyte-depleted marrow cells treated with a negative control antibody to set background fluorescence (Fig. 1). The Qa-m2$^+$ fraction (~3% of unfractionated marrow cells) contained 65% of sorted day 14 CFU-S (representing 15-fold enrichment compared with unfractionated marrow) but only 6% of sorted day 8 CFU-S (1.6-fold enriched) most of which were Qa-m2$^-$ (Table 3).

To further purify day 14 CFU-S, we used bone marrow obtained from mice treated 8 days previously with 5-fluorouracil (a cycle-active drug which preferentially kills proliferating cells) and endotoxin (which enhances marrow recovery[8]) (Fig. 1). This regenerating marrow (5FU8BM) contains supranormal numbers of CFU-S (ref. 8) and is enriched for these cells (Table 3). As found with normal marrow, >80% of day-8 and day-14 CFU-S were recovered in the lymphocyte-depleted 5FU8BM fraction. Single parameter (Qa-m2$^+$ fluorescence) sorting of viable lymphocyte-depleted cells resulted in 15-fold enrichment for day-14 CFU-S. However the fluorescent Qa-m2$^+$ fraction contained only 39% of sorted day-14 CFU-S (and <1% of sorted day-8 CFU-S) (Table 3). The reason for the smaller proportion of day-14 CFU-S in the Qa-m2$^+$ cell fraction from regenerating marrow compared with normal marrow is not known. It is possible that Qa-m2 expression by these cells may be related to their cell-cycle status. We are currently investigating this possibility. Based on prior studies of the light-scattering characteristics of CFU-S in 5FU8BM (unpublished observations), fluorescent (Qa-m2$^+$) cells were selected in a region gated according to forward and perpendicular light scatter (Region 2, Fig. 1*b*). This fraction was further enriched for day-14 CFU-S (to ~30-fold) but not for day-8 CFU-S, and contained 73% of the Qa-m2$^+$ day-14 CFU-S (24% of initial) (Table 3). The final recovery of day 14 CFU-S in this fraction was ~15–20% of the initial day 14 CFU-S in unfractionated 5FU8BM after all fractionation procedures (after taking into account losses of cells during sorting, washing and so on).

The 24-h spleen-seeding efficiencies (f) of day-8 and day-14 CFU-S in 5FU8BM, determined as described[3,9,10], were 0.03 and 0.035, respectively. The corresponding values for 2-h f were 0.09 and 0.106. If, as seems likely[11], f is the same for enriched populations of CFU-S as for those in unfractionated marrow

Table 2 Effect of anti-Qa-m2 and complement (C) on capacity of bone marrow cells to reconstitute haematopoiesis in lethally-irradiated mice

Treatment of cells	Day-14 post-825 rad: Platelet count ($\times 10^9$/l)	Viable nucleated cells per femur ($\times 10^{-6}$)	GM-CFC per femur ($\times 10^{-3}$)	MEG-CFC per femur ($\times 10^{-2}$)
Anti-Ly-2.1+C (1×10^6 cells per mouse)	335±27 (10)	14.1±2.5 (10)	8.7±2.1 (10)	6.0±1.2 (10)
Anti-Qa-m2+C (1×10^6 cells per mouse)	162±15 (10)	7.3±1.3 (10)	3.8±1.0 (10)	3.1±0.8 (10)
Radiation control (no cells)	40±3 (5)	1.2±0.3 (5)	0 (5)	0 (5)

C57BL/6 bone marrow cells were treated with anti-Qa-m2+C or anti-Ly-2.1 (negative control antibody)+C as described (Table 1) and injected into irradiated (825R) syngeneic hosts (1×10^6 cells per mouse). Fourteen days later, mice injected with cells and radiation controls receiving no cells were bled from the retroorbital plexus, then killed by cervical dislocation. Cells were obtained from the femurs of individual mice, counted, and assayed for megakaryocyte colony-forming cells (MEG-CFC) and granulocyte-macrophage colony-forming cells (GM-CFC) as described[5]. Femoral CFC content was calculated from the mean of four replicate culture plates per individual mouse cell suspension. Platelets were counted by phase microscopy. Results (mean ± 1s.e.m.) of a single experiment are shown. The numbers in parentheses indicate number of mice. Similar results were obtained in two additional experiments.

Table 3 Separation of CFU-S populations and enrichment for day-14 CFU-S using monoclonal anti-Qa-m2 and cell sorting

Cell fraction	Normal bone marrow: Initial viable cells %	CFU-S per 1×10^5 cells: Day 8	Day 14	5FU8 bone marrow: Initial viable cells %	CFU-S per 1×10^5 cells: Day 8	Day 14
Unfractionated unsorted marrow	100	17.4	18.5	100	97	110
Lymphocyte-depleted marrow	57	27.2	27.9	45	179	206
Qa-m2$^-$ viable cells (region 1, Fig. 1*a* and *b*)	53.7	25.9	9.8	42.8	172	111
Qa-m2$^+$ viable cells (region 1, Fig. 1*a* and *b*)	3.3	28.5	285	2.2	32	1,660
Qa-m2$^+$ cells selected by scatter characteristics for CFU-S (region 2, Fig. 1*b*)	ND	ND	ND	0.8	75	3,250

Bone marrow cells (NBM or 5FU8BM) were depleted of lymphocytes, treated with anti-Qa-m2, developed for indirect immunofluorescence, and sorted on the basis of fluorescence only (region 1) or by three parameter sorting (that is, forward and perpendicular light scatter characteristics and fluorescence (region 2)) as described (Fig. 1). The relative proportions of CFU-S in the various fractions (per cent of initial unfractionated unsorted marrow) were determined by multiplying the number of CFU-S per 1×10^5 cells by the proportion of viable cells in that fraction and dividing by the frequency of CFU-S in unsorted unfractionated marrow (for results see text). Results are typical of 3–6 similar experiments. ND, not determined.

and the 2-h *f* values are used, then at least 30% of cells in the most highly enriched fraction of 5FU8BM are day-14 CFU-S and ~0.8% are day-8 CFU-S. However, if the more generally accepted[9,10] 24-h values for *f* are used, ~90% of these cells are day-14 CFU-S (and ~2% are day-8 CFU-S). More than 98% of the cells in this fraction were undifferentiated mononuclear cells with moderately high nucleus-cytoplasm ratio, immature chromatin pattern, several nucleoli and agranular basophilic cytoplasm. Culture of these cells in agar[5] showed that ~5% were MEG-CFC, ~1–2% were GM-CFC and 2–3% were mixed E-CFC. Thus, highly purified multipotential stem cells (day-14 CFU-S), separated from the bulk of day-8 CFU-S, committed progenitor cells and other marrow cells, were obtained with reasonable yield from 5FU8BM.

Our results clearly demonstrate an antigenic difference between functionally distinct subpopulations of CFU-S, and strongly suggest that Qa-m2 is, or is part of, a differentiation antigen for multipotential haematopoietic stem cells. An intriguing but as yet untested possibility is that the Qa-m2 determinant may play a functional role in haematopoietic differentiation. Previous studies[5] and studies in progress (unpublished observations) indicate that differences in Qa-m2 expression can be used to separate MEG-GFC and mixed erythroid-CFC (Qa-m2$^+$) from GM-CFC and 7-day E-CFC (Qa-m2$^-$) suggesting that the former two types of CFC are more closely related to the multipotential stem cell than the latter committed progenitor cells. Thus the anti-Qa-m2 antibody should be a valuable tool for investigating the earliest steps in haematopoietic differentiation, including the order of restriction of potentialities in stem cell progeny[12]. Highly purified multipotential stem cells, obtained as described here, will be useful in investigation of the process of commitment to a line of differentiation, and in exploring the relationship between CFU-S and other primitive haematopoietic cells[7,13,14]. However, as day-14 CFU-S in 5FU8BM may have properties different from those in unstimulated normal bone marrow, we are currently attempting to further purify these cells from normal marrow using density gradients[15] and depletion of mature marrow cells with monoclonal antibodies[16] before cell sorting.

We thank Dr G. S. Hodgson for helpful advice. This work was supported by the National Health and Medical Research Council of Australia.

Received 25 July; accepted 18 November 1983.

1. Till, J. E. & McCulloch, E. A. *Radiat. Res.* **14,** 213–222 (1961).
2. Magli, M. C., Iscove, N. N. & Odartchenko, N. *Nature* **295,** 527–529 (1982).
3. Siminovitch, L., McCulloch, E. A. & Till, J. E. *J. cell comp. Physiol.* **62,** 327–336 (1963).
4. Hogarth, P. M., Crewther, P. E. & McKenzie, I. F. C. *Eur. J. Immun.* **12,** 374–379 (1982).
5. Harris, R. A., Hogarth, P. M., McKenzie, I. F. C. & Penington, D. G. *Expl Hemat.* **11,** 527–541 (1983).
6. Hogarth, P. M., Edwards, J., McKenzie, I. F. C., Goding, J. W. & Liew, F. Y. *Immunology* **46,** 135–144 (1982).
7. Hodgson, G. S. & Bradley, T. R. *Nature* **281,** 381–382 (1979).
8. Hodgson, G. S. & Bradley, T. R. *Cancer Treat. Rep.* **63,** 1761–1769 (1979).

9. Lahiri, S. K., Keizer, H. J. & van Putten, L. M. *Cell Tissue Kinet.* **3**, 355–362 (1970).
10. Monette, F. C. & de Mello, J. B. *Cell Tissue Kinet.* **12**, 161–175 (1979).
11. Visser, J. W. M. & Eliason, J. F. *Cell Tissue Kinet.* **16**, 385–392 (1983).
12. Nicola, N. A. & Johnson, G. R. *Blood* **60**, 1019–1029 (1982).
13. Nakahata, T. & Ogawa, M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3843–3847 (1982).
14. Boggs, D. R., Boggs, S. S., Saxe, D. F., Gress, L. A. & Canfield, D. R. *J. clin. Invest.* **70**, 242–253 (1982).
15. Visser, J. W. M. & Bol, S. J. L. *Stem Cells* **1**, 240–249 (1981).
16. Hoang, T. *et al. Blood* **61**, 580–588 (1983).

Glial heterogeneity may define the three-dimensional shape of mouse mesencephalic dopaminergic neurones

S. Denis-Donini*, J. Glowinski & A. Prochiantz

Groupe NB, INSERM U.114, Collège de France, 11 place Marcelin Berthelot, 75231 Paris Cedex 05, France

The shape of a neurone—the projection and branching pattern of axons and dendrites—appears to be determined by a combination of intrinisic and environmental influences[1–5]. We have previously shown that striatal target neurones influence the biochemical maturation of ascending mesencephalic dopamine (DA) cells in culture[6,7], as well as the elongation rate of DA neurites[8]. Using a similar approach in which the morphology of individual DA cells can be studied after ^{3}H-DA uptake and autoradiography, we now report on *in vitro* neurone-glia interactions and show that glial cells exert a morphogenetic effect on DA neurones. Dopaminergic neurones from the mesencephalon were plated on glial monolayers prepared either from the striatal or the mesencephalic region of the embryonic brain. On mesencephalic glial cells the majority of DA neurones develop a great number of highly branched and varicose neurites, whereas on striatal glia they only exhibit one long, thin and rather linear neurite. These results demonstrate that glial cells from two different brain regions have distinct properties which could be used to define neuronal polarity observed *in vivo*.

Cultures of glial cells from mouse embryos were generated from the mesencephalon (ED 13) and the striatum (ED 15) and grown for 3 weeks as described in detail in Fig. 1 legend. In such cultures, most neurones have died and the non-neuronal cells form a confluent monolayer (Fig. 3). Indeed autoradiography after ^{3}H-DA uptake or immunocytochemistry after incubation with an antibody against the 70 K subunit of the neurofilament triplet did not reveal the presence of surviving neurones. Mesencephalic cells (ED 13) containing a subpopulation of DA neurones were plated on these preformed monolayers and their morphology was examined after 1, 2, 3 and 10 days in culture following ^{3}H-DA uptake and autoradiography. Already after 1–2 days in culture DA neurones grown on glial cells from these two brain areas exhibit strikingly different morphologies. On a glial monolayer generated from the striatum, most cells are either ovoid or multipolar as described *in vivo* and *in vitro*[9–11], they usually extend one long linear process at one pole and one or a few shorter ones at the opposite pole or around the cell soma. This type of morphology is illustrated in Fig. 1*a* in which four cells have been photographed. However, when grown on a glial monolayer originating from the mesencephalon they have a great number of highly branched smooth or varicose processes (Fig. 1*b*). Figure 2 shows the differences in the morphology of DA neurones observed in both culture conditions. The number of neurites on glial mesencephalic monolayers as well as their thick varicose aspect has led us to call them 'highly branched' processes. In contrast, the thin and almost unique neurite that elongates on striatal glial cells will be called 'poorly branched'. Similar results were obtained when DA neurones were identified by immunocytochemistry using an antibody against tyrosine hydroxylase.

As already pointed out this dramatic difference between the morphologies of DA cells is clearly visible as early as one day after the beginning of the culture. Table 1 shows the number of neurones displaying one or the other configuration after various times in culture. Since in both culture conditions a small constant number (15%) of DA cells does not extend any neurite, we have only included neurites displaying cells in this table. The data show that the number of labelled DA neurones is identical after 2 days in culture on both monolayers. It slightly increases after 3 days probably because some neurones had not yet acquired the capacity to take up the neurotransmitter after 2 days *in vitro*. After 10 days, the number of surviving neurones is lower on both glial monolayers. The percentage of DA neurones presenting a 'highly branched' arborization remains constant on mesencephalic glial monolayers (60, 74 and 54% at 2, 3 and 10 days respectively). This is not the case on striatal glial monolayers, since, although 100% of the neurones develop one long neurite after 2 and 3 days *in vitro*, after 10 days this percentage falls to 34%, and 66% exhibit 'highly branched'

* Permanent address: Laboratory of Molecular Embryology, Via Toiano 2, 80072 Arco Felice, Naples, Italy.

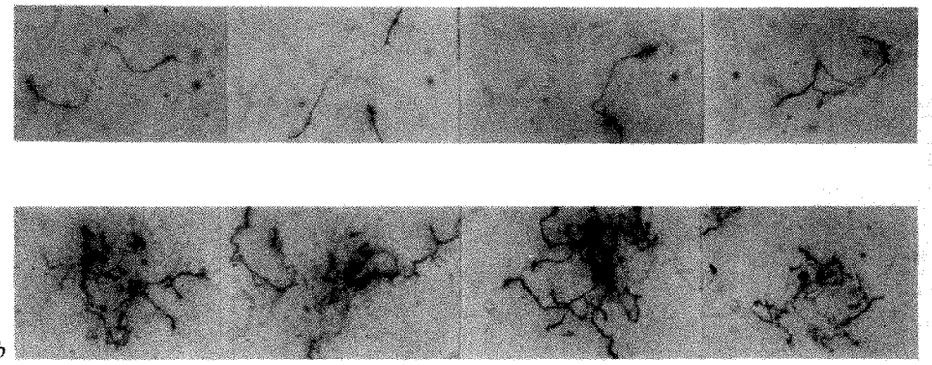


Fig. 1 *a*, Visualization of four dopaminergic neurones after 2 days *in vitro* on a monolayer of striatal glial cells (×46). *b*, Visualization of four dopaminergic neurones after 2 days *in vitro* on a monolayer of mesencephalic glial cells (×46).
Methods: Cells from the mesencephalon and the striatum of 13- and 15-day-old Swiss mouse embryos were prepared as already described[6–7], and plated at the concentration of 10^6 cells per dish in Falcon dishes coated with polyornithine (Sigma, 40,000 MW, 1.5 μg ml^{-1}). Glial monolayers were obtained by culturing the cells for 3 weeks in nutrient mixture F12, minimum essential medium (Gibco, 1:1) complemented with 10%-inactivated fetal calf serum (Serumaid), 3.3×10^{-2} M glucose, 2×10^{-3} M glutamine and 0.3×10^{-2} M sodium bicarbonate. The culture medium was not changed for 1 week and then changed 3 times a week. Freshly dissociated rostral mesencephalic 13-day-old embryonic cells (5×10^5 to 10^6) were seeded on these monolayers and the culture was pursued for 2 more days. Dopaminergic neurones were examined by autoradiography. Cells were incubated at 37 °C for 1 h with 5×10^{-7} M ^{3}H-DA (6 Ci mmol^{-1}, NEN) in 1-ml phosphate buffer supplemented with 10^{-3} M magnesium, 10^{-3} M calcium and 3.3×10^{-2} M glucose (PBS). After three washes with cold PBS, the cells were fixed for 1 h at room temperature with 1.5% glutaraldehyde (Taab Laboratories) in PBS, washed four times with PBS, air dried and covered with Kodak NTB2 emulsion. Autoradiograms were developed 2 weeks later using Kodak Dektol. No labelling was seen when the cells were incubated in the presence of 5×10^{-6} M benztropine (Merck), a potent inhibitor of ^{3}H-DA uptake into dopaminergic neurones.

0028-0836/84/070641—03$01.00
© 1984 Macmillan Journals Ltd

processes. The *in vitro* development of mesencephalic glial cells seeded together with the neurones could provide an explanation for this observation.

Since glial monolayers are important in neuronal morphogenesis, we analysed their cellular composition by immunocytochemistry using an antibody against the glial fibrillary acidic protein (GFAP), a specific marker for astrocytes. The morphology of mesencephalic and striatal non-neuronal cultures is not very different (Figs 3*a* and *b*): the cells are not equally stained with the antibody, a fact that demonstrates some heterogeneity in the level of expression of the glial filament. The weak fluorescence of some cells cannot be attributed to cross-reactions with vimentin. This was checked by the absence of GFAP labelling of several virally transformed cell lines known to synthesize high amounts of vimentin[12] as well as by immunoblotting experiments (not shown). We have shown that 8-day old mesencephalic primary cultures contain a large proportion of vimentin-positive cells which cannot be labelled with GFAP antisera in double-labelling experiments nor with anti-fibronectin or anti-galacto cerebrosides antibodies[13]. Although we do not exclude the presence of other cell types such as immature oligodendrocytes, microglia or epithelial cells we agree with several authors[14,15] that the non-neuronal monolayers are mainly composed of astroblasts at various maturation stages and astrocytes.

Glial cells, peripheral and central, play important roles in promoting neuronal survival[16,17], neurite outgrowth[18,19], differentiation[20,21] and neurotransmitter choice[22,23]. Our study demonstrates that glial cells from two different embryonic brain areas have distinct and specific morphogenetic effects on the polarity and arborization of mesencephalic ascending DA neurones.

The morphological differences between DA cells plated on mesencephalic or striatal glial substratum could reflect different

Table 1 Morphology of dopaminergic neurones

	Arborization of dopaminergic neurones in: Mesencephalic glial monolayer		Striatal glial monolayer	
Days *in vitro*	Poorly branched	Highly branched	Poorly branched	Highly branched
2	458 (40%)	705 (60%)	1,103 (100%)	—
3	340 (36%)	1,008 (74%)	1,790 (100%)	—
10	210 (46%)	251 (54%)	327 (34%)	548 (66%)

Number of dopaminergic neurones exhibiting different morphologies when grown on the two different types of glial cell monolayers. This experiment has been repeated three times and about the same percentages of the two morphologies have been found in all experiments at two concentrations of freshly dissociated mesencephalic cells (5×10^5 and 10^6).

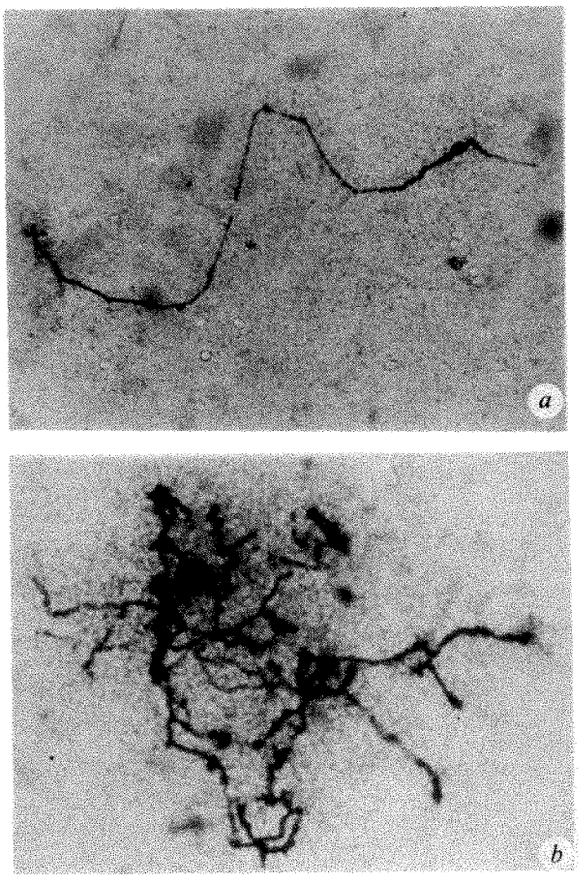


Fig. 2 Higher magnification of two neurones presented in Fig. 1. *a*, Dopaminergic neurones after 2 days *in vitro* on a monolayer of striatal glial cells (×300). *b*, Dopaminergic neurone after 2 days *in vitro* on a monolayer of mesencephalic glial cells (×300).

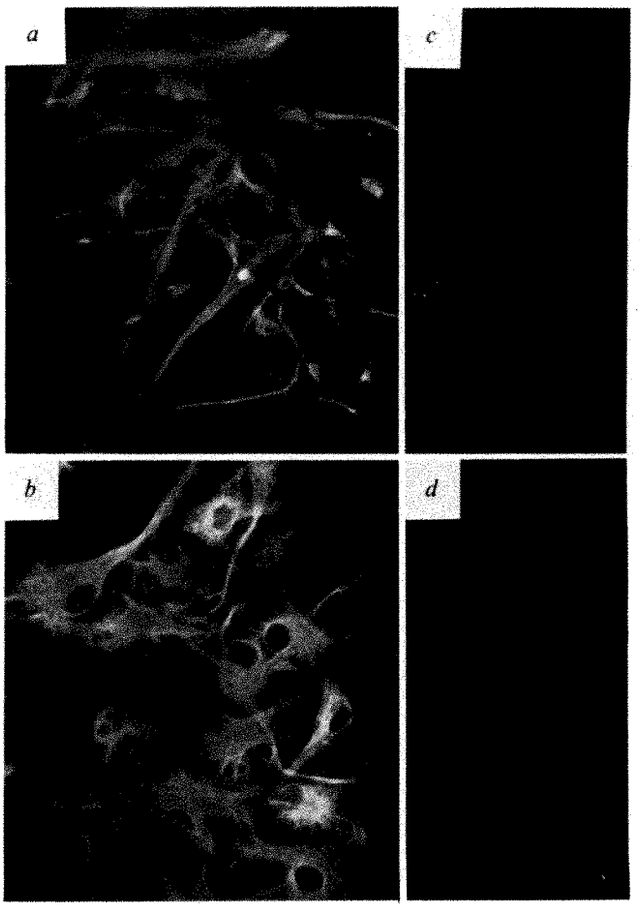


Fig. 3 Mesencephalic (*a*, *c*) and striatal (*b*, *d*) non-neuronal monolayers were prepared as described in Fig. 1. After 3 weeks in culture they were rinsed with PBS and permeabilized with a mixture of 3.7% formaldehyde and 1% methanol (Merck) for 1 h at room temperature. After several washes with PBS the cells were incubated 1 h at 37 °C with (*a*, *b*) or without (*b*, *d*) an antibody against glial fibrillary acidic protein (GFAP), raised in the rabbit. The second incubation was performed at 37 °C for 30 min with a solution of fluorescein-conjugated goat anti-rabbit immunoglobulins (1:50, Nordic). All incubations were in PBS containing 10% fetal calf serum. The cultures were mounted in PBS, glycerol (1:1) before examination under a Leitz HM-Lux microscope using a HBO 50-W mercury lamp. Magnification (×250).

developmental stages achieved by the neurones. This would mean that mesencephalic glial cells synthesize molecules that stimulate DA neurone development. Alternatively, the different morphologies of DA cells on both glial monolayers could arise by the specific selection by these monolayers of subpopulations of DA neurones. This seems not to be the case: the same number of DA cells is observed after 2 days *in vitro* on both substrata and since neurones growing a 'poorly branched' neurite can slowly develop a 'highly branched' arborization when mesencephalic glial cells have multiplied on the striatal monolayer. A third more interesting explanation comes from the observation that the morphological pattern obtained *in vitro* strikingly coincides with the geometry of the mesostriatal DA neurones. Indeed, during development, DA nigral neurones send their axons rostrally toward the striatum whereas the dendritic arborizations develop caudally in the nigral pars reticulata. It could be that on striatal as well as on a subpopulation of mesencephalic glia (40%), the unique neurite would represent an axon while on another subpopulation of mesencephalic non-neuronal cells (60%) DA neurones would be able to extend dendritic branches, with or without axon. Hemmendinger *et al.*[24] have already suggested that the formation of 'axon-like' processes depends on the presence of striatal or cortical target cells, whereas areas of extensive 'dendritic-like' arborizations are seen when DA neurones are in aggregates of mesencephalic cells only.

Since both neuronal morphologies can coexist in the same culture, either on mesencephalic glial cells or on striatal glial cells after 10 days when mesencephalic glia has been able to develop, diffusible molecules acting at long distances cannot be responsible for the observed phenomenon; close interactions between the neurite and its glial substratum are necessary.

In an earlier study we showed that striatal target cells are able to regulate *in vitro* the growth of DA neurones. In fact, DA processes transiently stop on these striatal target cells, while they ignore non-target mesencephalic or cerebellar cells[8]. It is, therefore, possible that direct contact is involved in stimulation and direction of axonal growth by striatal glial cells and recognition by axonal terminals of striatal target neurones. On the other hand the development of dendritic arborization could depend strictly on the signals provided by local mesencephalic glia.

We would like to thank M.-C. Daguet and A. Herbet for their critical and stimulating help. S.D.-D. was a recipient of a long-term EMBO fellowship. This work was supported by a grant from MRI no. 82 E 0125.

Received 31 March; accepted 5 December 1983.

1. Solomon, F. *Cell* **16**, 165–170 (1979).
2. Bray, D. *J. Cell Biol.* **56**, 702–712 (1973).
3. Benfey, M. & Aguayo, A. J. *Nature* **216**, 150–152 (1982).
4. Berry, M., McConnel, P. & Sievers, J. *Curr. Topics devl Biol.* **15**, 67–101 (1980).
5. Shankland, P., Bently, D. & Goodman, C. S. *Devl Biol.* **92**, 507–520 (1982).
6. Prochiantz, A., di Porzio, U., Kato, A., Berger, B. & Glowinski, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5387–5391 (1979).
7. di Porzio, U., Daguet, M.-C., Glowinski, J. & Prochiantz, A. *Nature* **288**, 370–373 (1980).
8. Denis-Donini, S., Glowinski, J. & Prochiantz, A. *J. Neuroscience* **3**, 2292–2299 (1983).
9. Bjorklund, A. & Lindvall, O. *Brain Res.* **83**, 531–537 (1975).
10. Fallon, J. H., Riley, J. N. & Moore, R. Y. *Neurosci. Letters* **7**, 157–162 (1978).
11. Berger, B. *et al. Neuroscience* **7**, 193–205 (1982).
12. Lazarides, E. *Nature* **283**, 249–256 (1980).
13. Prochiantz, A., Delacourte, A., Daguet, M.-C. & Paulin, D. *Expl Cell Res.* **139**, 404–410 (1982).
14. Sensenbrenner, M., Devilliers, G., Bock, E. & Porte, A. *Differentiation* **17**, 51–61 (1980).
15. Lindsay, R. M., Barber, P. C., Sherwood, M. R. C., Zimmer, J. & Raisman, G. *Brain Res.* **243**, 329–343 (1982).
16. Barde, Y. A., Edgar, D. & Thoenen, H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1199–1203, (1980).
17. Lindsay, R. M. *Nature* **282**, 80–82 (1979).
18. Adler, R. & Varon, S. *Devl Biol.* **86**, 69–80 (1981).
19. David, S. & Aguayo, A. J. *Science* **214**, 931–933 (1981).
20. Banker, G. A. *Science* **209**, 809–810 (1980).
21. Touzet, N. & Sensenbrenner, M. *Devl Neurosci.* **1**, 159–163 (1978).
22. Patterson, P. H. & Chun, L. L. Y. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3607–3610 (1974).
23. Patterson, P. H. & Chun, L. L. Y. *Devl Biol.* **56**, 263–280 (1977).
24. Hemmendinger, L. M., Garber, B. B., Hoffman, P. C. & Heller, A. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1264–1268 (1981).

A dynorphinergic pathway of Leu-enkephalin production in rat substantia nigra

Nadav Zamir, Miklós Palkovits, Eckard Weber*, Éva Mezey & Michael J. Brownstein

Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, Maryland 20205, USA
* Nancy Pritzker Laboratory of Behavioral Neurochemistry, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California 943305, USA

The amino acid sequence of the opioid peptide Leu-enkephalin[1–3] is found within several larger peptides[4–8], which are generated from the precursors proenkephalin and prodynorphin. Proenkephalin contains four copies of the sequence of Met-enkephalin, a single copy of the sequence of Leu-enkephalin and one copy each of two extended Met-enkephalin sequences[9–11]. Proenkephalin contains three peptides—α-*neo*-endorphin, dynorphin A and dynorphin B[12]—the N-terminal sequences of which are identical to that of Leu-enkephalin. There is good evidence that the large amounts of Leu-enkephalin found in the adrenal medulla are generated from the precursor proenkephalin[2,4,13,14], but as yet prodynorphin has not been shown to be processed to yield Leu-enkephalin. We show here that the relatively high levels of Leu-enkephalin found in the rat substantia nigra are supplied by striatonigral axons and generated from the precursor prodynorphin.

During our studies of the regional distributions of prodynorphin-related peptides, we observed that the concentrations of α- and β-*neo*-endorphin were considerably higher than the concentrations of dynorphin A (plus dynorphin A(1–8)) or dynorphin B[15,16]. This was especially striking in the substantia nigra and suggested to us that dynorphins A and/or B might be converted into smaller peptide species, including Leu-enkephalin. The fact that the level of Leu-enkephalin in the substantia nigra is more than twice as high as the level of Met-enkephalin-Arg-Gly-Leu, a peptide derived exclusively from proenkephalin (Table 1), also suggested that the Leu-enkephalin in this structure may not be derived exclusively from the proenkephalin precursor. Leu-enkephalin and Met-enkephalin-Arg-Gly-Leu are present in equimolar amounts in proenkephalin, and one may therefore expect their concentrations to be similar.

To determine the source of the Leu-enkephalin in the substantia nigra, we made unilateral knife cuts just rostral to the mesencephalon that transected descending fibres from the forebrain (Fig. 1, cut 2). These cuts resulted in a marked depletion of nigral α-*neo*-endorphin, dynorphin B and Leu-enkephalin, but did not affect levels of Met-enkephalin-Arg-Gly-Leu (Table 1). Thus, Met-enkephalin-Arg-Gly-Leu is probably not contained in descending inputs to substantia nigra. The dynorphin-related peptides and leu-enkephalin, on the other hand, must be in axons of neurones rostral to the substantia nigra.

In a second set of animals we made knife cuts that separate the globus pallidus from the caudate-putamen (Fig. 1, cut 1). These knife cuts also caused a substantial decrease in α-*neo*-endorphin, dynorphin B and Leu-enkephalin in the ipsilateral substantia nigra without altering the level of Met-enkephalin-Arg-Gly-Leu (Table 1). The same cuts resulted in decreases in

Table 1 Effects of various lesions on peptides in brain regions

Peptide	Treatment		Substantia nigra	Globus pallidus	Caudate
α-*neo*-endorphin	Rostral nigral	Intact	6,496.8 ± 652.5 (8)	962.3 ± 167.3 (8)	352.6 ± 48.6 (8)
	Deaf.	Lesioned	942.6 ± 291.6*(8)	951.1 ± 97.8 (8)	731.5 ± 113.9*(8)
			−85%		+107%
	GP	Intact	7,195.5 ± 953.5 (8)	981.2 ± 91.9 (8)	379.1 ± 47.1 (8)
	Deaf.	Lesioned	1,053.3 ± 353.0*(8)	324.9 ± 78.3*(8)	786.3 ± 125.7†(8)
			−85%	−67%	+107%
Dynorphin-B	Rostral nigral	Intact	2,019.8 ± 375.8 (8)	248.5 ± 54.0 (8)	173.8 ± 24.4 (5)
	Deaf.	Lesioned	300.4 ± 87.5*(8)	289.7 ± 65.4 (8)	339.8 ± 57.6‡(5)
	GP	Intact	2,133.7 ± 397.6 (8)		+95%
	Deaf.	Lesioned	291.9 ± 89.5*(8)	Not determined	
			−86%		
Leu-enkephalin	Rostral nigral	Intact	955.3 ± 99.8 (7)	3,525.6 ± 983.6 (7)	551.0 ± 70.2 (7)
	Deaf.	Lesioned	247.4 ± 62.7*(7)	4,744.0 ± 998.0 (7)	628.9 ± 66.4 (7)
			−74%		
	GP	Intact	784.6 ± 87.6 (7)	3,810.2 ± 746.4 (8)	420.4 ± 56.5 (8)
	Deaf.	Lesioned	211.1 ± 52.9*(8)	471.5 ± 66.8*(8)	793.9 ± 81.7†(8)
			−73%	−88%	+89%
Met-enkephalin-Arg-Gly-Leu	Rostral nigral	Intact	415.3 ± 82.9 (7)	5,338.6 ± 874.6 (7)	1,450.3 ± 291.8 (8)
	Deaf.	Lesioned	341.7 ± 74.1 (8)	5,836.5 ± 1,006.8 (8)	1,830.7 ± 350.3 (8)
	GP	Intact	334.9 ± 40.7 (8)	5,985.2 ± 755.4 (8)	1,001.1 ± 111.0 (8)
	Deaf.	Lesioned	385.1 ± 44.2 (8)	801.8 ± 144.3*(8)	2,039.9 ± 210.6*(8)
				−87%	+104%

Brain areas investigated were substantia nigra, globus pallidus and caudate nucleus. Values are expressed as fmol peptide per mg protein. Data are means ±s.e.m. Lesioned side is compared with intact side by paired *t*-test: * $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$. When a percentage change is indicated the lesioned side and control sides differed significantly. Numbers in parentheses indicate the number of separate animals studied. GP, globus pallidus; Deaf., deafferentation.

Methods: Male Sprague–Dawley rats (200–250 g) were killed by decapitation (10 days after operation) and their brains removed. Tissue samples were microdissected from frozen 300-μm sections of the brains[22] after inspection of the lesions. Individual tissue samples were taken from the lesioned and control sides of the same section. Tissue samples were placed in Eppendorf tubes containing 200 μl of 0.1 M HCl and homogenized by sonication. 20-μl aliquots of the homogenates were taken for protein determination[23]. The homogenates were centrifuged at 2,000*g* for 10 min at 4 °C, and the supernatants were transferred to 12 × 75 mm polysterene tubes and evaporated to dryness in a vacuum centrifuge. The radioimmunoassay (RIA) procedure was that described in ref. 17 with some modifications. Samples were rehydrated in phosphate-buffered saline (*p*H 7.6). Antisera were used at final dilutions of 1:60,000 for α-*neo*-endorphin, and Leu-enkephalin, 1:100,000 for dynorphin B and 1:75,000 for Met-enkephalin-Arg-Gly-Leu, which resulted in 30–45% noncompetitive binding of the trace. Each sample was incubated in a 500-μl volume containing 300 μl of sample in assay buffer, 100 μl of ^{125}I-labelled opioid peptide (~6,000 c.p.m.). The tubes were then incubated at 4 °C for 16–24 h. In RIAs for α-*neo*-endorphin and dynorphin B, separation of bound from free peptide was effected by addition of 1 ml of dextran-coated charcoal. A double-antibody RIA for Leu-enkephalin and Met-enkephalin-Arg-Gly-Leu was performed as described here. Normal rabbit serum (NRS) was also added to the first antibody solution to give a total NRS concentration of 1%. 100 μl of this solution were added to all except 'nonspecific binding' tubes which received 100 μl of 1% NRS in assay buffer. Reagents were mixed and the tubes incubated for 16–24 h. 100 μl of goat anti-rabbit γ-globulin diluted with assay buffer to a concentration sufficient for maximal precipitation were added to all tubes. Reagent was mixed and the tubes were incubated for 16–24 h. After incubation with the second antibody, all tubes were centrifuged for 20 min at 2,000*g* at 4 °C. The supernatant was aspirated and pellets were counted in a γ-counter. The RIA sensitivities were less than 4 pg per tube. The specificities of the antisera for dynorphin B[24], α-*neo*-endorphin[25], Leu-enkephalin[25] and Met-enkephalin-Arg-Gly-Leu[26] have been described elsewhere. The α-*neo*-endorphin antiserum does not recognize the enkephalins and dynorphins at 1 μM concentration. The dynorphin B antiserum does not cross-react with the enkephalins, dynorphin A, dynorphin A(1–8) or *neo*-endorphins at 1 μM concentration. The Leu-enkephalin antiserum cross-reacts with Met-enkephalin to 0.35% and shows <0.01% cross-reactivity with dynorphins or *neo*-endorphins at 1 μM concentration. The Met-enkephalin-Arg-Gly-Leu antiserum cross-reacts <0.005% with Met-enkephalin, Met-enkephalin-Arg, Met-enkephalin-Arg-Arg, Met-enkephalin-Arg-Phe, BAM 12P, dynorphin A(1–8), α-*neo*-endorphin and human β-endorphin at 1 μM concentration.

α-*neo*-endorphin, Leu-enkephalin and Met-enkephalin-Arg-Gly-Leu in the globus pallidus (Table 1). This finding confirms an earlier observation of the (pro)enkephalinergic striatopallidal pathway[17] and suggests that there are also dynorphinergic striatonigral and striato (or cortico)-pallidal pathways.

Dynorphin-containing cell bodies have been reported in the caudate[18]. Indeed, Vincent *et al.* and Palkovits *et al.* have described a striatonigral dynorphin system[19,20]. We sought to visualize such neurones after globus pallidus isolation (cut 2), reasoning that a dynorphin build-up in axons and perikarya (see Table 1) might facilitate immunocytochemical staining. In these brains we demonstrate dynorphin B immunoreactivity building up in axons on the proximal (caudate) side of the cut (Fig. 2*a*) and a dense network of stained nerve fibres and numerous cell bodies in the head of the caudate nucleus at some distance from the lesion (Fig. 2*b–d*). No cell bodies and very few immunoreactive fibres were observed on the intact side of the brain. There was a significant decrease in immunostaining in the substantia nigra on the side of the lesion (data not shown).

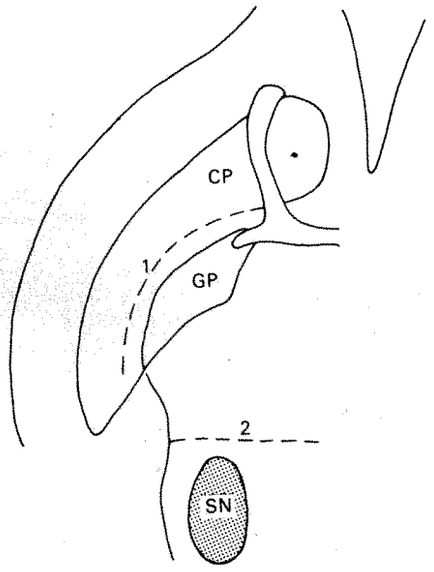


Fig. 1 Schematic representation of a horizontal section of the rat brain. CP, caudoputamen; GP, globus pallidus; SN, substantia nigra. 1, Unilateral knife cut isolating the caudate-putamen from globus pallidus. A Halasz knife[27] with a 2-mm radius and 3-mm height (the distance from the sleeve to the knife tip) was used to make this cut. The animal's head was fixed in a Kopf stereotaxic device (5°, nose down), and a rectangular flap of bone was removed from the skull. With the knife facing forward, the knife tip was placed 0.5 mm rostral to the bregma (~8.5 mm rostral to the interauricular line) and 2.5 mm lateral to the midline. The tip of the knife was lowered 7 mm beneath the surface of the dura and rotated 90° laterally. The knife was then moved caudally 2.5 mm, rostrally 2.5 mm and rotated medially 90° so that it was in its starting position in the brain. Finally, the knife was moved medially 2 mm, laterally 2 mm and withdrawn from the brain. 2, Unilateral mesencephalic cut transecting descending axons from caudate-putamen. This lesion was made with a 3-mm knife. The animal's head was fixed in a Kopf stereotaxic device (5°, nose down). The medial tip of the knife was placed 1 mm lateral to the midline 4.2 mm rostral to the interauricular line, and inserted ventrally until it touched the base of the brain. The animals were allowed to recover for 10 days, then they were decapitated and their brains were removed. Placement of all lesions was verified histologically.

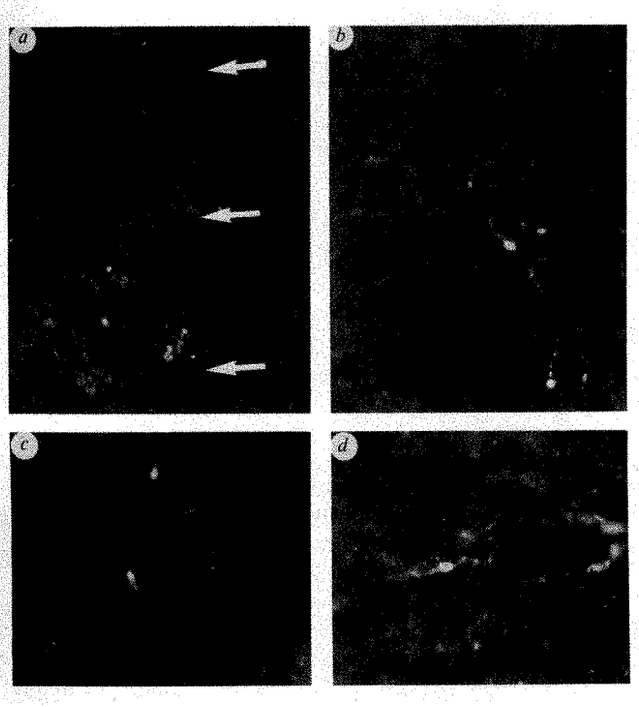


Fig. 2 *a*, Build-up of dynorphin B immunoreactivity in axons in the caudate nucleus proximal to a knife cut separating the globus pallidus from the striatum (arrow heads). ×75. *b–d*, Dynorphin B immunoreactive cell bodies in the head of the caudate nucleus. ×350.

Methods: Visualization of the dynorphin B immunoreactivity in brain tissue was carried out using the indirect fluorescent immunocytochemical technique[28]. The animals received an intraventricular injection of colchicine 24 h before they were anaesthetized with chloral hydrate and perfused with 4% phosphate-buffered paraformaldehyde solution. After a 90-min post-fixation the brains were washed in 0.1 M phosphate buffer overnight and then frozen on a liquid CO_2 stage. Cornal sections (10 μm thick) were cut in a microtome cryostat and mounted on gelatin-coated slides. The primary antibody, at a final dilution of 1:600, was incubated with the sections overnight at 4 °C. The sections were then incubated with rhodamine labelled secondary antibody (1:10) for 30 min at 37 °C. The slides were mounted in phosphate-buffered saline/glycerol and examined with a Zeiss microscope with epifluorescent illumination. There was no staining when the antibody was preabsorbed with an excess of dynorphin B.

Our data show that dynorphin-producing cells in the head of the caudate send fibres to the globus pallidus and substantia nigra. We cannot rule out the possibility that proenkephalin and prodynorphin coexist in the pallidal fibre population, but in the substantia nigra this does not seem to be the case, otherwise Met-enkephalin-Arg-Gly-Leu levels would decrease after striatonigral lesions, as do levels of *α-neo*-endorphin and dynorphin B. The most parsimonious explanation for the parallel decreases in *α-neo*-endorphin, dynorphin B and Leu-enkephalin is that in the striatonigral pathway Leu-enkephalin is made by dynorphinergic neurones.

Prodynorphin-derived peptides are potent κ-opiate receptor agonists[21] while Leu-enkephalin is a δ-opiate receptor agonist[21]. Thus, the same precursor (prodynorphin) may yield ligands for different opiate receptor subtypes that can exert different actions. The fact that Leu-enkephalin is derived from two separate precursors in brain may explain, in part, the different Leu-: Met-enkephalin ratios found in various brain regions.

Received 25 October; accepted 28 November 1983.

1. Hughes, J. *et al. Nature* **258,** 577–579 (1975).
2. Maysinger, D. *et al. Neuropeptides* **2,** 211–226 (1982).
3. North, R. A. & Egan, T. M. *Br. med. Bull.* **39,** 71–76 (1983).
4. Udenfriend, S. & Kilpatrick, D. L. *Archs Biochem. Biophys.* **221,** 309–323 (1983).
5. Kilpatrick, D. L., Wahlstrom, A., Lhm, H. W., Blacher, R. & Udenfriend, S. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6480–6483 (1982).
6. Kangawa, K., Minamino, N., Chino, N., Sakakibara, S. & Matsuo, H. *Biochem. biophys. Res. Commun.* **99,** 871–878 (1981).
7. Goldstein, A., Fischli, W., Lowney, L. I., Hunkapiller, M. & Hood, L. *Proc. natn. Acad. Sci. U.S.A.* **78,** 7219–7223 (1981).
8. Minamino, N., Kangawa, K., Chino, N., Sakakibara, S. & Matsuo, H. *Biochem. biophys. Res. Commun.* **99,** 864–870 (1981).
9. Noda, M. *et al. Nature* **295,** 202–208 (1982).
10. Gubler, U., Seeburg, P., Hoffman, B. J., Gage, L. P. & Udenfriend, S. *Nature* **295,** 206–208 (1982).
11. Comb, M., Seeburg, P. H., Adelman, J., Eiden, L. & Herbert, E. *Nature* **295,** 663–666 (1982).
12. Kakidani, H. *et al. Nature* **298,** 245–249 (1982).
13. Denis, D., Day, R. & Lemaire, S. *Int. J. Peptide Protein Res.* **19,** 18–24 (1982).
14. Hughes, J. *Br. med. Bull.* **39,** 17–24 (1983).
15. Zamir, N., Palkovits, M. & Brownstein, M. J. *Brain Res.* **280,** 81–93 (1983).
16. Zamir, N., Palkovits, M., Weber, E. & Brownstein, M. J. *Brain Res.* (in the press).
17. Cuello, A. C. & Paxinos, G. *Nature* **271,** 178–180 (1978).
18. Vincent, S. R., Hölkfelt, T., Christensson, I. & Terenius, L. *Neurosci. Lett.* **33,** 185–190 (1982).
19. Vincent, S. R., Hölkfelt, T., Christensson, I. & Terenius, L. *Eur. J. Pharmac.* **85,** 251–252 (1982).
20. Palkovits, M., Brownstein, M. J. & Zamir, N. *Neuropeptides* (in the press).
21. Paterson, S. J., Robson, L. E. & Kosterlitz, H. W. *Br. med. Bull.* **39,** 25–30 (1983).
22. Palkovits, M. *Brain Res.* **59,** 449–450 (1973).
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193,** 265–275 (1951).
24. Weber, E. & Barchas, J. D. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1125–1129 (1983).
25. Weber, E., Geis, R., Voigt, K. M. & Barchas, J. D. *Brain Res.* **260,** 166–171 (1983).
26. Weber, E., Roth, K. A., Evan, C. J., Chang, J-K. & Barchas, J. D. *Life Sci.* **31,** 1761–1764 (1982).
27. Halasz, B. & Pupp, L. *Endocrinology* **77,** 553–562 (1965).
28. Coons, A. H. in *General Cytochemical Methods* (ed. Danielli, J. F.) 399–422 (Academic, New York, 1958).

Dual role for *Dictyostelium* contact site B in phagocytosis and developmental size regulation

C. M. Chadwick, J. E. Ellison & D. R. Garrod

CRC Medical Oncology Unit, Centre Block, CF99,
Southampton General Hospital,
Southampton, Hants SO9 4XY, UK

Three cohesion molecules have been discovered in the slime mould *Dictyostelium discoideum*[1-4]. Two of these molecules are involved in morphogenesis at the aggregation stage of the life cycle and thereafter, and may also provide an essential trigger for late gene expression[5]. The third glycoprotein 126 (gp126) or contact site B, is present on axenically grown vegetative amoebae and persists to the aggregation stage where it is involved in the side-to-side cohesion of cells in aggregation streams[1,4]. It is puzzling that vegetative amoebae should possess a cohesion molecule because their solitary habit does not necessitate cohesion. However, they do need to adhere to the substratum and adhesion of bacteria to the cells is a prerequisite for phagocytosis. Vogel *et al.*[6] have proposed that the same receptor is involved in phagocytosis and cohesion. It has also been suggested that contact site B-mediated cohesion is a trigger for development[7]. Using a specific antibody against gp126, we now show that contact site B is a phagocytosis receptor. Furthermore, contact site B is involved in regulating the size of aggregates formed during morphogenesis; it also seems to be involved in cell–substratum adhesion but is not a developmental trigger.

A rabbit antibody (designated CMC13) was raised against gp126 purified to homogeneity by SDS-polyacrylamide gel electrophoresis. gp126 was the only cell surface protein to be immunoprecipitated by this antibody from vegetative cells of strain Ax2 (Fig. 1*a*). (Previously, gp126 was termed p126, but it has been shown to be glycosylated by periodic acid–dansyl hydrazine staining[8].)

The first question investigated using this antibody was whether gp126 is a phagocytosis receptor. A phagocytosis assay was carried out essentially according to Glynn[9] using ^{14}C-lysine-labelled *Escherichia coli* strain B/r. Univalent Fab′ fragments were prepared from both CMC13 and a control antibody, designated N. CMC13 Fab′ completely inhibits cell cohesion at a concentration of 0.7 mg ml^{-1}. Fab′ N provides a suitable control because it binds to the cell surface but does not block cohesion[4]. In the phagocytosis assay (Fig. 2) CMC13 blocked phagocytosis at 0.1 mg ml^{-1} whereas Fab′N had no effect on phagocytosis at eight times this concentration. We conclude that gp126 is the phagocytosis receptor as well as being the vegetative cohesion molecule. This conclusion offers an explanation for the anomaly that vegetative axenic *D. discoideum* cells are cohesive[10], whereas vegetative cells grown on bacteria are non-cohesive (ref. 11 and our unpublished observations). Thus, cells involved in phagocytosis could be depleted of cell surface receptor molecules because of receptor internalization whereas axenic cells would have a full complement of receptor molecules on their surfaces. To test this, we immunoprecipitated extracts of axenically grown Ax2 cells, bacterially grown Ax2 cells and NC-4 (wild type) cells. Figure 1*b* shows that gp126 is dramatically reduced in bacterially grown cells. This confirms the view that the cohesion receptor and the bacterial receptor are the same.

To determine whether gp126 is also involved in cell–substratum adhesion log phase axenic cells were suspended at 2×10^4 ml^{-1} in Bonner's solution containing 1 mg ml^{-1} of inhibitory Fab′. They were then allowed to settle onto a coverslip 30 min in the presence of Fab′, after which time the slide inverted and the percentage of cells remaining attached after further 30 min determined. Controls used Fab′ N and Bonner solution alone. The percentage of cells remaining attached the various treatments was: Bonner's solution, 94%; Fab′ CMC 13 (1 mg ml^{-1}), 13%; Fab′ N (1 mg ml^{-1}), 95%. Therefore inhibitory Fab′ greatly reduces adhesion of cells to a substratum.

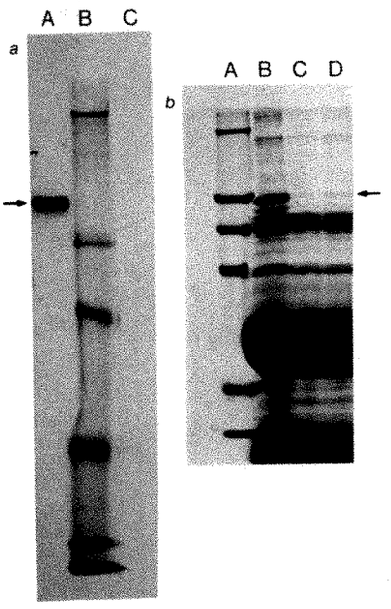


Fig. 1 *a*, Autoradiograph showing gp126 immunoprecipitate by CMC 13 IgG. Vegetative Ax2 cells were radioiodinated and detergent extract of the cells was immunoprecipitated with 150 μ CMC 13 IgG, using methods described elsewhere[4]. Lane A, ^{125} gp126 immunoprecipitated by CMC 13; B, ^{14}C-molecular weig (MW) markers (200,000, 92,000, 69,000, 46,000 and 30,000); pre-injection IgG. The same polypeptide (gp126) is the on Coomassie blue-stained band in immunoprecipitates of whole cel *b*, gp126 depleted in cells grown on *E. coli* B/r. Detergent extrac of 5×10^7 cells were immunoprecipitated by the anti-vegetati cell antiserum CMC 7 (see ref. 4). Lane A, MW markers (200,00 130,000, 97,400, 66,000, 45,000 and 29,000); B, axenic Ax2 cel C, Ax2 cells grown on *E. coli* B/r; D, NC4 cells grown on *E. c* B/r. The arrow denotes gp126.

Anti-gp126 Fab′ has allowed us to test whether contact B is a trigger for development[7]. Cells incubated in suspen in the presence of inhibitory Fab′ (1 mg ml^{-1}) became aggr tion-competent in 7–8 h. They did not cohere initially bu 7 h had developed the EDTA-resistant cohesion characte of the aggregation stage of the life cycle[1,2]. All cells posse stainable Fab′ on their surfaces after 7 h and the cell-free su natant was still able to inhibit the cohesion of vegetative Cells incubated on Millipore filters in the presence of inhib Fab′ developed synchronously to the fruiting body stage in as did control cultures; the only difference was that grexes fruiting bodies formed in the presence of inhibitory Fab′ much smaller than those in control cultures (Fig. 3). This d ence in morphology was similar to that shown by develc Ax2 cells grown in the absence and presence of glucose[12]. gp126 makes a significant contribution to the cohesivene developing cells and has a role in size regulation. Blocki gp126 with inhibitory Fab′ reduces cellular cohesiveness thereby reduces aggregate, and hence fruiting body, Development in the presence of inhibitory IgG was en normal, presumably because capping results in its removal the cell surface. Because cells can develop in conditions in

0028–0836/84/070646—02$01.00

© 1984 Macmillan Jourr

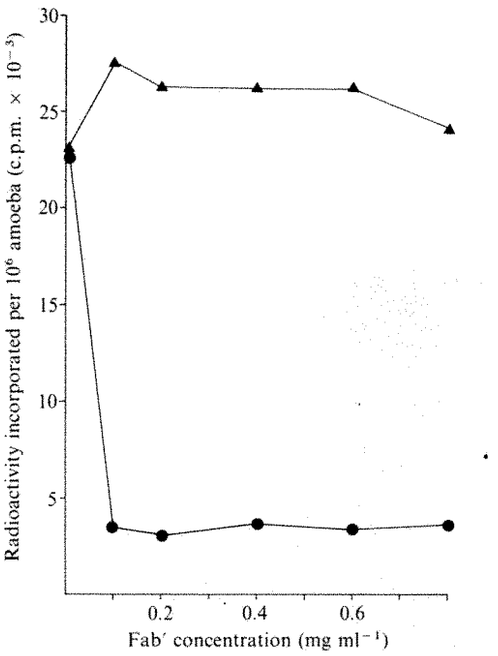


Fig. 2 Phagocytosis of ^{14}C-labelled *E. coli* B/r. ▲, Fab′ N; ●, CMC 13 Fab′. *E. coli* B/r was labelled by growth overnight in 100 ml of a shaken (140 r.p.m., 35 °C) solution of 0.05% sodium citrate, 0.1% $(NH)_2SO_4$, 0.3% KH_2PO_4, 0.7% K_2HPO_4, 0.01% $MgSO_4 \cdot 7H_2O$, 0.27% glucose containing 0.25 μCi ml^{-1} L-[U-^{14}C]lysine (Amersham). Bacteria were washed in 17 mm Na/K phosphate buffer, and suspended in buffer at 2×10^9 ml^{-1}. Log phase axenic amoebae were washed in phosphate buffer and suspended in buffer at 2×10^6 cells ml^{-1}. Samples (0.5 ml) of amoebae were mixed with 1 ml phosphate buffer containing Fab′ at the appropriate concentration. The Fab′ was allowed to bind to the cells for 10 min, then 0.5 ml of *E. coli* was added and the resulting suspension shaken for 90 min at 140 r.p.m., 22 °C. Phagocytosis was stopped with 2 ml of 4% glutaraldehyde. After 5 min of fixation, 5×10^7 stationary phase Ax2 cells were added as a carrier. Cells were collected by bench centrifugation then washed twice in 17 mM Na/K phosphate buffer to remove unbound *E. coli*. The pellet of washed cells was dissolved in 2 ml of scintillant (750 ml Triton X-100, 1,500 ml toluene, 6 g 2,5-diphenyloxazole). The radioactivity was measured with a Packard Tri Carb 300 β-counter. The specific radioactivity of the *E. coli* suspension was 34,500 c.p.m. per 10^8 bacteria. As a maximum of 27,000 c.p.m. was incorporated into 10^6 amoebae (in the presence of Fab′ N), each amoeba bound an average of 78 bacteria in permissive conditions. Glynn[9] obtained a value of 80 bacteria per cell.

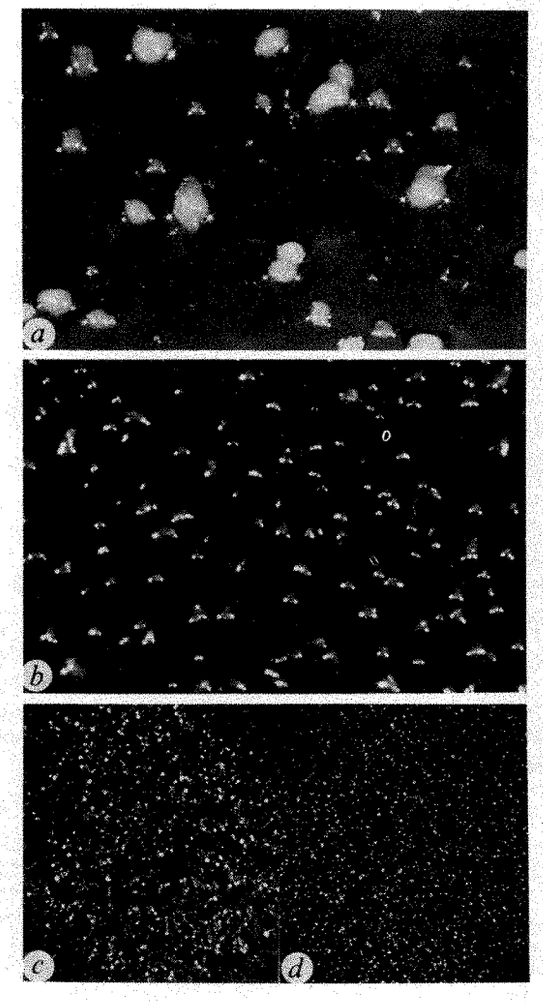


Fig. 3 Size regulation. *a*, Control grexes in Fab′ N (18 h). (×6.5.) *b*, Grexes in inhibitory Fab′ (18 h). (×6.5.) *c*, Control fruiting bodies in Fab′ N (24 h). (×1.2.) *d*, Fruiting bodies in inhibitory Fab′ (24 h). (×1.2.) Axenically grown cells were allowed to develop on Millipore filters according to the method of Sussman[13]. Inhibitory Fab′ (1.5 mg ml^{-1}) was added to the culture solution. The cells were allowed to develop for 24 h at 22 °C and were observed at intervals.

gp126-mediated cohesion is blocked, this molecule cannot be a developmental trigger.

Thus, the *Dictyostelium* cohesion molecule known as contact site B or gp126 has a dual role in phagocytosis and morphogenetic size regulation. We speculate that this is of evolutionary significance since the development of receptor-mediated phagocytosis could have provided the basis for cell–cell adhesion and hence the multicellularity displayed by higher organisms. It is possible that gp126 is related to the nonspecific receptor postulated by Vogel *et al.*[6], because it also seems to be involved in cell adhesion to glass.

This work was supported by the SRC. The Medical Oncology Unit at Southampton General Hospital is supported by the Cancer Research Campaign.

Received 13 October; accepted 1 December 1983.

1. Beug, H., Gerisch, G., Kempff, S., Riedel, B. & Cremer, G. *Expl Cell Res.* **63,** 147–158 (1970).
2. Beug, H., Katz, F. E. & Gerisch, G. *J. Cell Biol.* **56,** 647–658 (1973).
3. Steinemann, C. & Parish, R. W. *Nature* **286,** 621–623 (1980).
4. Chadwick, C. M. & Garrod, D. R. *J. Cell Sci.* **60,** 251–266 (1983).
5. Mangiarotti, G., Ceccarelli, A. & Lodish, H. F. *Nature* **301,** 616–618 (1983).
6. Vogel, G., Thilo, L., Schwarz, H. & Steinhart, R. *J. Cell Biol.* **86,** 456–465 (1980).
7. Marin, F. T., Goyette-Boulay, M. & Rothman, F. G. *Devl Biol.* **80,** 301–312 (1980).
8. Eckhardt, A. E., Hayes, C. E. & Goldstein, L. J. *Analyt. Biochem.* **73,** 192–197 (1976).
9. Glynn, P. J. *Cytobios* **30,** 153–166 (1981).
10. Swan, A. P. & Garrod, D. R. *Expl Cell Res.* **93,** 479–484 (1975).
11. Garrod, D. R. *Expl Cell Res.* **72,** 588–591 (1972).
12. Garrod, D. R. & Ashworth, J. M. *J. Embryol. exp. Morph.* **28,** 463–479 (1972).
13. Sussman, M. in *Methods in Cell Physiology* Vol. 2, Ch. 14, 397–410 (Academic, New York, 1966).

Induction of human vascular endothelial stress fibres by fluid shear stress

R.-P. Franke, M. Gräfe, H. Schnittler, D. Seiffge & C. Mittermayer

Department of Pathology, Technische Hochschule Aachen, D-5100 Aachen, FRG

D. Drenckhahn*

Department of Anatomy and Cell Biology, Universität Marburg, D-3550 Marburg, FRG

Endothelial cells of the arterial vascular system and the heart contain straight actin filament bundles, of which there are few, if any, in the venous endothelium[1–4]. Since stress fibre-containing endothelial cells within the vascular system tend to be located at sites exposed to particularly high shear stress of blood flow, we have investigated, in an experimental rheological system (Fig. 1), the response of the endothelial actin filament skeleton to controlled levels of fluid shear stress. Here we report that endothelial stress fibres can be induced by a 3-h exposure of confluent monolayer cultures of human vascular endothelium to a fluid shear stress of 2 dynes cm^{-2}, approximately the stress occurring in human arteries *in vivo*. Fourfold lower levels of shear stress that normally occur only in veins, had no significant effect on the endothelial actin filament system. The formation of endothelial stress fibres in response to critical levels of fluid shear stress is probably a functionally important mechanism that protects the endothelium from hydrodynamic injury and detachment.

Endothelial cells were collected from human umbilical vein[5] and grown on glass coverslips previously coated with a layer of extracellular matrix[6]. This coating (achieved by precultivation of the coverslips with bovine corneal endothelial cells) was important in that it allowed the formation of confluent human endothelial monolayer cultures that were firmly attached to the substrate (Fig. 2). Cells grown on glass were generally less firmly attached to the surface as revealed by occasional detachment of individual cells and even large groups of cells during exposure to fluid shear stress. Fluorescent staining of the confluent cultures with rhodamine-labelled phalloidin (a specific probe for polymerized actin[7]) and with antibodies to calf thymus myosin[2] and chicken gizzard and pectoral muscle α-actinin[8] showed a rather uniform pattern of stress fibres (Fig. 3). The stress fibres displayed the typical continuous staining for actin and an interrupted pattern for myosin and α-actinin similar to the descriptions of stress fibres found in cultured aortic endothelium[1,9] and other tissue culture cells (for a recent review, see refs 10, 11). Stress fibre staining was most prominent in the peripheral cytoplasm close to the margin of the polygonally-shaped cells. We often observed intensely stained focal areas from which numerous stress fibres were seen to radiate in various directions. Outside the marginal zone intensely stained stress fibres were rather infrequent. Most of the relatively few stress fibres projecting over the central parts of the cells appeared to be rather thin and were only faintly stained.

Controlled levels of shear stress were then applied by using a transparent rotating cone placed with its tip on the centre of the coverslips (Fig. 1). Rotation of the cone caused the culture medium to flow between the cone and the surface of the coverslip. In this study a lamina flow was chosen, producing a shear stress of 2 dynes cm^{-2}, which is about the minimum level in human arteries for basal conditions (2–20 dynes cm^{-2}, locally up to 100 dynes cm^{-2})[12]. No changes in cellular shape or position occurred during exposure to shear stress, as judged by phase contrast microscopy and TV recording of the endothelium throughout the exposure period (1, 2, 3 and 4 h). Temperature and *p*H of the medium were kept constant during the experiments.

After a 3-h exposure to shear stress, the quantity and staining intensity of stress fibres had dramatically increased (Fig. 3). No preferential alignment or direction of the newly formed stress fibres was seen. The exact time course of induction of stress fibres has so far not been determined. At present we can only state that after a 1-h exposure to shear stress there was no increase in the amount of stress fibres, but a slight increase was observed after 2 h. After 4 h the quantity and density of stress fibres were about the same as after 3 h of exposure to shear stress. Thus most stress fibres seemed to have developed during the second and third hour of shear stress treatment. When the shear stress was reduced to fourfold lower levels (0.5 dynes cm^{-2}), a level normally found only in the venous system, no significant increase in the quantity of stress fibres was seen after 3 h of exposure.

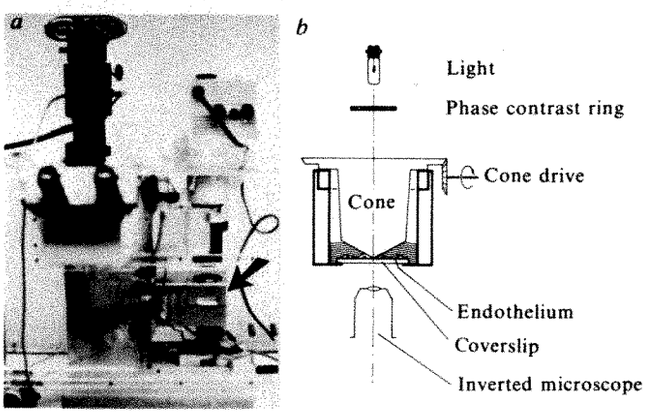


Fig. 1 *a*, Rheoscope used in this study. The construction is based on a cone and plate mechanism originally developed for microrheological studies of blood cell aggregations[20]. An arrow indicates the rheoscope chamber, which is shown in *b* in simplified cross-sectional form (not to scale). A transparent polycarbonate cone having an angle of 2.5° is fitted into a Plexiglas holder and connected to a speed-controlled motor with variable rotational velocities. The cone is placed with its tip on the surface of the coverslip under microscopic control. The direction and magnitude of shear stress were determined precisely both by direct measurements and from theory (ref. 21 and our unpublished data).

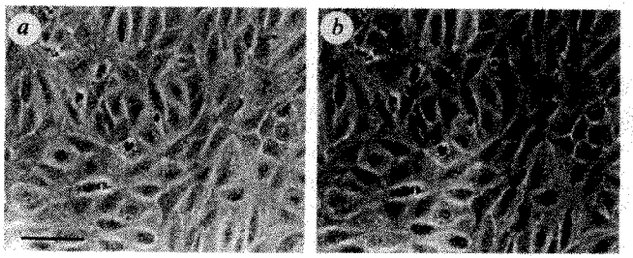


Fig. 2 *a*, *b*, Phase contrast micrographs of confluent monolayer cultures of human vascular endothelial cells collected from the umbilical vein[5], cultured in medium TC 199 containing 20% pooled human serum and 1 mg ml^{-1} endothelial growth supplement extracted from bovine brain[22]. Cells were seeded on coverslips previously coated with an extracellular matrix layer consisting of bovine corneal endothelium[6], and were grown for 8–10 days. The same area of the monolayer was monitored throughout the exposure to fluid shear stress (2 dynes cm^{-2}). *a*, Before shear stress exposure; *b*, after 3 h of exposure. No changes in cellular shape or position have occurred. Scale bar, 100 μm.

* To whom reprint requests should be addressed.

0028-0836/84/070648—02$01.00

© 1984 Macmillan Journals Ltd

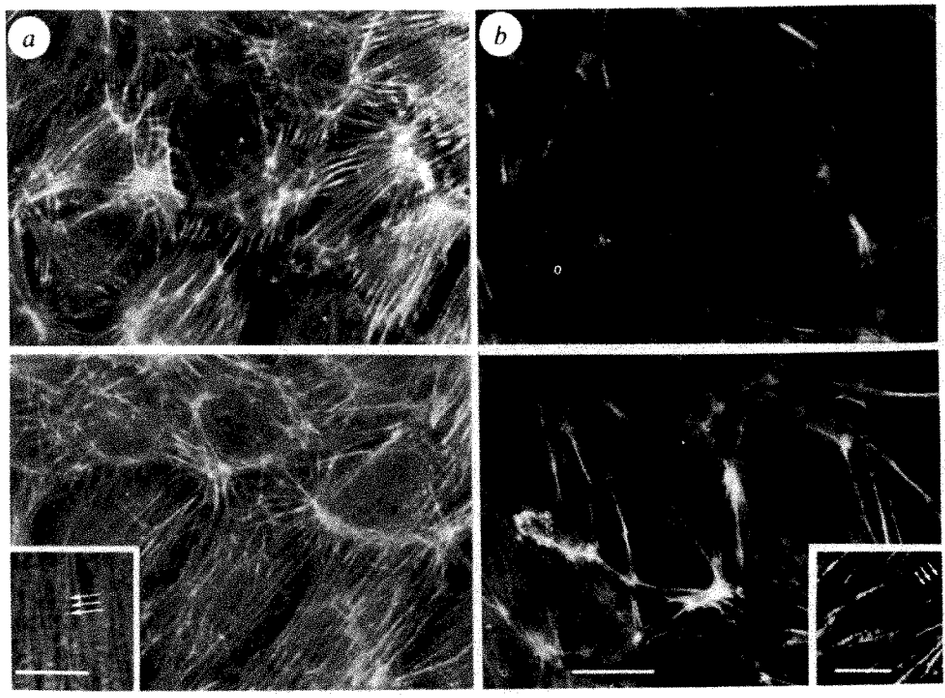


Fig. 3 *a*, Pattern of actin filament bundles (stress fibres) in human endothelial monolayer cultures (two examples are shown) after 3 h of exposure to low arterial levels of fluid shear stress (2 dynes m^{-2}). *b*, Corresponding controls. Scale bar, 20 μm. Only the central parts of the monolayers (area of ~1 cm^2) were examined, because endothelial cells located close to the margin of the coverslips were still in a subconfluent state and thus contained varying amounts of stress fibres. Each shear stress experiment was followed by a control experiment in which corresponding cultures were placed in the rheoscope chamber for the same length of time as for exposure to shear stress, but without rotation of the cone. Stress fibres were visualized by fluorescent staining with 1.4 μg ml^{-1} tetramethylrhodaminyl- which was applied to the monolayers after 5 min of fixation with 2% paraformaldehyde in phosphate-buffered saline and 3 min of permeabilization with 80% acetone (all steps were done at room temperature). The insets show stress fibres at higher magnification (scale bars, 10 μm) stained with antibodies to calf thymus myosin (inset in *a*) and chicken gizzard α-actinin (inset in *b*) using the indirect immunofluorescence technique described elsewhere[2].

Although no attempts were made to determine the threshold value of shear stress that induces stress fibre formation in this *in vitro* system, the experiments reported here clearly show that at a critical level of fluid shear force (which seems to be much lower *in vitro* than *in situ*) endothelial cells will respond by forming stress fibres. These stress fibres probably protect the cells against the shear stress of fluid flow. In view of the observation that isolated stress fibres can contract in appropriate conditions[13,14], it seems reasonable to assume that endothelial stress fibres may apply tension to resist the shear forces acting on the cells, thus allowing the cells to maintain their flattened phenotype and to remain firmly attached to the substratum. To a certain degree, stress fibre formation may prevent hydrodynamic damage to the endothelium and thus protect the vascular wall from certain pathological stimuli such as those thought to be involved in the initiation of arteriosclerosis[15].

The shear-induced stress fibres were more or less randomly distributed with respect to the direction of fluid flow. This pattern apparently differs from the alignment of stress fibres in arterial endothelial cells *in situ*, which is characterized by orientation of the fibres parallel to the direction of blood flow[1–4,16]. However, as the arterial endothelium consists of elongated cells having their long axis oriented in the direction of blood flow (this kind of orientation is probably caused by long-term shear stress in one direction)[17], and in view of the fact that in elongated, cultured cells stress fibres are mainly aligned parallel to the cellular long axis[18], it seems likely that the uniform alignment of arterial endothelial stress fibres parallel to the blood flow depends on the particular orientation of arterial endothelial cells rather than on direct actions of fluid shear stress on the orientation of stress fibres. In the experimental conditions of the present study, the confluent polygonal endothelial cells did not change their shape during shear stress; this is probably the reason why the induced stress fibres did not display a uniform alignment in the direction of the fluid flow. Support for this view comes from regenerating arterial endothelial cells of the rabbit aorta: at the edges of the outgrowing endothelium, stress fibres were frequently found oriented diagonally or even perpendicularly to the blood flow but the fibres were always aligned parallel to the cellular long axis[3]. Similar observations were obtained with subconfluent and confluent cultures of endothelium exposed to long-term (1–2 days) shear stress. Reorientation of endothelial cells in the direction of the fluid flow was accompanied by re-alignment of the stress fibres which were always (before and after the shear treatment) oriented roughly parallel to the cellular long axis (ref. 19 and our unpublished observations). However, such changes in the alignment of stress fibres seem to be only secondary effects brought about by shear stress-induced reorientation of endothelial cells.

The present study provides the first evidence that in human vascular endothelium, fluid shear stress may act directly on the stress fibre system without affecting cellular shape and orientation. The factors mediating this response of the endothelial actin filament system remain to be determined.

Supported by the Deutsche Forschungsgemeinschaft (Dr 91/3-2, 91/4-1, SFB 106) and by the Bundesministerium für Forschung und Technologie (GSF/CMT21).

Received 28 September; accepted 23 November 1983.

1. Drenckhahn, D. *Prog. appl. Microcirculation* **1,** 53–70 (1983).
2. Drenckhahn, D., Gröschel-Stewart, U., Kendrick-Jones, J. & Scholey, J. *Eur. J. Cell Biol.* **30,** 100–111 (1983).
3. Gabbiani, G., Gabbiani, F., Lombardi, D. & Schwartz, S. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2361–2364 (1983).
4. Wong, A. J., Pollard, T. D. & Herman, I. *Science* **219,** 867–869 (1983).
5. Jaffé, E. A., Nachman, R. L., Becker, D. G. & Minick, C. R. *J. clin. Invest.* **52,** 2745–2756 (1973).
6. Gospodarowicz, D., Vlodavsky, J. & Savion, N. *J. supramolec. Struct.* **13,** 339–372 (1980).
7. Faulstich, H., Trischmann, H. & Mayer, D. *Expl Cell Res.* **144,** 73–82 (1983).
8. Drenckhahn, D. & Mannherz, H. *Eur. J. Cell Biol.* **30,** 167–176 (1983).
9. Kalnins, V. I. & Subrahmanyan, L. *Eur. J. Cell Biol.* **24,** 36–44 (1981).
10. Goldman, R. D., Milsted, A., Schloss, J. A., Starger, J. & Yerna, M. J. *A. Rev. Physiol.* **41,** 703–722 (1979).
11. Gröschel-Stewart, U. & Drenckhahn, D. *Collagen Rel. Res.* **2,** 381–463 (1982).
12. Dewey, C. F., Bussolari, S. R., Gimbrone, M. A. & Davies, P. F. *J. biomech. Eng.* **103,** 177–185 (1981).
13. Isenberg, G., Rathke, P. C., Hülsmann, N., Franke, W. W. & Wohlfarth-Bottermann, K. E. *Cell Tissue Res.* **166,** 427–428 (1976).
14. Kreis, T. E. & Birchmeyer, W. *Cell* **22,** 555–561 (1980).
15. Ross, R. & Glomset, J. A. *New Engl. J. Med.* **295,** 369–377, 420–425 (1976).
16. Hammersen, F. *Adv. Microcirculation* **9,** 95–134 (1980).
17. Langille, B. L. & Adamson, S. L. *Circulation Res.* **48,** 481–488 (1981).
18. Buckley, I. K. & Porter, K. *Protoplasma* **64,** 350–380 (1967).
19. White, G. E., Fujiwara, K., Shefton, E., Dewey, C. F. & Gimbrone, M. A. *Fedn Proc.* **41,** 321 (Abstr.) (1982).
20. Schmidt-Schönbein, H., Gosen, J. V., Heinrich, L., Klose, H. J. & Vogeler, E. *Microvasc. Res.* **6,** 366–376 (1973).
21. Kiesewetter, H. *et al. Biomed. Techn* **27,** 209–213 (1982).
22. Maciag, T., Cerundolo, J., Ilfley, S., Kelley, P. R. & Forand, R. *Proc. natn. Acad. Sci. U.S.A.* **76,** 5674–5678 (1979).

Mapping by monoclonal antibody detection of glycosaminoglycans in connective tissues

John R. Couchman*, Bruce Caterson†, James E. Christner‡ & John R. Baker‡

* Unilever Research, Colworth Laboratory, Sharnbrook, Bedfordshire MK44 1LQ, UK
† Biochemistry Department, West Virginia University, Medical Center, Morgantown, West Virginia 26506, USA
‡ Diabetes Research and Training Center, University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294, USA

Chondroitin sulphate proteoglycans are widespread connective tissue components[1–4] and chemical analysis of cartilage and other proteoglycans has demonstrated molecular speciation involving the degree and position of sulphation of the carbohydrate chains[5,6]. This may, in turn, affect the properties of the glycosaminoglycan (GAG), particularly with respect to self-association and interactions with other extracellular matrix components[6,7]. Interactions with specific molecules from different connective tissue types, such as the collagens[8,9] and their associated glycoproteins[10,11], could be favoured by particular charge organizations on the GAG molecule endowed by the sulphate groups. So far, it has not been possible to identify and map chondroitins of differing sulphation in tissues, but we have now raised three monoclonal antibodies which specifically recognize unsulphated, 4-sulphated and 6-sulphated chondroitin and dermatan sulphate. These provide novel opportunities to study the *in vivo* distribution of chondroitin sulphate proteoglycans. We demonstrate that chondroitin sulphates exhibit remarkable connective tissue specificity and furthermore provide evidence that some proteoglycans may predominantly carry only one type of chondroitin sulphate chain.

The characteristics, specificity and designation of each antibody are given in Table 1 and Fig. 1. Details of the methodology and protocols used are given elsewhere[12]. Rat tissue sections for indirect immunofluorescence staining with these antibodies required pretreatment with GAG-degrading enzymes to liberate the specific antigen as listed in Table 1. The omission of enzyme treatment in the staining schedule served as a control revealing nonspecific staining and was negative for each monoclonal antibody. Figure 1*c* shows that 3-B-3 antibody has minor activity against unsulphated chondroitin (C0-S) in addition to the major determinant, chondroitin 6-sulphate (C6-S). However, the comparative use of both the 1-B-5 and 3-B-3 antibodies allows the distribution of C0-S and C6-S to be easily determined.

In the rat we found that the distribution of C0-S was virtually restricted to cartilage (Fig. 2*a*) since the 1-B-5 antibody stained articular and hyaline cartilage brightly, but this antibody did not stain dermis or other connective tissues apart from a faint staining in the intima of rat aorta (Fig. 2*b*). However, chondroitin 4-sulphate (C4-S) and/or dematan sulphate (DS) was more widely distributed than unsulphated chondroitin. In addition to cartilage staining by the 9-A-2 antibody (Fig. 3*a*), fluorescence was widely distributed in loose connective tissue. The dermis, in particular, was well stained in a fibrillar pattern suggesting some association with dermal collagen fibres (Fig. 3*b*) as was the connective tissue supporting blood vessels, nerve fibres and striated muscle from a variety of locations (Fig. 3*c*). The media and adventitia of the aorta stained positively as did the kidney mesangium (Figs 3*d*, *e*). Epithelia and endothelial components of the skin (Fig. 3*b*), aorta (Fig. 3*d*) and kidney were themselves negative as were their supporting basement membrane zones (BMZ), including those of the glomerulus. The indications are that a BMZ population of C4-S or DS was not detectable. A further series of experiments was performed to establish whether some or all of the loose connective tissue staining was in fact chondroitin 4-sulphate (C4-S) or dermatan sulphate (DS). Accordingly, tissue sections were pretreated with

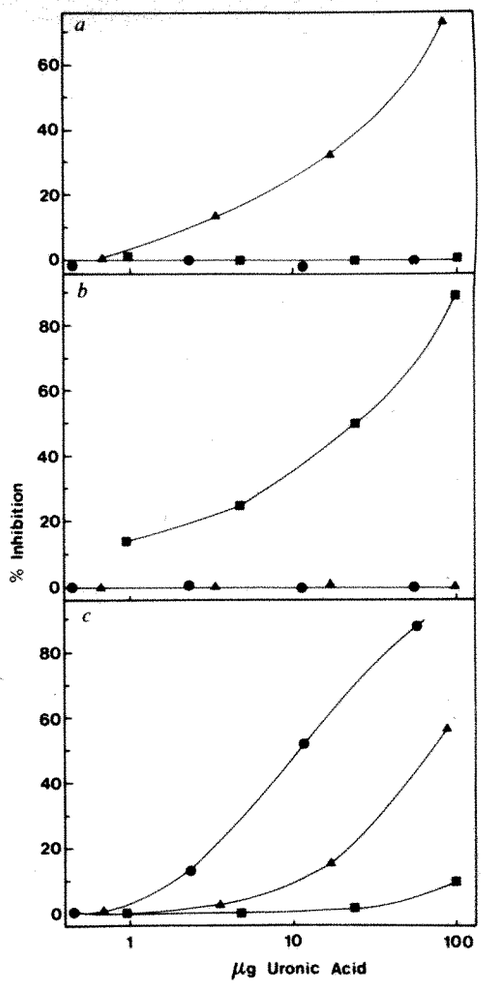


Fig. 1 Antibody characterization by inhibition of the binding of ^{125}I-labelled chondroitinase ABC-digested rat chondrosarcoma proteoglycan to monoclonal antibodies as a function of the concentration of chondroitin sulphate disaccharides. The disaccharides tested were 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulpho-D-galactose (■), the corresponding 6-sulphated disaccharide (●), and the nonsulphated disaccharide (▲). ^{125}I-antigen (about 1 ng of protein containing 3×10^4 c.p.m.) and chondroitin sulphate disaccharides in the amounts indicated were incubated with *a*, antibody 1-B-5 (1/400 dilution of ascites fluid), *b*, antibody 9-A-2 (1/100 dilution), or *c*, antibody 3-B-3 (1/400 dilution) in a total volume of 200 μl of buffer containing 0.5% deoxycholate, 0.1% Nonidet P-40, 0.1% bovine serum albumin, 0.15 M NaCl, 0.03% NaN_3, 0.01 M sodium phosphate, *p*H 8.0. After incubation at room temperature (22–25 °C) for 90 min the antibody-bound antigen was separated from the unbound using heat- and formalin-inactivated *Staphylococcus aureus* cells as described previously[30]. Per cent inhibition was calculated relative to the amount of ^{125}I-labelled antigen bound to the antibody in the absence of added chondroitin sulphate disaccharides. The conditions for use of the antibodies are shown in Table 1.

chondroitinase ACII in place of chondroitinase ABC. Under these conditions C4-S but not DS is digested[13]. We found that cartilage staining persisted (Fig. 3*f*) but the dermis of skin and other loose connective tissue was now weakly stained or unstained (Fig. 3*g*). We, therefore, deduce that the staining depicted in Figs 3*a* and *b* is largely derived from DS.

Chondroitin 6-sulphate (C6-S) distributions were revealed by the 3-B-3 antibody after chondroitinase ABC or testicular hyaluronidase pretreatment of tissue sections. In addition to cartilage staining (Fig. 4*a*), very heavy staining of the intima and media of the aorta was detected (Fig. 4*c*) as was a remarkable specificity for a number of BMZ in the rat including those of blood vessel endothelia, muscle, peripheral nerve fibres, the

0028-0836/84/070650—03$01.00

© 1984 Macmillan Journals Ltd

dermal/epidermal junction, kidney tubules and the glomerulus (Figs 4*a–f*). Further tests were carried out by enzyme-linked immunosorbent assay and no cross-reactivity (not shown) between the antibody and other previously characterized BMZ components such as laminin[14] and type IV collagen[15] was detected. Antigen adsorption with rat liver heparan sulphate proteoglycan[16] did not block BMZ staining. In addition, the tissue-staining patterns seen with two antisera against heparan sulphate proteoglycan[17,18] were quite distinct from that with the 3-B-3 antibody (not shown). The presence of a BMZ population of C6-S proteoglycan has not previously been shown by immunohistochemical means, but chemical analysis of glomerular BMZ has indicated a minor amount of chondroitin sulphate in addition to the predominant GAG, heparan sulphate[19,20]. The presence of a chondroitin sulphate proteoglycan in the supernatants of vascular endothelial cell cultures has recently been reported and is consistent with our results[21]. The precise localization of C6-S within BMZ is currently under investigation by electron microscopy.

Although the antigens for monoclonal antibody production were in each case of cartilage origin, the epitopes recognized by the antibodies are common to other connective tissue locations and are revealed by chondroitinase ABC pretreatment. The possibility that masking of other potentially antigenic sites occurs through the interaction with other components cannot be discounted but seems unlikely in view of the specificity of staining patterns shown here. Moreover, our findings agree in principle with previous chemical analyses of whole tissues. For example, chondroitin chains with differing sulphation (C0-S, C4-S and C6-S) have been reported from cartilage[22], and we found that all three monoclonals stained both articular and hyaline cartilage intensely. Similarly, the predominant GAG types in aorta have been reported to be C6-S and DS[23], results entirely consistent with our immunofluorescence localizations. An advantage of our methods is, however, that we have shown that these two chondroitin subspecies do not share the same tissue locations (Figs 3, 4). While C6-S was present only in the intima and media, C4-S was prominent in the adventitia region and also the media but not the intima. This is just one example of the much higher resolution that can be achieved by immunohistochemical detection of glycosaminoglycans over previous methods. Some similarity in properties between cartilage and aorta proteoglycans have also been noted before[24,25]. In contrast to cartilage it seems that for other tissue locations chondroitin sulphate chains of only one major type are covalently bound to a specific protein core. Other reports have

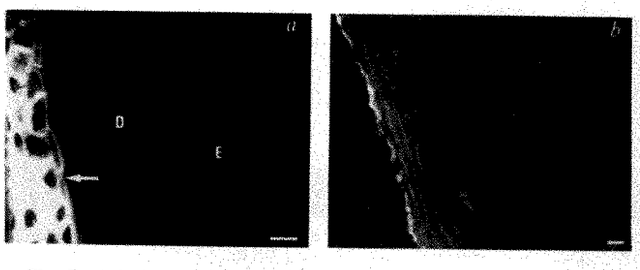


Fig. 2 1-B-5 antibody staining showing the distribution of unsulphated chondroitin in chondroitinase ABC treated (0.2 U ml^{-1} in 0.1 M Tris-acetate *p*H 7.3 containing 1 mg ml^{-1} bovine serum albumin) rat ear (*a*) and aorta (*b*) sections. Cartilage was heavily stained (arrow), but the epidermis (E) and dermis (D) with its associated muscle fibres, blood vessels and nerve fibres were unstained. The intima of the rat aorta was weakly positive with the 1-B-5 antibody. Scale bars, 15 μm. For this and all other staining, small pieces of freshly excised tissue from rats were snap-frozen in isopentane chilled in liquid nitrogen. Cryostat sections (6–8 μm) were briefly air-dried, fixed for 15 min in 3% formaldehyde, then washed repeatedly in Dulbecco's phosphate-buffered saline, including one 15-min wash supplemented with 0.1 M ammonium chloride. Antibody staining was for 30–45 min at 37 °C in a wet-box with several washes between applications.

suggested that chondroitin sulphates of different types may occur within a single chain[26]. One possibility for future consideration in this regard is that the striking tissue specificity of chondroitin sulphates shown here may reside in the disaccharides adjacent to the protein core of the proteoglycan, these remaining after enzyme treatment. Mixed forms of chondroitin sulphate, if present in more distal regions of the chondroitin chains would then be preferentially removed by the enzyme before antibody application. Recently, we have characterized an additional monoclonal antibody which specifically recognizes keratan sulphate and which could also be used for immunohistochemical analyses by techniques similar to those reported here[12].

This work has two important implications. First, the different forms of chondroitin recognized by the three monoclonal antibodies have distinct and often separable distributions. Our observations further indicate that the proteoglycans involved may predominantly carry only one type of chondroitin chain with respect to sulphation. If these deductions are correct it is possible that the core proteins of the chondroitin proteoglycans are distinct gene products expressed in a tissue or cell-specific manner with a concomitant specificity of chondroitin sulphate

Table 1 Conditions for use of the antibodies

Monoclonal antibody designation	Antigen	Antibody source for staining	Antibody class and working dilution	Second antibody and working dilution	Antibody specificity
1-B-5	Chondroitinase ABC digested rat chondrosarcoma proteoglycan	Ascites fluid	IgG1 1:50	Affinity purified goat anti-mouse IgG1-FITC or TRITC 0.05 mg ml^{-1}	Unsaturated uronic acid* residue linked to *N*-acetylgalactosamine
9-A-2	Chondroitinase ABC digested bovine articular cartilage proteoglycan	Ascites fluid	IgG1 1:50	Affinity purified goat anti-mouse IgG1-FITC or TRITC 0.05 mg ml^{-1}	Unsaturated uronic acid* residue linked to *N*-acetylgalactosamine 4-sulphate
3-B-3	Chondroitinase ABC digested rat chondrosarcoma proteoglycan	Ascites fluid	IgM 1:50	Affinity purified goat anti-mouse IgM-FITC or TRITC 0.1 mg ml^{-1}	Unsaturated* or saturated uronic acid residue linked to *N*-acetylgalactosamine 6-sulphate Some activity against unsulphated derivative

FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate.
*These represent the residues from chondroitinase ABC digestion of chondroitin, chondroitin 4-sulphate and chondroitin 6-sulphate respectively.

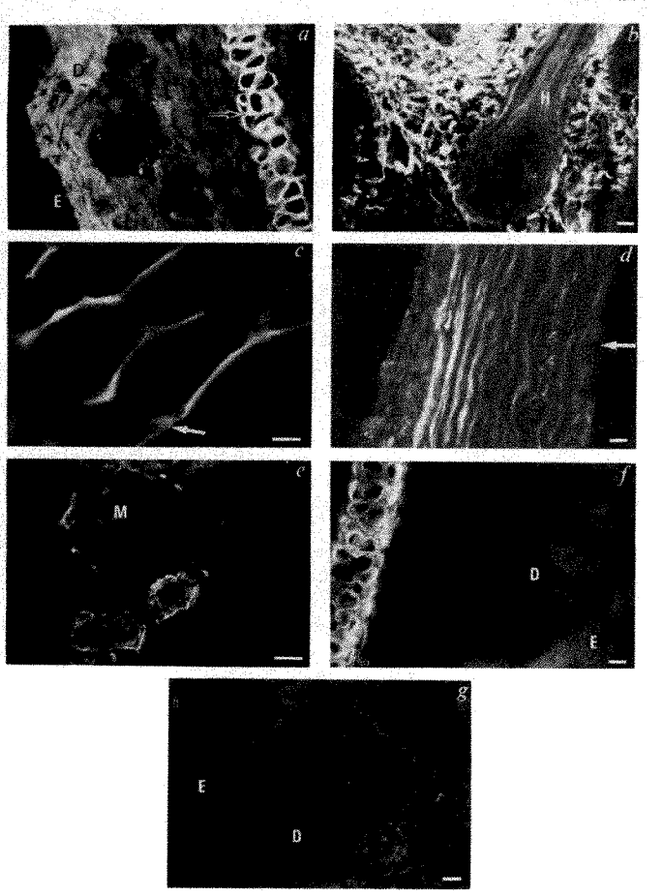


Fig. 3 9-A-2 antibody staining on rat tissue sections pretreated with chondroitinase ABC (*a–e*) to stain C4-S and DS, or chondroitinase AC II (*f*, *g*) to stain C4-S only[12]. *a*, Ear cartilage (arrow) and dermal connective tissue (D) were heavily stained, but the epidermis (E) was negative. Dorsal rat skin dermis (*b*) had a fibrillar staining pattern and the epithelia of the hair shaft (H) were unstained. *c*, Connective tissue surrounding striated muscle and blood vessels (arrow) were also positively stained with this antibody. *d*, Staining was seen through most of the cross-section of rat aorta but no staining of the endothelial basement membrane zone was visible (arrow). *e*, Connective tissue surrounding kidney blood vessels and Bowman's capsule were positively stained together with weak staining of the mesangium (M). The tubular and glomerular basement membrane zones were apparently unstained. *f*, *g*, Sections of rat ear and dorsal skin respectively after chondroitinase AC II pretreatment (compare *a* and *b*). Only cartilage staining persisted indicating the presence of C4-S. Dermal connective tissue staining was lost indicating that it contained DS. Scale bars, 15 μm.

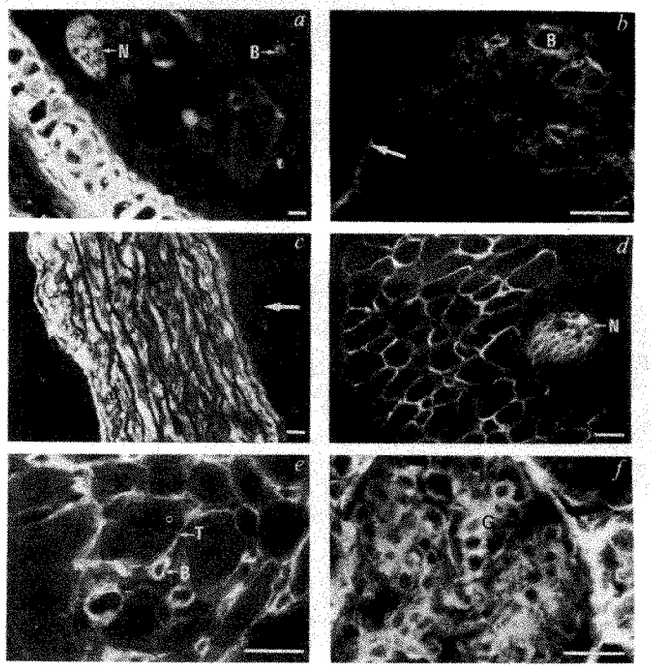


Fig. 4 Distribution of C6-S in chondroitinase ABC-treated rat tissue sections as revealed by the 3-B-3 antibody. In addition to ear cartilage (*a*), this antibody stained basement membrane zones of dermal nerve fibres (N) and blood vessels (B) (*a*, *b*), the dermo-epidermal junction (arrow) (*b*), intima and media of aorta but not adventitia (arrow) (*c*), basement membrane zones of striated muscle and associated nerve fibres (*d*) and those of kidney tubules (T) and glomerulus (G) (*e*, *f*). Scale bar, 15 μm.

chains. Our data therefore provide further evidence for the specificity of connective tissue types, seen previously in terms of both collagenous[15,27,28] and non-collagenous glycoproteins such as laminin[14] and chondronectin[29]. The specificity of sulphation of chondroitin sulphate proteoglycan in connective tissues may facilitate interactions with other matrix components.

We believe that these antibodies can be used to isolate and characterize the chondroitin sulphate proteoglycans and their protein cores from a variety of tissue sources. In particular, distinct protein cores can be isolated from mixed populations of proteoglycans; at present very little is known regarding the relationship between them and their interactions with other matrix components. Our preliminary findings also suggest that the antibodies have wide cross-species reactivity, as might be expected from the highly conserved nature of GAG chains. Antibodies such as these will therefore be valuable in the elucidation of the functions of chondroitin sulphate proteoglycans in human connective tissue synthesis and assembly as well as their roles in many pathological conditions.

We thank Dr J. F. Kearney (University of Alabama) for purified fluorochrome-conjugated antibodies and Ms L. Lewis for technical assistance. This work was supported by NIH grants HL 28195, AM 27308, AM 32474.

Received 5 September; accepted 23 November 1983.

1. Muir, H. & Hardingham, T. E. *MTP Int. Rev. Sci. Biochem. Ser. 1* **5**, 153–222 (1975).
2. Dietrich, C. P., Sampaio, L. O. & Toledo, O. M. S. *Biochem. biophys. Res. Commun.* **71**, 1–10 (1976).
3. Hascall, V. C. & Hascall, G. K. in *Cell Biology of Extracellular Matrix* (ed. Hay, E. D.) 39–63 (Plenum, New York, 1981).
4. Caterson, B., Baker, J. R., Christner, J. E. & Couchman, J. R. *J. invest. Derm.* **79**, 45s–50s (1982).
5. Rodén, L. & Schwartz, N. B. *MTP Int. Rev. Sci. Biochem. Ser. 1* **5**, 96–152 (1975).
6. Lindahl, U. & Höök, M. *A. Rev. Biochem.* **47**, 385–417 (1978).
7. Fransson, L.-Å. *Biochim. biophys. Acta* **437**, 106–115 (1976).
8. Toole, B. P. *J. biol. Chem.* **251**, 895–897 (1976).
9. Scott, J. E. & Orford, C. R. *Biochem. J.* **197**, 213–216 (1981).
10. Ruoslahti, E. & Engvall, E. *Biochim. biophys. Acta* **631**, 350–358 (1980).
11. Del Rosso, M., Cappelletti, R., Viti, M., Vannucchi, S. & Chiarugi, V. *Biochem. J.* **199**, 699–704 (1981).
12. Caterson, B., Christner, J. E. & Baker, J. R. *J. biol. Chem.* **258**, 8848–8856 (1983).
13. Yamagata, T., Saito, H., Habuchi, O. & Suzuki, S. *J. biol. Chem.* **243**, 1523–1535 (1968).
14. Timpl, R. *et al. J. biol. Chem.* **254**, 9933–9937 (1979).
15. Kefalides, N. A., Alper, R. & Clark, C. C. *Int. Rev. Cytol.* **61**, 167–228 (1979).
16. Kjellén, L., Pettersson, I. & Höök, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5371–5375 (1981).
17. Hassell, J. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 4494–4498 (1980).
18. Woods, A., Kjellén, L., Höök, M., Smith, C. G. & Rees, D. A. *J. Cell biol.* (in the press).
19. Kanwar, Y. S., Hascall, V. C. & Farquhar, M. G. *J. Cell Biol.* **90**, 527–532 (1981).
20. Parthasarathy, N. & Spiro, R. G. *J. biol. Chem.* **256**, 507–513 (1981).
21. Oohira, A., Wight, T. N. & Bornstein, P. *J. biol. Chem.* **258**, 2014–2021 (1983).
22. Rodén, L., Baker, J. R., Cifonelli, J. A. & Mathews, M. B. *Meth. Enzym.* **28**, 73–140 (1972).
23. Toledo, O. M. S. & Dietrich, C. P. *Biochim. biophys. Acta* **498**, 114–122 (1979).
24. Antomopoulos, C. A., Axelsson, I., Heinegård, D. & Gardell, S. *Biochim. biophys. Acta* **338**, 108–119 (1974).
25. Gardell, S., Baker, J. R., Caterson, B., Heinegård, D. & Rodén, L. *Biochem. biophys. Res. Commun.* **95**, 1823–1831 (1980).
26. Seno, N., Akiyama, F. & Anno, K. *Biochim. biophys. Acta* **362**, 290–298 (1974).
27. Linsenmayer, T. F. in *Cell biology of Extracellular Matrix* (ed. Hay, E. D.) 5–37 (Plenum, New York, 1981).
28. Martinez-Hernandez, A., Gay, S. & Miller, E. J. *J. Cell. Biol.* **92**, 343–349 (1982).
29. Hewitt, A. T. *et al. J. biol. Chem.* **257**, 2330–2334 (1982).
30. Christner, J. E., Caterson, B. & Baker, J. R. *J. biol. Chem.* **255**, 7102–7105 (1980).

Is stepwise sarcomere shortening an artefact?

John D. Altringham*, Roberto Bottinelli† & Joan W. Lacktis‡

Department of Anesthesiology and Division of Bioengineering, and ‡ Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195, USA
* Department of Physiology, Bute Medical Building, St Andrews, Fife KY16 9TS, UK
† Istituto di Fisiologia Umana, Via Forlanini 6, 27100 Pavia, Italy

A report[1] in 1977 raised the intriguing possibility that sarcomere shortening in muscle may occur in a stepwise fashion, in which episodes of shortening are interrupted by periods of little or no movement. This was taken by its authors to imply the synchronous activity of cross-bridges over a large volume of tissue—behaviour which cannot easily be reconciled with commonly accepted views of muscle contraction[2–5]. Stepwise shortening has also been reported recently in relaxed muscle fibres on which length changes were externally imposed[4,6,7], and that system has allowed us to define more rigorously the circumstances in which stepwise shortening is observed. Here we report a high correlation between the frequency of 'steps' or 'pauses' and the translation velocity of the fibre past the measuring system, suggesting that stepwise shortening is not a physiological property of muscle but an instrumentation artefact.

To measure sarcomere length, we used two independent methods which have previously been used to study stepwise shortening: diffraction of laser light by the periodic arrangement of sarcomeres along the length of a muscle fibre[8], and an optical imaging technique using a phase-locked loop (PLL)[9]. Changes of sarcomere length were brought about in single frog muscle fibres (see Fig. 1 legend) by anchoring one end and stretching and releasing the other. The effects of different translation velocities were studied either (1) by releasing at a constant speed but observing at different points along the fibre (exploiting the gradient of velocity from the fixed to the moving end of a fibre), or (2) by varying the speed of the release and observing at a fixed point. In each case, natural markers on the fibres were used as points of reference. Both methods yield records showing the variation with time of sarcomere length at the point observed, from which the frequency of steps (evident in a variable proportion of experiments) could be calculated.

The results are summarized in Fig. 1 (laser diffraction) and Fig. 2 (optical imaging). In most experiments, the 'pauses' were inclined to the horizontal, giving the records a 'saw-tooth' appearance, but we have seen no justification for selecting only flat pauses in our analysis, as flat and inclined pauses often occurred on the same record. With each method, plots of frequency against the translation velocity of the point of the fibre observed fall on straight lines with correlation coefficients near unity. Extrapolation to zero shortening velocity predicts smooth shortening. The frequency of the oscillations we have observed appears to be determined only by the translation velocity of the fibre at the point observed, and not by the rate of change of sarcomere length. The latter is determined by the velocity of the moving end of the fibre, and was found to be very nearly constant along the length of the fibre, exhibiting only minor random variation.

The scatter in the data of Fig. 1 results, to a large extent, from errors in frequency estimation. With laser diffraction, steps appeared most often in areas of the fibre giving less than optimum signal quality and could not be found in most fibres. It was difficult to obtain cascades of clear steps throughout the length change. The PLL almost invariably produced regular cascades of steps, and frequency estimation was correspondingly accurate. Recent reports[4,10] have also noted the greater abundance and regularity of steps with the PLL. Sarcomere length records were usually repeatable over multiple 'contractions' as in previous experiments[1,3,11].

In search of a mechanism which might generate these oscillations, the first-order diffraction line was monitored during sarcomere length changes. As the duration of a pause could be extended from <50 ms to >1 s simply by decreasing the length

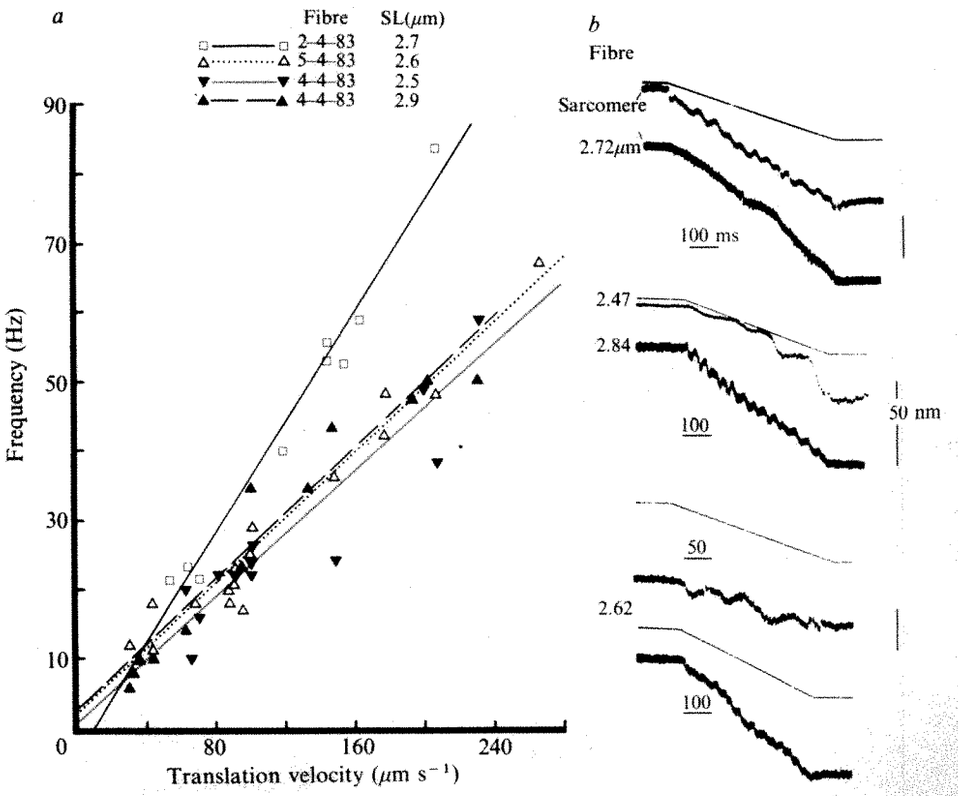


Fig. 1 *a*, Step frequency plotted against translation velocity; results from laser diffraction[8], beam diameter 100–200 μm. The lines were fitted by least-squares regression, $r = 0.94$–0.98. Frequency was measured by obtaining cascades of steps throughout the length change, and counting the number of cycles throughout. Translation was measured by following the movement of natural markers during the length change. Translation velocity was varied in two ways (see text). Method (1) was used on fibres of 2-4-83 and 4-4-83 and a combination of (1) and (2) on fibre of 5-4-83. *b*, Typical records from graph data. Upper records: translation velocity varied by studying two different points on the fibre, muscle release unchanged. The lower sarcomere length trace of each pair was obtained from the point nearer to the moving end of the fibre (note the high frequency of this record). Middle records: both the sarcomere length and the point along the fibre were changed; release velocity constant. Lower records: the position along the fibre, and the velocity and amplitude of length change varied from the same initial sarcomere length. All experiments were done on single anterior tibialis fibres of *Rana temporaria*, at 6–8 °C, in standard Ringer's solution. All fibres gave reproducible tetanic force records before and after the experiment. Fibres were viewed at all times through a ×40 water immersion objective (Zeiss, n.a. 0.75).

© 1984 Macmillan Journals Ltd

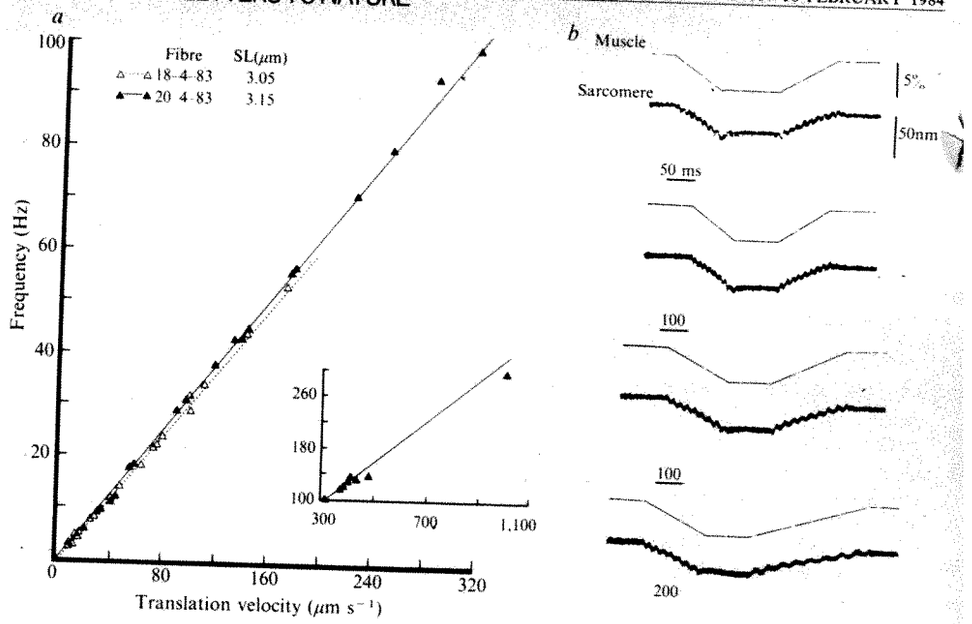


Fig. 2 *a*, Step frequency plotted against translation velocity. Inset shows continuation of data from fibre of 20-4-83. The lines were fitted by least-squares regression, $r = 1.0$ in both cases. Sarcomere length was determined by an on-line, direct imaging technique[9], which uses a PLL to measure the spatial frequency of the microscopic image of the striation pattern, over 12–14 sarcomeres in series (30–40 × 5 × 5 μm). Representative records are shown in *b*; the translation velocity was varied by changing the velocity of release and stretch (fibre of 20-4-83). The data for 18-4-83 were derived from changing the velocity of release/stretch, and by moving the sensor along the fibre. The data points at 18, 22, 29, 32, 34 and 53 Hz were obtained by method (1) (see text), with all sarcomeres shortening at the same velocity.

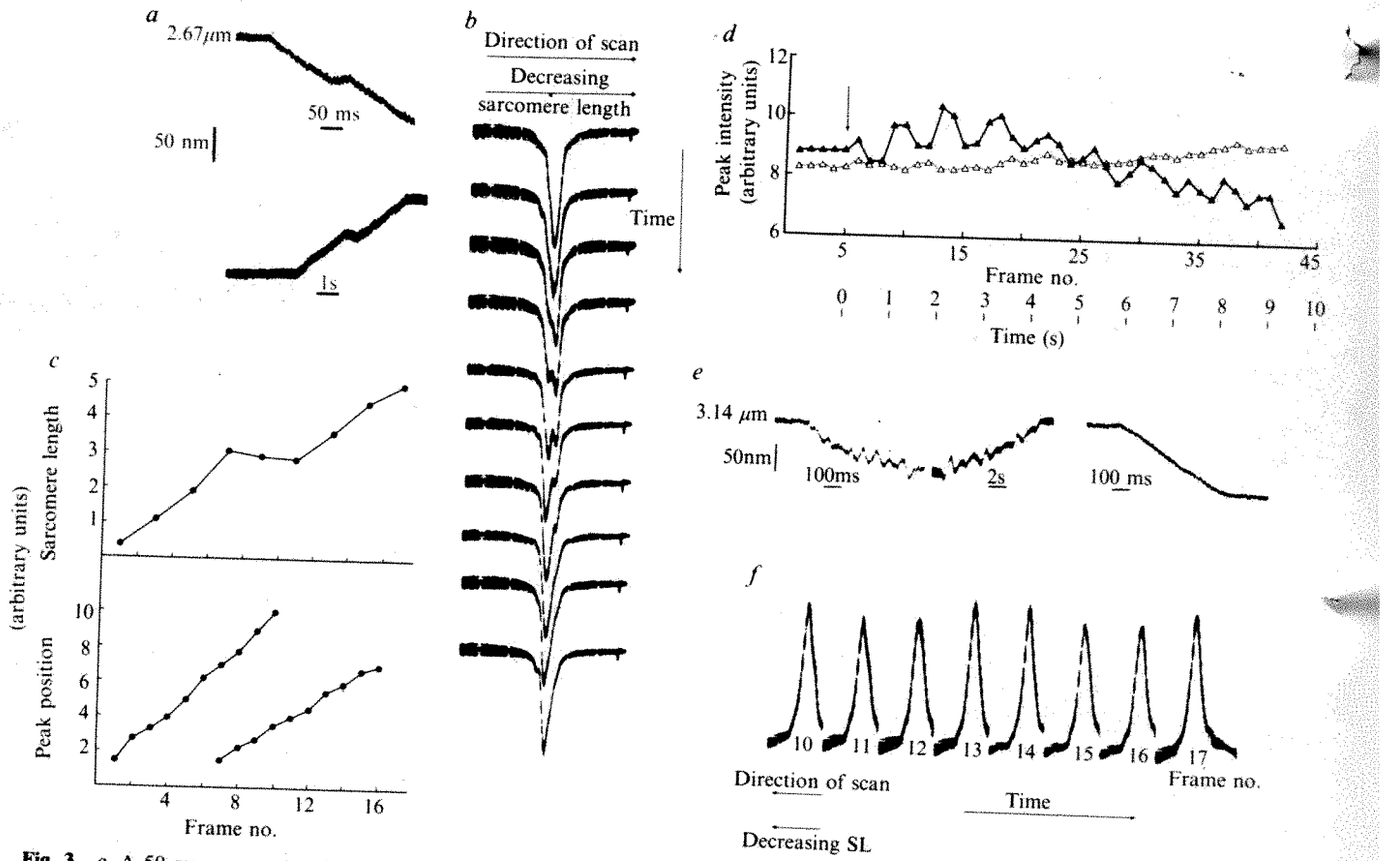


Fig. 3 *a*, A 50-ms pause, extended on the restretch to 1 s by slowing the length change. *b*, The first-order diffraction peak, as seen by the photodiode array, photographed at 4 frames per s during the extended pause. *c*, Sarcomere length, and the position of the two peaks of the first-order diffraction line (relative to an arbitrary reference line) plotted against time. Sarcomere length was plotted by electronically scanning the photodiode array from left to right (once every 256 μs), integrating the light intensity, and computing the distance along the array at which 50% of the integral was reached. The 50% integral value from each scan was carried over to the next, and logic circuits were switched when the integral reached this value—the sarcomere length. Thus, each sarcomere length computation used the first-order light intensity value from the previous scan. It was assumed that the intensity does not change significantly from scan to scan. This assumption is valid for most purposes, but when used close to the resolution limit, as here, the errors may be significant. In this case, the appearance of a secondary peak increased the area under the curve at long sarcomere lengths, switching the logic prematurely, and resulting in an apparent halt in sarcomere lengthening, despite the fact that both peaks moved continuously, as shown in *c*. *d–f* Shows a second example in which the steps/oscillations are the result of periodic intensity changes in an otherwise smooth, near-symmetrical first-order diffraction line. In *d*, the peak intensity of the diffraction line is plotted against time for data taken during the slow restretch of the release/stretch sequence illustrated in the left-hand and centre records of *e*. Note how symmetrical the sarcomere length changes are, despite the difference in time base. The number of intensity change cycles equals the number of sarcomere length oscillations, and the earlier, larger sarcomere length changes on the stretch are associated with the greatest intensity changes. Consistent with the method of sarcomere length determination, an increase in intensity leads to an overestimate of sarcomere length. Frames 10–17 of the diffraction line are shown in *f*. For comparison the open triangles in *d* are from data taken during a typical smooth sarcomere length change (*e*, right-hand record).

change velocity, it was possible to photograph the diffraction line during a prolonged pause (Fig. 3*a*). Here the apparent pause can be accounted for by the formation of a secondary peak (Fig. 3*b*), leading to a mis-computation of sarcomere length, as the method is based on the assumption that there are no significant changes in peak intensity and symmetry between computations (see Fig. 3 legend). Small changes in first-order intensity alone may also produce steps (Fig. 3*d–f*). The analyses in Fig. 3 are representative of a total of 14, from three fibres, at sarcomere lengths of 2.5–3.9 μm. In all cases steps were associated with detectable changes in the diffraction line.

Thus, changes in intensity and the formation of secondary peaks in the first-order line are possible causes of steps/oscillations. Both phenomena might arise from Bragg diffraction[2,12], while myofibrillar sliding, resulting in periodic variation in myofibrillar skew, might also contribute to intensity fluctuations[13,14]. Diffraction steps may therefore have several sources, explaining their often irregular appearance. The slope of the regression line for the PLL data indicates that one oscillatory cycle corresponds to the translation of one sarcomere past a given point in the optical field. This, and the regular appearance of PLL steps, suggests a single mechanism of generation, inherent in the method of sarcomere length determination.

Due to the rapid time course of a twitch, attempts to correlate step frequency with translation in active contractions would be difficult. However, one can estimate the upper and lower limits of translation velocity if fibre length and the extent and time course of internal shortening are known; all are readily available from our own and published data. From these calculations, translations of <20–70 μm in 50–150 ms might be expected; very similar values were calculated by Jacobson *et al.*[15]. From Figs 1 and 2, frequencies of <20–1,000 Hz are predicted, that is, half periods or pause durations of 1 to >25 ms. This range is consistent with that of published durations[1,3–7,11,15]. The wide variety of step patterns observed in active contractions might be expected, because translation will be random relative to that occurring during the linear length changes imposed on resting fibres. Without studying active contractions, the above must remain speculative.

Many published properties of the stepwise shortening phenomenon can be explained on the basis of a translation-dependent oscillation. The increase in pause duration observed under: increased load[11], decreased sarcomere length (and concomitant decrease in shortening velocity)[11] and near the peak of a twitch[3,11], can all be accounted for by the decrease in internal movements in these conditions. Decreasing translation velocity would decrease the oscillation frequency and hence increase pause duration.

That step generation may result from changes in the diffraction pattern is consistent with the observation that steps are more common in cardiac than in skeletal preparations[1,3]; it is well known that cardiac muscle gives poorer diffraction patterns.

Our studies revealed other features suggesting a non-physiological origin for steps. A 20 °C change in temperature had no effect on steps during passive length changes. The occurrence of steps at sarcomere lengths around 4.0 μm, indistinguishable from those at 2.4 μm (ref. 7), clearly indicates that steps do not result from acto-myosin interaction, unless there is a substantial change in filament length during the passive stretch of sarcomeres beyond 3.65 μm.

We thank Dr A. M. Gordon and Professor A. F. Huxley for their comments on the manuscript and Drs A. M. Gordon, M. E. Cantino and F. Fristoe for many stimulating and helpful discussions. This work was supported by postdoctoral fellowships from the SERC (J.D.A.), the Rotary Foundation (R.B.) and the AHA of Washington (J.W.L.).

Received 25 August; accepted 22 November 1983.

1. Pollack, G. H., Iwazumi, T., ter Keurs, H. E. D. J. & Shibata, E. F. *Nature* **268,** 757–759 (1977).
2. Rüdel, R. & Zite-Ferenczy, F. *Nature* **278,** 573–575 (1979).
3. Pollack, G. H., Vassallo, D. V., Jacobson, R. C., Iwazumi, T. & Delay, M. J. in *Cross-bridge Mechanism in Muscle Contraction* (eds Sugi, H. & Pollack, G. H.) 23–40 (University of Tokyo Press, 1979).
4. Pollack, G. H. *et al.* in *Contractile Mechanisms in Muscle* (eds Pollack, G. H. & Sugi, H.) (Plenum, New York, in the press).
5. Delay, M. J., Ishide, N., Jacobson, R. C., Pollack, G. H. & Tirosh, R. *Science* **213,** 1523–1525 (1981).
6. Lacktis, J. W., Brozovich, F. V. & Pollack, G. H. *Biophys. J.* **37,** 358a (1982).
7. Lacktis, J. W., Brozovich, F. V. & Pollack, G. H. *Biophys. J.* **41,** 264a (1983).
8. Iwazumi, T. & Pollack, G. H. *I.E.E.E. Trans. Biomed. Eng.* **26,** 86–93 (1979).
9. Myers, J., Tirosh, R., Jacobson, R. C. & Pollack, G. H. *IEEE Trans. biomed. Engng* **29,** 463–466 (1982).
10. Jacobson, R. C., Tirosh, R., Delay, M. J. & Pollack, G. H. *Biophys. J.* **33,** 223a (1981).
11. Vassallo, D. V. & Pollack, G. H. *Circulation Res.* **51,** 37–42 (1982).
12. Rüdel, R. & Zite-Ferenczy, F. *J. Physiol., Lond.* **290,** 317–330 (1979).
13. Oba, T., Baskin, R. J. & Lieber, R. L. *J. Muscle Res. Cell Motility* **2,** 215–218 (1981).
14. Yeh, Y., Baskin, R. J., Lieber, R. L. & Roos, K. P. *Biophys. J.* **29,** 519–522 (1980).
15. Jacobson, R. C., Tirosh, R., Delay, M. J. & Pollack, G. H. *J. Muscle Res. Cell Motility* (in the press).

● A REPLY from Dr H. E. Pollack has been received and is being considered.

Glycophorin is linked by band 4.1 protein to the human erythrocyte membrane skeleton

Richard A. Anderson* & Rex E. Lovrien

Department of Biochemistry, University of Minnesota, 140 Gortner Laboratory, St Paul, Minnesota 55108, USA

The cytoskeleton underlying the membrane of erythrocytes is thought to control changes in cell shape such as the diskocyte to the echinocyte. Since the binding of lectins to the transmembrane protein glycophorin blocks the cell shape change, we have proposed that the cytoplasmic end of glycophorin is linked to the cytoskeleton[1]. Here we show that the cytoskeletal protein known as band 4.1 specifically associates with the cytoplasmic domain of glycophorin on inside-out erythrocyte membrane vesicles and also with glycophorin reconstituted into phosphatidylcholine vesicles. The binding of band 4.1 to glycophorin is saturable and inhibitable by antibodies which bind specifically to the cytoplasmic domain of glycophorin. We therefore believe that band 4.1 protein provides the link between glycophorin and the cytoskeleton.

Glycophorin was isolated[2,3], desialated and incorporated into phosphatidylcholine vesicles (dGP-vesicles) by dialysis of a mixed micellar solution of octyl glucoside, phosphatidylcholine and desialoglycophorin[4]. The resulting dGP-vesicles are large enough to be easily sedimented using the ultracentrifuge. It is this property which allows proteins that associate with the vesicles to be separated from proteins that remain free in solution.

Membrane skeletal proteins were sequentially solubilized from isolated erythrocyte membranes[5] by extracting with buffered solutions of various ionic strengths[6,7]. The membrane skeletal extracts are enriched in specific proteins (refs 7, 8 and Fig. 1). The extracted proteins were combined with dGP-vesicles and incubated at 37 °C. The dGP-vesicles with bound proteins were then sedimented in the ultracentrifuge and the supernatant was removed. Protein from the vesicle pellet was analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE, Fig. 1). As a control an equivalent amount of phosphatidylcholine vesicles (PC-vesicles) prepared identically to dGP-vesicles but without glycophorin were combined with membrane skeletal proteins and analysed in an identical fashion (Fig. 1). The SDS-PAGE shows that only one membrane skeletal protein (band 4.1) specifically bound to the dGP-vesicles. Although several proteins bound nonspecifically to both kinds of vesicles, only band 4.1 showed a large relative increase in the amount bound to dGP-vesicles. Quantification of the amount of band 4.1 bound[8] shows that dGP-vesicles bound 16-fold more band 4.1 (4.7 μg) than did PC-vesicles (0.3 μg) (Fig. 1, lanes *f*, *g*).

In a similar experiment, membrane skeletal proteins extracted with 1 M KCl (mainly spectrin, bands 2.1, 6, 4.9 and 4.1) were

* Present address: Department of Pathology, Yale University School of Medicine, 310 Cedar Street, New Haven, Connecticut 06510, USA.

0028-0836/84/070655-04$01.00

© 1984 Macmillan Journals Ltd

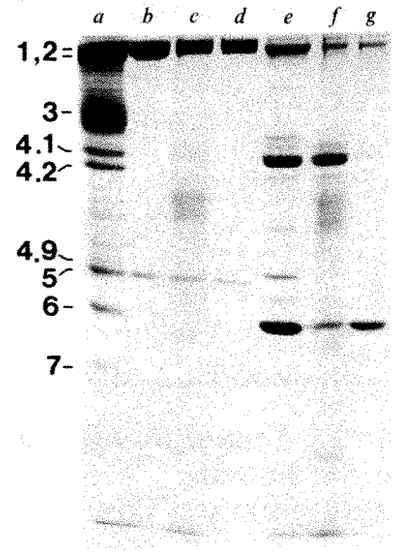


Fig. 1 SDS-PAGE of membrane skeletal protein extracts and membrane skeletal proteins bound to dGP- and PC-vesicles. Samples are: *a*, membrane proteins, 25 μg; *b*, low ionic strength extract, 7 μg protein; *c*, dGP-vesicles + low ionic strength extracted protein; *d*, PC-vesicles + low ionic strength extracted protein; *e*, 1 M KCl extracted protein, 14 μg protein; *f*, dGP-vesicles + 1 M KCl extracted protein; *g*, PC-vesicles + 1 M KCl extracted protein.

Methods: Isolated glycophorin[2] (15 mg ml^{-1}) was desialated with *Clostridium perfringens* neuraminidase (0.1 U ml^{-1}) at *p*H 5.0 for 1 h at 37 °C. The desialoglycophorin was then reconstituted into large phosphatidylcholine vesicles[4]. To a thin film of phosphatidylcholine was added octyl-β-D-glucopyranoside (octyl glucoside) and desialoglycophorin in water. The resulting mixed micellar solution of phosphatidylcholine (36 mM), octyl glucoside (340 mM) and desialoglycophorin (0.12 mM) was extensively dialysed against 140 mM NaCl, 5 mM sodium phosphate, 3.0 mM NaN_3, 0.5 mM DL-dithiothreitol (DTT) at *p*H 7.6. The solution of dGP-vesicles was applied to a 2.0 × 38 cm Sepharose CL-4B column equilibrated with 130 mM KCl, 10 mM NaCl, 5 mM sodium phosphate, 3 mM NaN_3, 1 mM EDTA, 0.5 DTT at *p*H 7.6 (130 mM KCl buffered). Vesicles with reconstituted desialoglycophorin eluted in the column void. Control vesicles (PC-vesicles) were prepared identically, except without desialoglycophorin. Membrane skeletal proteins were extracted from isolated erythrocyte membranes using: (1) low ionic strength buffer to extract spectrin and actin and (2) 1 M KCl buffer to extract primarily bands 2.1, 4.1, 6 and residual spectrin (refs 5 and 6 describe the complete buffers). The cytoskeletal protein solutions were dialysed against 130 mM KCl, 10 mM NaCl, 5 mM sodium phosphate, 0.5 mM DTT, 0.1 mM phenylmethylsulphonyl fluoride (PMSF) and then combined with dGP- and PC-vesicles. The final concentration of membrane skeletal protein was 20 μg ml^{-1}; glycophorin concentration was 50 μg ml^{-1}. The concentration of phosphatidylcholine was 250 μg ml^{-1} for both dGP- and PC-vesicles. Solutions of membrane skeletal proteins and vesicles were incubated at 37 °C for 20 min then centrifuged at 150,000*g* for 1 h at 2 °C. The vesicle pellets were solubilized in sample buffer, applied to a SDS-PAGE and developed according to Laemmli's method[22].

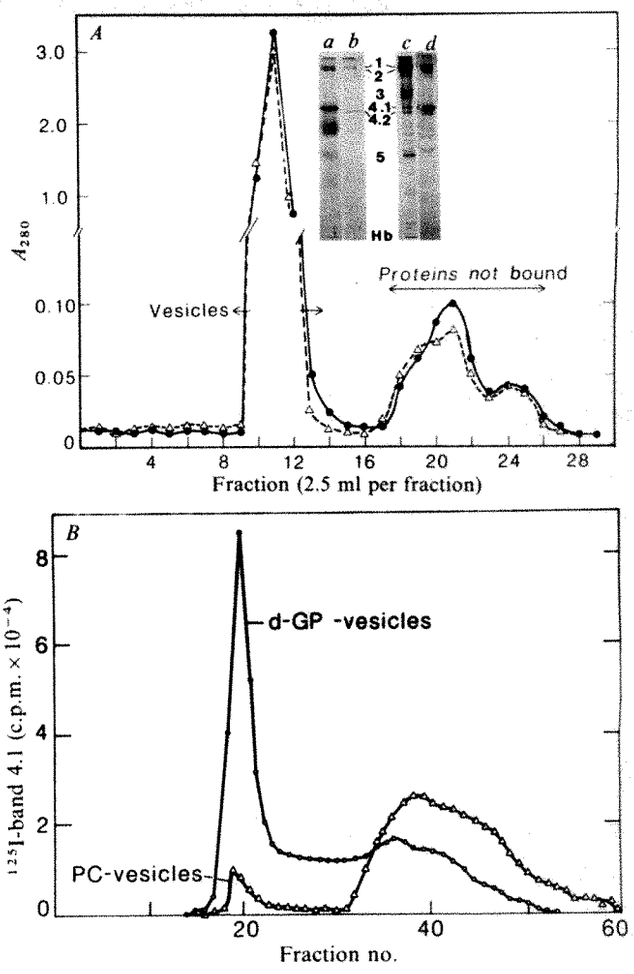


Fig. 2 *A*, Band 4.1 remains bound to dGP-vesicles (△) after chromatography of Sepharose CL-4B, but not to PC-vesicles (●). The samples on SDS-PAGE are: *a*, proteins bound to dGP-vesicles; *b*, proteins bound to PC-vesicles; *c*, 20 μg of total membrane proteins; *d*, 12 μg of protein extracted from membranes with 1 M KCl buffer. *B*, Elution profile of ^{125}I-protein 4.1 which was combined with dGP-vesicles (○) or with PC-vesicles (△).

Methods: *A*, The 1 M KCl extracted membrane skeletal proteins (100 μg ml^{-1}) were combined with dGP-vesicles (100 μg ml^{-1} glycophorin) or PC-vesicles. The solutions, after incubation at 37 °C for 20 min, were applied to a 2 × 38 cm Sepharose CL-4B column. Vesicles eluted from the column in the void together with bound protein. The vesicles which eluted from the column were concentrated by centrifugation (as in Fig. 1) and the protein in the vesicle pellet was analysed by SDS-PAGE. *B*, Purified band 4.1 was labelled with ^{125}I Bolton–Hunter reagent[6,7]. The ^{125}I-labelled band 4.1 (50 μg ml^{-1}) was combined with dGP-vesicles (200 μg ml^{-1} glycophorin) or an equivalent amount of PC-vesicles; after incubation the solution was applied to a 2 × 38 cm Sepharose CL-4B column as above. The elution profile was monitored by following the ^{125}I label. The vesicle peak was determined by turbidity measurements at 280 nm.

combined with either dGP-vesicles or PC-vesicles. Following incubation at 37 °C the vesicle solutions were applied to a Sepharose 4B-CL column. Both kinds of vesicles eluted from the column in the void and were completely separated from proteins which were not bound (Fig. 2*A*). The vesicles were then analysed by SDS-PAGE as above, comparing the proteins remaining bound to dGP-vesicles (Fig. 2*A*, lane *a*) with proteins binding to PC-vesicles (lane *b*). In these conditions dGP-vesicles retained about 40-fold more band 4.1 than did PC-vesicles: 3.9 μg compared with <0.1 μg of bound band 4.1, respectively. Spectrin was the only other protein which bound to the vesicles. The dGP-vesicles also retained more spectrin than did PC-vesicles, possibly due to the spectrin–band 4.1 association[6,7].

To establish that band 4.1 associates specifically with dGP-vesicles without participation of other proteins, band 4.1 was purified and labelled with ^{125}I using the Bolton–Hunter reagent[7,8]. Purified band 4.1 showed the characteristic major doublet on Laemmli SDS-PAGE, with two minor bands[9], above and below band 4.1, representing about 15% of the total applied proteins. All three bands are immunologically cross-reactive with anti-band 4.1 antibody[9]. The ^{125}I-labelled band 4.1 was combined with dGP-vesicles or PC-vesicles and the vesicle solutions were applied to a Sepharose 4B column. Elution of band 4.1 was monitored by following the ^{125}I label. The elution profile of band 4.1 from the Sepharose column (Fig. 2*B*) demonstrates that a large fraction (37%) of the ^{125}I-band 4.1 remained bound

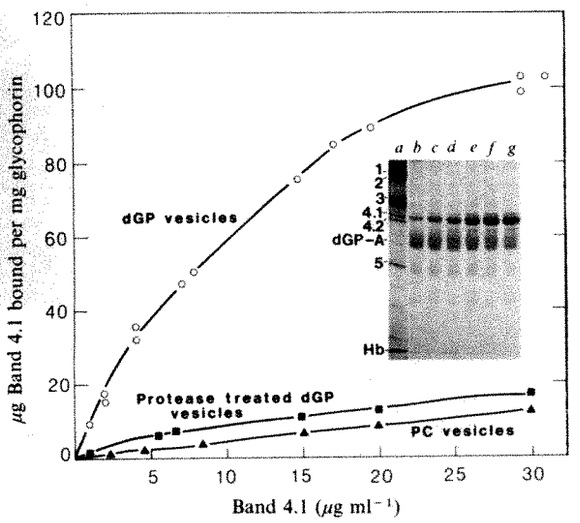


Fig. 3 Binding isotherms of native protein 4.1 to dGP-vesicles (○), protease-treated dGP-vesicles (■) and PC-vesicles (▲). An SDS-PAGE of band 4.1 bound to dGP-vesicles is shown. Samples are: *a*, 30 μg of total membrane protein; *b–g*, protein from the dGP-vesicle pellet, the band 4.1 concentration being varied over 2–30 μg ml^{-1}.

Methods: The binding isotherms were determined by combining dGP-vesicles (60 μg ml^{-1} glycophorin) with increasing amounts of native band 4.1. The solutions were incubated as described in Fig. 2 legend, followed by centrifugation of the dGP-vesicles with bound protein. The resulting vesicle pellet was prepared for SDS-PAGE as in Fig. 1. To the SDS-PAGE (separate lanes) was applied between 1 and 10 μg per lane of ultra-pure bovine serum albumin (BSA) as a protein standard. The amount of band 4.1 in each lane of the SDS-PAGE was quantitated by extracting and quantitating the Coomassie blue stain. The amount of dye bound was compared with a standard curve generated with known amounts of BSA applied to the same SDS-PAGE[8]. From the standard curve the amount of band 4.1 bound was determined as a ratio of μg band 4.1 bound per mg glycophorin. The SDS-PAGE shows that the desialoglycophorins all migrate well below band 4.1 on the SDS-PAGE and thus do not interfere with dye extraction from the 4.1 band; ~70 μg per lane glycophorin was applied. Binding is plotted in terms of μg band 4.1 bound per mg reconstituted glycophorin versus the total band 4.1 concentration in μg ml^{-1} (○). Nonspecific binding of band 4.1 was determined using an equivalent amount of phosphatidylcholine vesicles without incorporated desialoglycophorin (▲). Desialoglycophorin reconstituted into phosphatidylcholine vesicles was cleaved with a 20:1 weight ratio of desialoglycophorin to both trypsin and chymotrypsin at 37 °C for 4 h. Protease activity was destroyed by addition of 0.1 mM diisopropyl fluorophosphate (DFP). Binding of band 4.1 to protease-treated dGP-vesicles is plotted in terms of μg band 4.1 bound per mg of original glycophorin (■).

and co-eluted with the dGP-vesicles. In contrast, only a small fraction (2.5%) of the ^{125}I label co-eluted with PC-vesicles. Analysis of these vesicles by SDS-PAGE showed that 85–90% of the ^{125}I label bound to dGP-vesicles migrated with band 4.1. The majority (70–80%) of the ^{125}I label bound to PC-vesicles migrated with the small molecular weight minor protein band on SDS-PAGE, not with band 4.1. The dGP-vesicles in these conditions retained 60-fold more band 4.1 than did an equivalent amount of PC-vesicles.

To demonstrate further that band 4.1 associates specifically with reconstituted glycophorin, a binding isotherm of band 4.1 to dGP-vesicles was determined. The binding isotherm (Fig. 3), determined using native band 4.1, illustrates that the association of 4.1 with dGP-vesicles is saturated at ~110 μg of band 4.1 bound per mg glycophorin. The binding constant is $1.1 \pm 0.4 \times 10^7$ M^{-1}. Nonspecific binding was determined using an equivalent amount of PC-vesicles (Fig. 3). The band 4.1 binding sites on dGP-vesicles are removed by proteolysis with trypsin and chymotrypsin, indicating that band 4.1 binding requires a protease-sensitive peptide (Fig. 3).

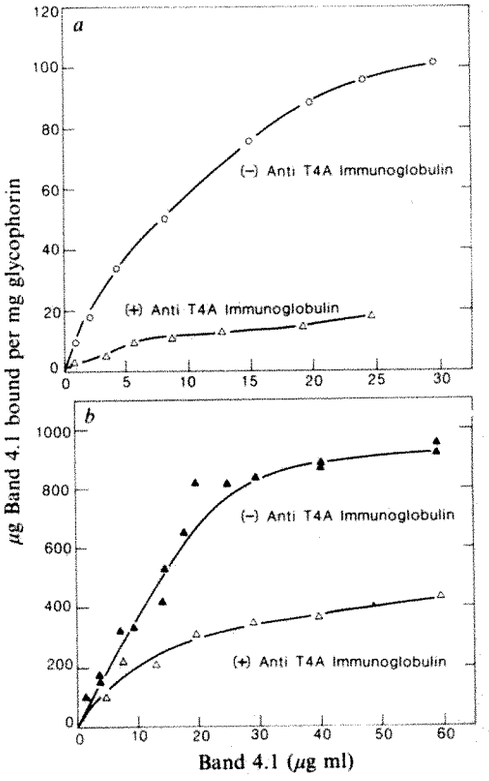


Fig. 4 *a*, Binding isotherm of ^{125}I-Bolton–Hunter labelled band 4.1 to dGP-vesicles (○) and inhibition of ^{125}I-band 4.1 binding to dGP-vesicles by anti-glycophorin cytoplasmic domain antibody (anti-T4A antibody) (△). *b*, Binding of ^{125}I-band 4.1 to IOVs (▲) and inhibition of ^{125}I-band 4.1 binding to IOVs using anti-T4A antibody (△).

Methods: *a*, Band 4.1 (200 μg) was labelled with 0.2 mCi of ^{125}I-Bolton–Hunter reagent[7]. Labelled band 4.1 (0.8 mCi mg^{-1}) with full biological activity was isolated by binding ^{125}I-band 4.1 to dGP-vesicles, separating the biologically active ^{125}I-band 4.1 from inactive ^{125}I-band 4.1 by centrifugation or chromatography as in Fig. 2*B* and then eluting the bound ^{125}I-band 4.1 from the dGP-vesicles with 1 M KCl buffer. The ^{125}I-band 4.1 was dialysed against 130 mM KCl buffer and diluted with cold band 4.1 to a specific activity of ~1 μCi per mg. This ^{125}I-band 4.1 was used for band 4.1 binding to dGP-vesicles. (Sheep antiserum raised against the cytoplasmic domain of glycophorin A[11] (cyanogen bromide fragment 2) was a gift from Drs Marchesi and Furthmayr, Yale University.) Pure antibody was isolated by affinity chromatography on the T4A(B) tryptic fragment of glycophorin A which was coupled to Sepharose-4B with cyanogen bromide[23]. To inhibit band 4.1 binding to dGP-vesicles, the vesicles were pretreated with antibody at a 1:1 antibody/glycophorin molar ratio. However, as glycophorin is asymmetrically incorporated into phospholipid vesicles it is likely that at most only 20% of the glycophorins have available cytoplasmic domains[4], thus a 5:1 molar ratio is more likely. At such a molar ratio of antibody to glycophorin cytoplasmic domain the antibody binding sites are likely to be saturated. *b*, IOVs were prepared according to refs 14 and 15. The amount of glycophorin in these IOVs was determined by assaying for sialic acid[24], assuming 70% of total sialic acid is attached to glycophorins and that glycophorins are 30% sialic acid by weight. The concentration of glycophorin in IOVs used for binding was 4 μg ml^{-1} (30 μg ml^{-1} total protein). The amount of bound ^{125}I-band 4.1 was determined by counting the vesicle pellet. Inhibition of band 4.1 binding to IOVs was accomplished by using a fivefold antibody excess as above.

Reconstitution of glycophorin into phosphatidylcholine vesicles results in an asymmetric orientation of glycophorin across the lipid bilayer. The majority of the carbohydrate-bearing domains are located on the outside surface of the vesicles[4,10]. Such asymmetric orientation corresponds well with the binding isotherm, as asymmetric orientation of glycophorin would result in the low band 4.1:glycophorin stoichiometry that is observed

at saturation, if band 4.1 associates with the cytoplasmic domain of glycophorin.

Further evidence that band 4.1 associates with the cytoplasmic domain of glycophorin is provided by antibodies directed against the cytoplasmic domain of glycophorin A. These antibodies were used to inhibit band 4.1 binding to vesicles containing glycophorin. Antiserum was raised in sheep immunized against the cyanogen bromide fragment (CB2) containing the cytoplasmic domain of glycophorin A (ref. 11). Pure antibody specific for the cytoplasmic domain of glycophorin was isolated by affinity chromatography using the T4A fragment of glycophorin A[11,12] as the affinity ligand attached to Sepharose 4B. The T4A fragment is the major portion of the glycophorin A cytoplasmic domain[11,12].

Band 4.1 binding and inhibition of binding was monitored with ^{125}I-labelled band 4.1 (refs 7, 8). The binding isotherm of ^{125}I-band 4.1, with full biological activity towards dGP-vesicles (Fig. 4*a*), compares well with that determined using native band 4.1 (Fig. 3). Anti-T4A antibody, preincubated with dGP-vesicles before addition of protein 4.1, blocked 70–80% of the band 4.1 binding sites on dGP-vesicles. However, when pure glycophorin A was reconstituted into phosphatidylcholine vesicles, band 4.1 specifically bound to these vesicles and anti-T4A antibody completely inhibited (>90%) the band 4.1 binding. Moreover, when glycophorin B was reconstituted into phosphatidylcholine vesicles, band 4.1 specifically bound to these vesicles, but the binding was not inhibited by anti-T4A antibody (unpublished results). Such behaviour is expected, as anti-T4A antibody does not cross-react with glycophorin B[13].

Inverted erythrocyte membrane vesicles (IOVs) were isolated from fresh erythrocyte membranes[5] and stripped of membrane skeletal proteins[14,15]. The stripped IOVs were used to establish a binding isotherm of ^{125}I-labelled band 4.1 to the cytoplasmic surface of the erythrocyte membrane (Fig. 4*b*). The binding isotherm shows saturation and approximately the same binding affinity as band 4.1 binding to dGP-vesicles. At saturation 880 μg of band 4.1 are bound per mg of glycophorin. Pretreatment of stripped IOVs with anti-T4A antibody blocked at least 60% of the band 4.1 binding sites (Fig. 4*b*). Only the cytoplasmic domain of glycophorin A was blocked by anti-T4A antibody. Thus, at least 60% of the band 4.1 binding sites are contributed by glycophorin A. The remaining binding sites may be contributed by the cytoplasmic domains of glycophorin B and glycophorin C which would still be available for binding[16]. Thus in En(−a) erythrocytes, which are thought to be devoid of glycophorin A[17], glycophorins B and C could still contribute enough membrane attachment sites to accommodate all of the protein 4.1 molecules in the cell.

The above results provide evidence that band 4.1 specifically associates with the cytoplasmic domain of glycophorin A, and also possibly glycophorin B. Previously, band 4.1 was shown to associate tightly with spectrin[6,7]. The binding site for band 4.1 on spectrin is located in close proximity to the spectrin–actin association site[6,7]. Furthermore, association of band 4.1 with spectrin markedly enhanced the interaction between spectrin and actin protofilaments[18–21]. As such, the effect of band 4.1 on the spectrin–actin association could have a regulatory role in controlling the dynamics of membrane skeleton change. Control of membrane skeletal change may in turn be mediated by externally bound lectins via the interaction between transmembrane glycophorin and band 4.1.

Received 23 October; accepted 28 November 1983.

1. Anderson, R. A. & Lovrien, R. *J. Cell Biol.* **85**, 534–548 (1980); *Nature* **292**, 158–161 (1981).
2. Marchesi, V. T. & Andrews, E. P. *Science* **174**, 1247–1248 (1971).
3. Furthmayr, H., Tomita, M. & Marchesi, V. T. *Biochem. Biophys. Res. Commun.* **65**, 113–121 (1975).
4. Mimms, L. T., Zampighi, G., Nozaki, Y., Tanford, C. & Reynolds, J. A. *Biochemistry* **20**, 833–840 (1981).
5. Dodge, J. T., Mitchell, C. & Hanahan, D. J. *Archs Biochem. Biophys.* **100**, 119–130 (1963).
6. Tyler, J. M., Hargreaves, W. R. & Branton, D. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5192–5196 (1979).
7. Tyler, J. M., Reinhardt, B. N. & Branton, D. *J. biol. Chem.* **255**, 7034–7039 (1980).
8. Fenner, C., Traut, R. R., Mason, D. T. & Wikman-Coffel, J. *Analyt. Biochem.* **63**, 595–602 (1975).
9. Cohen, C. M., Foley, S. F. & Korsgren, C. *Nature* **299**, 648–650 (1982).
10. Ong, R. L., Marchesi, V. T. & Prestegard, J. H. *Biochemistry* **20**, 4283–4292 (1981).
11. Cotmore, S. F., Furthmayr, H. & Marchesi, V. T. *J. molec. Biol.* **113**, 539–553 (1977).
12. Tomita, M., Furthmayr, H. & Marchesi, V. T. *Biochemistry* **17**, 4756–4770 (1978).
13. Furthmayr, H. *Nature* **271**, 519–524 (1978).
14. Hargreaves, W. R., Giedd, K. N., Verkleij, A. & Branton, D. *J. biol. Chem.* **255**, 11965–11972 (1980).
15. Steck, T. L., Weinstein, R. S., Strauss, J. H. & Wallach, D. F. H. *Science* **168**, 255–257 (1970).
16. Mueller, T. J. & Morrison, M. in *Erythrocyte Membranes* Vol. 2, 95–112 (Liss, New York, 1981).
17. Dahr, W., Uhlenbruck, G., Wagstaff, W. & Leikola, J. J. *J. Immunogenet.* **3**, 383–393 (1976).
18. Ungewickell, E., Bennett, P. M., Calvert, R., Ohanian, V. & Gratzer, W. G. *Nature* **280**, 811–814 (1979).
19. Fowler, V. & Taylor, D. L. *J. Cell Biol.* **85**, 361–376 (1980).
20. Brenner, S. L. & Dorn, E. D. *J. biol. Chem.* **254**, 8620–8627 (1979).
21. Cohen, C. M. & Korsgren, C. *Biochem. biophys. Res. Commun.* **97**, 1429–1435 (1980).
22. Laemmli, V. *Nature* **227**, 680–681 (1970).
23. March, S. C., Parikn, I. & Cuatrescasas, P. *Analyt. Biochem.* **60**, 149–152 (1974).
24. Warren, L. *J. biol. Chem.* **234**, 1971–1975 (1959).

Activation of the mouse cellular Harvey-*ras* gene in chemically induced benign skin papillomas

Allan Balmain, Martin Ramsden, George T. Bowden* & John Smith

Beatson Institute for Cancer Research, Garscube Estate, Bearsden Road, Glasgow G61 1BD, UK
* Division of Radiation Oncology, College of Medicine, University of Arizona, Tucson, Arizona 85724, USA

An important feature of the development of many human and animal tumours is the appearance of pre-malignant benign lesions, some of which undergo further changes during progression to malignancy[1–4]. Many of the currently accepted concepts of multi-stage carcinogenesis have been developed using an experimental model based on the chemical induction of tumours in mouse skin[3,4]. In this system, many of the pre-malignant papillomas which arise are promoter-dependent, and appear to regress if promoter treatment is interrupted, whereas others progress to form autonomous benign lesions and, in some cases, malignant carcinomas[5]. Although the number and nature of the events leading to malignancy are not known, DNA transfection experiments have led to the identification of several genes which may be qualitatively altered in tumour cells (see ref. 6 for review). We have previously shown that DNA from transplantable mouse skin carcinomas induced by chemical carcinogens has the ability to transform NIH/3T3 cells, and that the gene responsible for the transformation is an activated form of the mouse cellular Harvey-*ras* gene (c-*ras*^{H})[7]. We have now investigated the stage of carcinogenesis at which the proto-oncogene acquires transforming activity. We demonstrate that primary papillomas induced by chemical carcinogens in two different mouse strains have an activated c-*ras*^{H} gene. This constitutes the first report of a benign tumour which contains DNA with detectable transforming activity. In addition, steady-state levels of c-*ras*^{H} gene transcripts are elevated in the papillomas as compared with normal epidermis.

Both benign and malignant skin lesions can be induced in mouse skin by sequential treatment with initiators and promoters of carcinogenesis[3,4]. Tumours were induced in the dorsal skin of Sencar mice, a strain bred for sensitivity to chemical carcinogens[8,9], by initiation with dimethylbenzanthracene (DMBA) followed by multiple treatments with the tumour promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA). In this strain individual papillomas and carcinomas are large enough to provide sufficient DNA for analysis by transfection into NIH/3T3 cells. Table 1 shows the results obtained when several primary tumours were tested for transforming activity. Of five DNA samples prepared from papillomas, four showed positive results in transfection assays. Similarly, two out of three carcinomas induced foci of transformed cells in NIH-3T3 fibroblasts. In the same series of experiments, DNA from normal

0028-0836/84/070658—03$01.00

© 1984 Macmillan

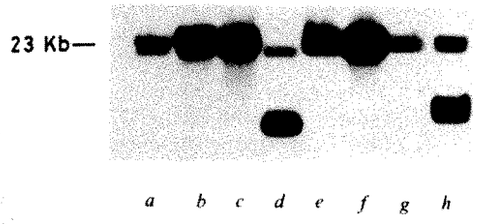


Fig. 1 Southern blot analysis of sequences related to the v-*ras*^{H} gene in NIH/3T3 transformants induced by primary mouse papilloma and carcinoma DNAs. The DNAs were from the following sources: *a*, NIH/3T3; *b*, NIH/3T3 transformant obtained by transfection with primary carcinoma DNA; *c–f*, *h*, transformants induced by different papilloma DNAs; *g*, spontaneously arising transformant.

Methods: Tumours were induced on the dorsal skins of Sencar or NMRI mice by sequential treatment with DMBA and TPA[3,4]. High molecular weight DNA was isolated as previously described[7]. DNA (20 μg) from NIH/3T3 transformants was digested with *Eco*RI, electrophoresed through a 1% agarose gel, blotted onto nitrocellulose and hybridized with a nick-translated v-*ras*^{H} specific probe (BS9)[22]. Blots were washed to a final stringency of 0.5 × SSC at 65 °C and exposed to Kodak XAR5 film with intensifying screens at −70 °C.

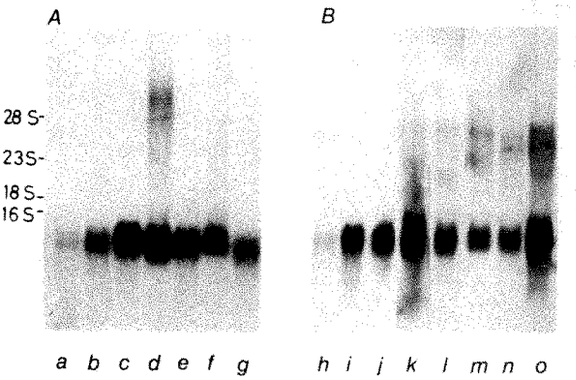


Fig. 2 *A*, Northern blot of c-*ras*^{H} transcripts in primary tumours. Total cellular RNAs were isolated from the following sources: *a*, normal epidermis; *b*, TPA-treated epidermis; *c–e*, individual papillomas from Sencar mice; *f*, pooled small papillomas from NMRI mice; *g*, Sencar carcinoma. *B*, Transcripts of c-*ras*^{H} sequences in NIH/3T3 transformants. Sources of the RNA were: *h*, NIH/3T3 cells; *i*, *j*, NIH/3T3 transformants induced by transplanted NMRI carcinoma DNA[7]; *k–n*, transformants induced by papilloma DNA; *o*, transformant induced by primary carcinoma DNA.

Methods: Frozen tumours or epidermis were ground in liquid nitrogen and lysed in 5 M guanidinium thiocyanate, 50 mM Tris-HCl (*p*H 7.0), 50 mM EDTA and 5% (v/v) 2-mercaptoethanol. Total cellular RNA was isolated from the lysate by centrifugation through a 5.7 M CsCl, 50 mM EDTA (*p*H 7.0) cushion, followed by ethanol precipitation. Aliquots (10 μg) of RNA were heated to 60 °C for 5 min in 50% deionized formamide, 2.2 M formaldehyde, 20 mM MOPS buffer, cooled rapidly, electrophoresed through 1% agarose gels in MOPS buffer (20 mM morpholinopropanesulphonic acid, 5 mM Na acetate, 1 mM EDTA *p*H 7.0), blotted onto nitrocellulose and hybridized to the ^{32}P-labelled v-*ras*^{H}-specific probe.

epidermis or from epidermis induced to proliferate by a single treatment with TPA failed to induce any stable transformants. Preliminary experiments using NIH mice (A.B. and J.S., unpublished results) also suggest that chronic treatment with TPA (three times weekly for 7 weeks) does not cause activation of transforming genes to a level detectable by the NIH/3T3 transfection assay. When primary transformants induced either by papilloma or carcinoma DNAs were grown in bulk culture, all the high molecular weight DNAs from these cells were positive when tested in second rounds of transfection into NIH/3T3 fibroblasts, indicating that a stable transforming gene was being transferred from both papillomas and carcinomas.

It has previously been noted that some papillomas can develop on the dorsal skin of Sencar mice after repeated promoter treatment but without any prior initiation[9]. This suggested that these mice may have a constitutive hereditary mutation which predisposes to the development of skin tumours. However, the lack of transforming ability of DNA from normal Sencar epidermis or from proliferating epidermis treated with TPA suggests that the transforming activity of the papilloma DNA cannot be attributed to a constitutive mutation in this mouse strain.

It remained possible, however, that Sencar papillomas might be abnormally 'advanced' in terms of tumour progression, and that transforming activity in other less sensitive mouse strains might only be acquired at the carcinoma stage. For this reason, papillomas were induced in NMRI mice and tested for transforming activity. Two independent DNA preparations from pooled small papillomas were positive in transfection assays, whereas liver or brain DNAs from the same inbred mouse strain were negative.

The transformation efficiencies with all of the primary tumour DNAs, including both papillomas and carcinomas, were about 5–10-fold lower than DNAs from the human EJ bladder carcinoma cell line[10–13] or a transplanted mouse carcinoma[7]. Similar low efficiencies have also been reported for a number of primary human tumours[14]. Note that the transforming efficiencies of all papilloma DNA samples were at least as high as that of the primary carcinomas, indicating that the results cannot be attributed to the presence of a small, histologically undetectable proportion of malignant carcinoma cells within some of the papillomas. In addition, the similar efficiencies obtained with the pooled small NMRI papillomas and the individual large Sencar papillomas demonstrate that papilloma size *per se* is not an important determinant of transforming gene concentration.

We have previously shown that transplanted carcinomas of NMRI mice contain an activated form of the c-*ras*^{H} gene[7]. To determine whether the *ras*^{H} gene is reproducibly activated during chemical carcinogenesis in the mouse skin model system, DNA from transformants induced by papilloma or carcinoma DNAs was isolated and analysed by Southern blotting and hybridization with a *ras*^{H}-specific probe. Newly acquired *ras*^{H} genes in transformed foci can be detected either by comparing band intensities with appropriate controls on genomic Southern blots or, more convincingly, by the presence of additional restriction fragments containing the extra gene(s) due to loss of a restriction site on integration. Figure 1 shows that DNAs from all of the foci induced by both papillomas and carcinomas exhibit a large increase in the intensity of hybridization with a *ras*^{H} probe, compared with normal 3T3 controls. In addition, some of the foci (lanes *d*, *h*) have an additional restriction fragment containing the extra *ras*^{H} genes, with the NIH/3T3 gene visible at the normal 23-kilobase (kb) position. No evidence for increased intensity of hybridization or additional restriction fragments were observed in spontaneously arising foci isolated from the same experiments (lane *g*). The amplification of the *ras*^{H} gene in the foci is not attributable to transfer of multiple copies from the orginal tumour cells, as these appear to contain only single copy *ras*^{H} sequences (data not shown). On probing the DNA of the foci with a Kirsten-*ras* specific probe, the intensity and pattern of hybridization in all foci was virtually identical to that in the 3T3 controls (data not shown), suggesting that the transfer and amplification had probably only occurred with the *ras*^{H} gene.

To determine whether c-*ras*^{H} transcripts are elevated in mouse skin tumours, RNA was prepared from a series of primary tumours and hybridized on Northern blots with a *ras*^{H}-specific probe. Figure 2*A* shows that the concentration of the major 1.4-kb transcript is increased in a series of papillomas relative to normal epidermis. This increase is apparent in individual Sencar papillomas (lanes *c–e*), in pooled small papillomas from NMRI mice (lane *f*), and, to a lesser extent, in a Sencar primary carcinoma (lane *g*). In view of the difficulties involved in estimating RNA concentrations by Northern blot hybridizations with a single probe, the blot shown in Fig. 2*A* has been re-hybridized with a cloned probe which is complementary to the ubiquitous, highly abundant cytoplasmic 7S RNA[15]. This demonstrated that

Table 1 Transforming activity of chemically induced skin papillomas and carcinomas

Source of DNA	Mouse strain	Samples tested	Positive samples‡
Normal epidermis	NIH/Swiss	2	0
	Sencar	2	0
TPA-treated epidermis	NIH/Swiss	1	0
	Sencar	1	0
Papilloma	Sencar	5*	4*
Carcinoma	Sencar	3	2
Papilloma	NMRI	2†	2†
Liver	NMRI	1	0
Brain	NMRI	1	0

Frozen tissues were ground in liquid nitrogen and DNA samples were prepared by resuspension in guanidinium thiocyanate and spinning through CsCl as previously described[7]. Transfections were carried out by adding 20 μg high molecular weight DNA to 25 cm^2 flasks seeded 24 h earlier with 3×10^5 cells per flask. Cells were maintained and foci scored as described earlier[7]. Epidermal cells were prepared from groups of 10–20 mice by scraping with a rounded scalpel after heating the skins in water at 55 °C for 30 s. Epidermal fractions were immediately frozen in liquid nitrogen.

* One DNA sample was prepared from three pooled papillomas.

† Both DNA samples were prepared from 8–10 pooled papillomas.

‡ Samples positive in transfection assay.

with the exception of the RNA from the papilloma in lane *e* and from the TPA-treated epidermis, approximately equivalent amounts of undegraded RNA were present in each lane (data not shown). Similar relative transcript levels have been found using RNA dot hybridization with probes specific for ras^H, 7S RNA and ribosomal RNA sequences (data not shown). We conclude that ras^H transcripts are elevated to a varying degree in different papillomas. The elevation is not due simply to an increase in the proportion of proliferating cells within the tumours because the hyperplastic epidermis induced by TPA does not contain substantially higher ras^H transcript levels.

An increase in the concentration of c-ras^H trancripts with respect to NIH/3T3 controls was also observed in transformed foci induced by transfection of DNA from papillomas or carcinomas (Fig. 2*B*). No reproducible correlation was found between steady-state concentrations of ras^H-related transcripts and the source of DNA used to induce the transformants.

The elevation of c-ras^H transcripts which is observed in the chemically induced primary tumours contrasts with the observations reported on the transcription of the equivalent human gene in the EJ/T24 bladder carcinoma cell line. Tabin *et al.*[16] compared c-ras^H transcript levels in EJ cells and in a normal human bladder cell line, and found no detectable differences. A possible explanation of this apparent contradiction is that the establishment of normal cells in culture leads to elevated ras^H gene expression, thus disguising any true increase during tumour development *in vivo*. In this context, it is interesting that elevated ras^H transcription has recently been found in several primary human tumours of the colorectum[17]. Alternatively, prolonged culture of tumour cells may result in lower levels of ras^H transcripts. It is also possible, if somewhat less likely, that differential extraction of RNA from basal or supra-basal cells could have occurred in our experiments, leading to artificially low ras^H transcript levels in control epidermis.

The number of independent genetic events involved in the transformation of a normal cell into a malignant tumour cell is unknown, but estimates range from two[18,19] to about seven[20]. It seems likely that the acquisition of dominant transforming properties by the DNA of malignant cells constitutes an important step in tumour progression. It has previously been speculated that this event occurs relatively late in tumour development[21]. This was based on the assumption that NIH/3T3 cells may already exist in an 'initiated' state, and require only one additional (late) event to acquire malignant properties. The data presented here, however, demonstrate that during chemical carcinogenesis *in vivo*, activation of the ras^H gene is a relatively early event. The proportion of papilloma DNAs which exhibited transforming activity (>85%) is much higher than the percentage of benign tumours which progress to carcinomas in this system (5–7%)[5]. This indicates that the mutational event which induces the transforming capacity of the DNA does not take place at the transition to malignancy, but already exists in most or all of the pre-malignant lesions.

Our results demonstrate that no significant differences are found between papillomas and carcinomas with respect to transforming activity of high molecular weight DNA and the degree of transcription of the activated c-ras^H gene. The progression of some, but not all, papillomas to malignant carcinomas may therefore be determined by another genetic event which does not involve the c-ras^H gene.

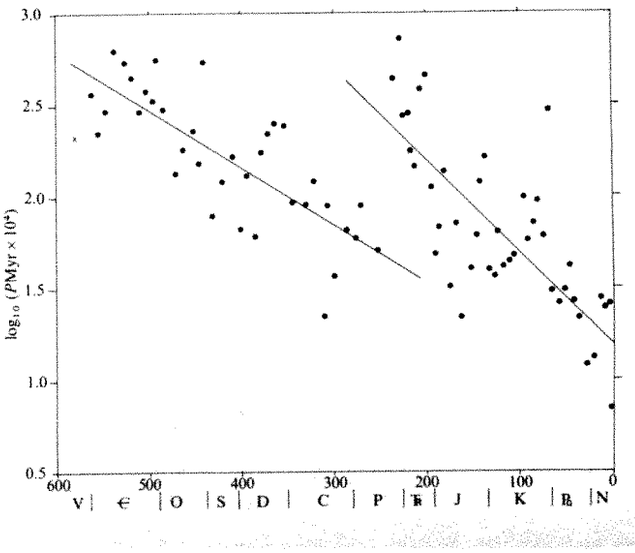
We thank Kl. Goerttler, H. Loehrke and T. Slaga for supplying tumour material, E. Hecker and G. Furstenberger for TPA, Gail Cole for technical assistance, and I. Kerr, G. D. Birnie and J. Paul for discussions and support. The Beatson Institute is supported by grants from the Cancer Research Campaign.

Received 28 July; accepted 15 December 1983.

1. Pierce, G. B. & Fennell, R. H. in *Cancer Medicine* (eds Holland, J. F. & Frei, E.) 149–167 (Lea & Febiger, Philadelphia, 1982).
2. Knudson, A. G. *Cancer Invest.* **1,** 187–193 (1983).
3. Hecker, E., Fusenig, N. E., Kunz, W., Marks, F. & Thielmann, H. W. (eds) *Carcinogenesis* Vol. 7 (Raven, New York, 1982).
4. Boutwell, R. K. *Crit. Rev. Tox.* **2,** 419–443 (1974).
5. Burns, F. J., Vanderlaan, M., Snyder, E. & Albert, R. E. In *Carcinogenesis* Vol. 2 (eds Slaga, T. J., Sivak, A. & Boutwell, R. K.) 91–96 (Raven, New York, 1978).
6. Cooper, G. M. Science **217,** 801–806 (1982).
7. Balmain, A. & Pragnell, I. B. *Nature* **303,** 72–74 (1983).
8. Digiovanni, J., Slaga, T. J. & Boutwell, R. K. *Carcinogenesis* **1,** 381–389 (1980).
9. Hennings, H. *et al. Cancer Res.* **41,** 773–779 (1981).
10. Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S. & Barbacid, M. *Nature* **298,** 343–347 (1982).
11. Der, C. J., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79,** 3637–3640 (1982).
12. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* **297,** 474–478 (1982).
13. Shimizu, K., Goldfarb, M., Perucho, M. & Wigler, M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 383–387 (1983).
14. Pulciani, S. *et al. Nature* **300,** 539–542 (1982).
15. Balmain, A., Krumlauf, R., Vass, J. K. & Birnie, G. D. *Nucleic Acids Res.* **10,** 4259–4277 (1982).
16. Tabin, C. J. *et al. Nature* **300,** 143–149 (1982).
17. Spandidos, D. & Kerr, I. B. *Br. J. Cancer* (submitted).
18. Armitage, P. & Doll, R. *Br. J. Cancer* **11,** 161–169 (1957).
19. Moolgavkar, S. H. & Knudson, A. G. *J. natn. Cancer Inst.* **66,** 1037–1051 (1981).
20. Cook, P. J., Doll, R. & Fellingham, S. A. *Int. J. Cancer* **4,** 93–112 (1969).
21. Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. *Nature* **300,** 149–152 (1982).
22. Ellis, R. W. *et al. J. Virol.* **36,** 408–420 (1980).

Errata

IN the letter 'A resetting of Phanerozoic community evolution' by L. M. Van Valen, *Nature* **307,** 50–52 (1984), the words 'It is therefore not feasible' in paragraph 5 should be replaced by 'This sort of analysis cannot be used'; and the reference to the 'Red Queen hypothesis' on page 51 should read 'Red Queen's hypothesis'. In addition, Fig. 1 was poorly reproduced: a correct version is shown below.



Lunar magnetism

IN a recent review of lunar palaeomagnetic investigations, Runcorn[1] has emphasized the possibility that an ancient core dynamo was the dominant source of the observed crustal magnetization by generating surface fields of 0.01–1.0 G in the $3.2–4.0 \times 10^3$ Myr time period. Major implications for lunar internal and dynamical history were discussed in part on the basis of both palaeomagnetic data and electromagnetic sounding data. Here we emphasize aspects of the lunar palaeomagnetic and electromagnetic sounding results which appear to be inconsistent with the core dynamo hypothesis; a more complete discussion can be found elsewhere[2].

First, various geophysical measurements limit the radius of a present-day lunar metallic core to a maximum of 400–500 km and do not require a metallic core of any radius. Therefore, the statement "evidence for an iron core in the Moon today (has) been found" (ref. 1) is somewhat misleading. Low-frequency electromagnetic sounding measurements for times when the Moon was in the solar wind or terrestrial magnetosheath[3,4] have been shown by Hobbs *et al.*[5], using inverse theory, to imply only an upper bound of 435 km on the radius of a possible metallic core; the data are equally consistent with a core radius of 0 km. Although Russell *et al.*[6], in an analysis of magnetometer records from periods when the Moon was in the lobes of the geomagnetic tail, obtained tentative evidence for a core with conductivity $\geq 10\ S\ m^{-1}$ and radius ≥ 400 km, a molten silicate core could equally well provide a conductivity of this magnitude so an iron core with conductivity $\geq 10^5\ S\ m^{-1}$ is not required. In addition, remaining questions about the possible effects of tail-lobe plasma on the induced moment measurements preclude acceptance of these results as definitive. Recent summaries of lunar seismic investigations conclude that seismic evidence for the existence of a metallic core is unresolved[7,8] with earlier suggestions of a possible molten core of radius ≤ 350 km continuing to depend on the interpretation of a single data point[9]. Other independent constraints are model-dependent to equal or greater extents as noted by Runcorn.

Anderson[10], using Busse's theory of planetary magnetism[11], has calculated that a lunar spin rate 20 times the present value combined with a fluid conducting core radius of 750 km would be necessary to produce surface palaeofields of 0.02 G, that is, approximately corresponding to minimum palaeointensities during the 3.2–4.0 Myr time period. To produce surface fields of ~1 G, corresponding to the high-field epoch discussed by Runcorn and Cisowski *et al.*[12], a core radius exceeding 1,000 km would be required. Therefore, assuming the first-order validity of Busse's model, the geophysical constraints on the radius of a metallic core summarized above are inconsistent with an iron core dynamo origin for lunar palaeomagnetism. In order to overcome these difficulties, Cisowski *et al.* have suggested a possible close approach of the Moon to the Earth at $\sim 3.8 \times 10^3$ Myr acting to amplify a marginal lunar dynamo according to a mechanism considered theoretically by Levy[13]. However, even accepting the latter hypothesis, it is doubtful that a core dynamo can explain all of the magnetization that is inferred from both orbital and sample studies to have occurred over a period exceeding 10^3 Myr. An additional mechanism is needed as has also been stressed by Anderson.

Circumstantial evidence for the identity of this mechanism comes from orbital magnetometer and electron-reflectance studies of the distribution of lunar magnetic anomalies and their correlation with surface geological features as well as from returned sample studies and surface magnetic field investigations. On the near side, most magnetic anomalies detected at low altitudes with the Apollo subsatellite magnetometers correlate with exposures of the Fra Mauro and Cayley Formations, primary and secondary basin ejecta materials[14]. Additional anomalies detected across part of the far side using the electron reflectance technique have been shown to occur peripheral to ringed impact basis where basin ejecta units are observed or are inferred to be present[15]. Two separate exposures of the Fra Mauro Formation peripheral to the Imbrium basin, separated by ~100 km, have been inferred to be magnetized in very different directions, indicating a local scale size for the magnetizing field[16]. High-amplitude orbital anomalies detected via both techniques generally correlate with surficial concentrations of apparently relatively young Reiner γ-type units which most probably have an impact-related origin[14,17–19]. In combination with the relatively high intrinsic magnetization levels that are characteristic of some returned breccias and soil samples as compared with mare basalts[20,21], these correlations strongly suggest that surface ejecta materials produced in large-scale meteoroid impacts are primary sources of the observed orbital magnetic anomalies. The lack of large-scale anomalies associated with major structural features such as the mascons argues against thermal remanence or chemical remanence acquisition by thick crustal layers cooling over long time periods in the presence of a large-scale steady magnetic field. The lack of clear evidence for thermal remanence acquisition for at least some fine-grained mare basalts[22] and the 0.025 G Thellier palaeointensity obtained for a young (<200 Myr) Apollo 17 impact-produced glass sample[23] are also problematical or contradictory observations for the core dynamo hypothesis.

Because many orbital anomalies are closely associated with relatively surficial impact-produced materials and since impacts are the dominant sources of localized, near-surface energy during lunar history, an impact-related origin for lunar magnetizing fields has been repeatedly proposed. The possibility that transient magnetic fields may be generated as a result of the interaction of highly electrically conducting ionized ejecta with a weak ambient interplanetary magnetic field was first suggested by Hide[24] with specific reference to lunar palaeomagnetism. Spontaneous generation of transient magnetic fields in energetic plasma clouds produced in hypervelocity meteoroid impacts was later suggested by Srnka[25] based on theoretical models for magnetic field observed in laser-heated plasmas. More recently, Cerroni and Martelli[26] have theoretically investigated field generation by thermally driven currents and compression of a pre-existing magnetic field at the boundary of an expanding ionized ejecta cloud with application to lunar palaeomagnetism. Field compression mechanisms involving cometary impacts have also been proposed on several occasions[17,27]. Although the kinetic energies of major impacts on planetary surfaces far exceed those achievable in laboratory experiments, some progress has been made towards experimental detection of plasma, magnetic field and rock magnetization effects associated with hypervelocity impacts onto basalt targets[28–30]. In this context, it is of interest that lunar palaeointensity data indicate that the more intense palaeofields (~1 G) were present near the termination of the basin-forming epoch[12]. Since impact-generated fields are expected to scale as a positive power of the scale of the impact and since the basin-forming impacts were the most energetic in lunar history, this result may support the impact model rather than refute it as was argued by Runcorn.

Thus, while it is not yet possible clearly to identify the mechanism(s) responsible for generation of strong magnetic fields at the lunar surface implied by the Apollo palaeomagnetic investigations, the possible importance of local mechanisms involving meteoroid impact processes is suggested by a large fraction of the available evidence. In such conditions, it is

0028–0836/84/070661—02$01.00
© 1984 Macmillan Journals Ltd

advisable to consider cautiously the proposed implications of a former lunar dynamo.

L. L. HOOD
C. P. SONETT
Lunar and Planetary Laboratory, The University of Arizona, Tucson, Arizona 85721, USA

L. J. SRNKA*
Lunar and Planetary Institute†, 3303 NASA Road 1, Houston, Texas 77058, USA

* Permanent address: Exxon Production Research Company, PO Box 2189, Houston, Texas 77001, USA.
† Operated by the Universities Space Research Association (NASA Contract NASW 3389), Lunar and Planetary Institute Contribution No. 523.

1. Runcorn, S. K. *Nature* **304,** 589–596 (1963).
2. Hood, L. & Cisowski, S. *Rev. Geophys. Space Phys.* **21,** 676–684 (1983).
3. Hood, L. L., Herbert, F. & Sonett, C. P. *J. geophys. Res.* **87,** 5311–5326 (1982).
4. Sonett, C. P. *Rev. Geophys. Space Phys.* **20,** 411–455 (1982).
5. Hobbs, B., Hood, L., Herbert, F. & Sonett, C., *J. geophys. Res.* (in the press).
6. Russell, C. T., Coleman, P. J. & Goldstein, B. E., *Geochim. cosmochim. Acta Suppl.* **12,** 831–836 (1981).
7. Goins, N. R., Toksoz, M. N. & Dainty, A. M. *Geochim. cosmochim. Acta Suppl.* **9,** 2421–2439 (1979).
8. Nakamura, Y., Latham, G. V. & Dorman, H. J. *J. geophys. Res.* **87,** A117–A123 (1982).
9. Nakamura, Y. *et al. Geophys. Res. Lett.* **1,** 137–141 (1974).
10. Anderson, K. A. *J. geophys. Res.* **88,** A588–A590 (1983).
11. Busse, F. *Ann. Rev. Fluid Mech.* **10,** 435–462 (1978).
12. Cisowski, S. M., Collinson, D. W., Runcorn, S. K., Stephenson, A. & Fuller, M. *J. geophys. Res.* **88,** A691–A704 (1983).
13. Levy, E. H. *Geochim. cosmochim. Acta Suppl.* **10,** 2335–2342 (1979).
14. Hood, L. L., Coleman, P. J. & Wilhelms, D. E. *Science* **204,** 53–57 (1979); *Geochim. cosmochim. Acta Suppl.* **10,** 2235–2257 (1979).
15. Anderson, K. A. & Wilhelms, D. E. *Earth planet. Sci. Lett.* **46,** 107–112 (1979).
16. Hood, L. L. *Geochim. cosmochim. Acta Suppl.* **11,** 1879–1896 (1980).
17. Schultz, P. H. & Srnka, L. J. *Nature* **248,** 22–26 (1980).
18. Hood, L. L. *Nature* **487,** 86 (1980); *Geochim. cosmochim. Acta Suppl.* **12,** 817–839 (1981).
19. Hood, L. L., Lin, R. P. & Metzger, A. E. *Lunar planet. Sci.* **14,** 323–324 (1983).
20. Strangway, D. W., Gose, W., Pearce, G. & McConnell, R. K. *Nature* **246,** 112–113 (1973).
21. Fuller, M. *Rev. Geophys. Space Phys.* **12,** 23–70 (1974).
22. Fuller, M., Cisowski, S. M. & Hale, C. J. *Geochim. cosmoschim. Acta Suppl.* **10,** 2211–2233 (1979).
23. Sugiura, N. *et al. Geochim. cosmochim. Acta Suppl.* **10,** 2189–2198 (1979).
24. Hide, R. *The Moon* **4,** 39 (1972).
25. Srnka, L. J. *Geochim. cosmochim. Acta Suppl.* **8,** 785–792 (1977).
26. Cerroni, P. & Martelli, G. *Planet. Space Sci.* **30,** 395–398 (1982).
27. Gold, T. & Soter, S. *Planet. Space Sci.* **24,** 45–54 (1976).
28. Martelli, G. & Newton, G. *Nature* **269,** 478–480 (1977).
29. Srnka, L. J. *et al. Earth planet. Sci. Lett.* **42,** 127–137 (1979).
30. Bianchi, R. *et al. Nature* (submitted).

RUNCORN REPLIES—Hood, Sonett and Srnka's conclusion that "the possible importance of local mechanisms involving meteoroid impact processes is suggested" largely rests (in their two penultimate paragraphs) on the observed correlation between the magnetic anomalies, mapped by the subsatellite magnetometers and by the reflected electron technique, and the ejecta blankets from impact events. This argument is scarcely logical: one might just as well argue that as lava flows are the most strongly magnetized rocks on the Earth and that as volcanoes are "the dominant sources of localized near surface energy during (Earth) history" the remanent magnetization of lavas must be produced by electric phenomena in the eruptions and has nothing to do with the geomagnetic field.

Hood *et al.* refer to two sources 100 km apart with "very different directions of magnetizations"; my Fig. 3*b* (ref. 1) shows that these (C1, C2) are roughly opposite. I explained that this is entirely consistent with the ejecta having a uniform direction of magnetization on an even larger scale. The ejecta, like the lavas, can only become magnetized with uniform direction after final emplacement in the presence of a uniform ambient field. No experimental demonstration has yet been made of magnetization produced during hypervelocity impacts on basalts that is attributable to transient plasma fields rather than the Earth's field, but such processes may well account for some magnetized material on the Moon, and elsewhere in the Solar System. My review was concerned with the test of the rival hypotheses. The bipolar clustering of north magnetic poles along three different axes, the proximity of the multi-ring basins to, and the parallelism of the velocities of the bodies that created them with the palaeoequators of corresponding age, strongly support magnetization by an ancient core dynamo field and cannot be understood on the magnetization by impact hypothesis. In Table 1 (ref. 1), the axis of rotation for the mean poles of Lower Nectarian age should be 50.4° S 162° E and for the Pre-Nectarian poles 7.8° N and 92.3 °E.

That the lavas in the maria basins, especially the thick ones in the circular maria (which account for the mascons), do not show up by the magnetic mapping is not a mystery as we have Apollo lava samples and their intensity of magnetization is 10^{-5} EMU cm^{-3}—an order of magnitude less than that of breccia. Such uniformly magnetized layers of lava produce less than 0.1 nT near their surfaces, except at their edges where the fields are about 10 nT but fall off so rapidly with height as to be undetectable by the subsatellite. By contrast, the magnetic effect of the Rima Sirsalis graben feature 10 km across and 300 km long has been detected[2,3]. That the intensity of magnetization of ejecta and breccia is an order of magnitude greater than that of lavas is explained by difference in the amount of free iron: it seems likely[4] that in the impacts reduction of silicates occurs due to rise in temperature with the release of Fe metal grains.

Since I wrote my review, two further lines of evidence for a lunar iron core have been found. First, lunar laser ranging has determined that the axis of rotation makes an angle of 0.23 arc s with the Cassini plane, the mean dissipation within the Moon corresponds to $Q = 28$. Seismic studies show that the Q of the outer parts of the Moon is 1,000, so Yoder[5] interprets the result in terms of an iron core. Second, Newsom and Drake[6] find that tungsten is depleted more in the lunar rocks than in the Earth's mantle, where it is 10^{-4} of Solar System abundances. As the latter depletion is ascribed to the Earth's core and as general abundance patterns between the Earth and Moon are similar, indicating a common origin, a further extraction of siderophile elements is required for the Moon: thus Newsom and Drake argue for an iron core. I wrote about the (now six) independent lines of evidence: "None of these is conclusive evidence for an iron core, in each case other models can be generated to fit the observations"; thus the free iron Drake and Newsom require might be distributed throughout the Moon like currants in a cake. That Hood *et al.* consider my account as "somewhat misleading" can only mean that they do not appreciate that even though each line of evidence is not conclusive the fact that all point to the existence of a lunar core lends much greater weight to the hypothesis than any one line could: a venerable principle of reasoning called Occams razor. In fact other models would be mutually inconsistent: the 'currants in the cake' model would not explain the low moment of inertia or the delayed seismic P wave.

Anderson's argument is based on Busse's theory in which the strength of a dynamo field is independent of the energy source driving the convection: experiments on dynamos and all other theories gave a contrary result[7]. Busse's result was obtained by equating the Coriolis term and the Lorentz force in the dynamo equation. I conclude that the dynamo theory, which in spite of its attractiveness did not in fact predict any of the many phenomena discovered in Earth and planetary magnetic fields, is not understood sufficiently well to be a secure basis for Anderson's argument.

S. K. RUNCORN

1. Runcorn, S. K. *Nature* **304,** 589–596 (1983).
2. Anderson, K. A., Lin, R. P., McGuire, R. E. & McCoy, J. E. *Earth planet. Sci. Lett.* **34,** 141–151 (1977).
3. Srnka, L. J., Hoyt, J. L., Harvey, J. V. S. & McCoy, J. E. *Phys. Earth planet. Inter.* **20,** 281–290 (1979).
4. King, E. A. *J. geophys. Res.* **87,** Suppl., A429–A434 (1982).
5. Yoder, C. F. *Phil. Trans. R. Soc.* A**303,** 327–338 (1981).
6. Newsom, H. E. & Drake, M. J. *Nature* **297,** 210–212 (1982).
7. Soward, A. M. & Roberts, P. H. *Rotating Fluids in Geophysics* (Academic, New York, 1974).

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

ANNOUNCING

NEW FOR 1984

Pharmaceutical Medicine

The contents will include:

★ **Original papers**
- on methods used to evaluate the efficacy and safety of drugs in man
- with emphasis on the validation of these methods
- covering human pharmacology, clinical research, drug epidemiology, and safety surveillance
- and especially the application of new methods to drug testing

★ **Abstracts of methodological studies published elsewhere**
- either used already in the context of drug investigation
- or methods suitable for drug evaluation

★ **Invited reviews on topics related to pharmaceutical medicine**

★ **Editorials and Commentaries**
- offering opinions
- high-lighting problems

★ **Correspondence, Book Reviews, and Calendar**

Editor
Robert N Smith MD FRCP
Director of the Medical Division, Glaxo Group Research Limited, Greenford; formerly Senior Lecturer and Consultant Physician, Clinical Pharmacology and Therapeutics, University of Sheffield

Editorial Advisory Board
Daniel L Azarnoff MD FACP
President, Research and Development, G D Searle & Company, Chicago; formerly Professor of Medicine and Pharmacology, University of Kansas Medical Center
Michael G Carter MB BPharm
Director of Pharmaceutical Division, Roche Products Limited, Welwyn Garden City
Arthur H Hayes, Jr, MD
Dean and Provost, New York Medical College, New York City; formerly FDA Commissioner, Washington DC
William P Maclay MB DTM&H
Director of Medical Affairs, Sandoz Products Limited London
Sir Abraham Goldberg MD FRCP
Regius Professor of the Practice of Medicine, University of Glasgow; Chairman, Committee on Safety of Medicines
J Desmond Fitzgerald MD FRCP
Medical Director, Imperial Chemical Industries plc, Macclesfield; formerly Professor of Medicine, MacMaster University, Hamilton, Ontario
Cyril Maxwell MB ChB
Medical Director and Chief Executive, Clinical Research Services Limited, London; formerly Medical Director, Richardson-Merrell Limited
Paul Turner MD FRCP
Professor of Clinical Pharmacology, St Bartholomew's Hospital Medical College, London
Derek C Quantock PhD MD
Director of Science and Technology, Fisons Limited, Loughborough; formerly Medical Director, Association of the British Pharmaceutical Industry
Peter S Schönhöfer MD
Research Coordination, Institute für Arzneimittel, Bundesgesundheitsamt, Berlin; formerly Professor of Pharmacology, Medizinsche Hochschule, Hannover
Julian H Shelley BSc MRCP
Head of Medical Research, Boehringer Ingelheim International GmbH, Bracknell; Visiting Lecturer in Pharmacology, King's College, London
Geoffrey R Venning BM FRCP
Director of Research and Development, Janssen Pharmaceutical Limited, Marlow; formerly Senior Medical Officer, Medicines Division, DHSS
William M Wardell DM DPhil
Associate Professor of Pharmacology and Assistant Professor of Medicine; Director, Center for the Study of Drug Development, University of Rochester Medical Center

☐ Please send me a guide to contributors
☐ Please send me further information
☐ Please reserve me a sample copy of issue No. 1

NAME _____

ADDRESS _____

_____

_____

_____

To: David N Guthrie,
Scientific and Medical Division,
The Macmillan Press Ltd.,
Houndmills, Basingstoke, Hampshire
RG21 2XS, U.K.

Subscription Information
Frequency: 4 issues per year
Language: English
Rates per volume of 4 issues:
UK & Eire: £45.00
USA/Canada (surface), US$95.00
Rest of the World (surface), £63.00

SCIENTIFIC & MEDICAL | M | MACMILLAN PRESS LTD

Circle No.67 on Reader Service Card.

Wiley

NEW YORK · CHICHESTER
BRISBANE · TORONTO · SINGAPORE

REPRODUCTIVE BIOLOGY OF INVERTEBRATES

Volume II: Spermatogenesis and Sperm Function

edited by **K.G. Adiyodi** and **R.G. Adiyodi,** *Vatsyayana Centre of Invertebrate Reproduction, Calicut University, Kerala, India*

This volume summarizes variations in sperm morphology and cytology, and also provides information on cytochemistry including enzymology of the respiratory metabolic pathways of sperm cells where such data are available. It examines aspects of motility, metabolism, behaviour, transport, storage and survival of spermatozoa, spermiation and spawning, sperm absorption, and the physiology of spermatozoa within the male and female reproductive tracts. Details and sequences of processes and the levels of complexity involved in spermatogenesis are described. Attempts have been made to identify the morphogenetic factors at work in the production of spermatozoa and to describe the endogenous and exogenous influences in spermatogenesis and sperm function. The taxonomic and phylogenetic implications of the structural relationships of spermatozoa are also discussed.

Series: Reproductive Biology of Invertebrates, Volume II

January 1984 — 718 pages
0471 90071 0 — (Single volume price) $93.75/£49.95
(Subscription price) $80.00/£42.45

METHODS FOR NEURONAL RECORDING IN CONSCIOUS ANIMALS

by **R. Lemon,** *Department of Anatomy, Erasmus Universiteit, Rotterdam* and **A. Prochazka,** *St. Thomas's Hospital Medical School*

This book provides an up to date review of the current methodology for recording from single neurons in the central nervous system of conscious animals. This technique is now being widely used to explore the relationship between the activity of single neurons and behaviour, and has provided valuable insights into the function of the conscious brain.

IBRO Handbook Series: Methods in the Neurosciences, Volume 4

January 1984 — 174 pages
0471 90174 1 — (cl) $34.50/£18.00
0471 90237 3 — (pr) $17.95/£9.50

ADVANCES IN ANALYSIS OF BEHAVIOUR

Volume 3: Biological Factors in Learning

edited by **M.D. Zeiler,** *Department of Psychology, Emory University, Atlanta, USA* and **P. Harzem,** *Department of Psychology, Auburn University, Alabama, USA*

This volume deals with those biological factors that enter the learning situation. It brings together the recent thinking on the topic by major researchers in the area. It will be of value therefore to advanced students and researchers whether their interest in learning stems from psychology or other biological sciences of behaviour.

Wiley Series on Advances in Analysis of Behaviour

November 1983 — 420 pages
0471 90137 7 — $75.00/£39.00

DORSAL VENTRICULAR RIDGE

A Treatise on Forebrain Organization in Reptiles and Birds

by **P.S. Ulinski,** *The University of Chicago*

This book provides the first critical review and synthesis of ongoing research on the structure and function of the dorsal ventricular ridge (DVR), a characteristic brain component of birds and reptiles. It outlines the major features of the DVR and explains its role in reptilian and avian sensory processes and behavior.

Wiley Series in Neurobiology

November 1983 — 300 pages
0471 87612 7 — $51.95/£36.95

THE CHEMISTRY OF PEROXIDES

edited by **S. Patai,** *Department of Organic Chemistry, The Hebrew University, Jerusalem, Israel*

The series 'The Chemistry of Functional Groups' is planned to cover in each volume all aspects of the chemistry of compounds containing one of the important functional groups in organic chemistry.
This volume contains material on functional groups which include the -O-O- bond, i.e., organic peroxides, hydroperoxides, acyl peroxides, peroxy acids and esters and ozonides. None of these groups have been treated in the volumes which appeared up to now in the series.

Series: The Chemistry of Functional Groups

December 1983 — 1020 pages
0471 10218 0 — $225.00/£120.00

SOLID PHASE BIOCHEMISTRY

Analytical and Synthetic Aspects

edited by **W.H. Scouten,** *Bucknell University*

A comprehensive introduction to all major applications of solid phase biochemistry, including affinity chromatography, immobilized enzymes, solid phase synthesis of proteins and nucleic acids. It discusses matrices used in affinity chromatography and other analytical processes based on solid phase systems. The book covers genetic engineering, borate chromatography and experts review significant achievements in the field and discuss the most useful techniques.

Chemical Analysis: Volume 66

October 1983 — 796 pages
0471 08585 5 — $99.75/£71.25

TOPICS IN PHOSPHORUS CHEMISTRY, Volume 11

edited by **M. Grayson** and **E.J. Griffith,** *Monsanto Company, St. Louis.*

This continuing series presents critical evaluations and reviews of progress in phosphorus chemistry, contributed by leading experts in the field. Articles are up-to-date and present enough background material for the reader to cross disciplinary lines.

Topics in Phosphorus Chemistry, Volume 11

October 1983 — 462 pages
0471 89628 4 — $113.00/£80.75

John Wiley & Sons Limited

Baffins Lane · Chichester · Sussex PO19 1UD · England

Circle No.10 on Reader Service Card.

Turing and the computer

I.J. Good

Alan Turing: The Enigma. By Andrew Hodges.
Burnett Books (distributed by Hutchinson)/Simon & Schuster: 1983. Pp.587. £18, $21.75.

A SHORT biography of Alan Turing was written by his mother but this new one, by Andrew Hodges, is much more complete, objective and illuminating. Turing was one of the pioneers of computer science. His most notable work in this area was related to the foundations of mathematics, appreciation of which requires some relevant background.

A set whose elements can be put into one-to-one correspondence with the positive integers 1, 2, 3, . . . is said to be *countable*. For example, the even numbers 2, 4, 6, . . . form a countable set. The mathematician David Hilbert invented a hotel in which the rooms were numbered 1, 2, 3, . . . to infinity. One day all the rooms were occupied, but a countable number of additional people wanted rooms. So, for each n, the resident in room n moved to room $2n$, thus vacating the odd-numbered rooms for accommodating the newcomers.

A number of the form m/n, where m and n are integers, is said to be *rational*. At first sight, the set of rational numbers seems to be "larger" than the set of integers, but it is still countable. For we can imagine the positive rational numbers "counted" in the order 1/1; 2/1; 1/2; 3/1, 2/2, 1/3; 4/1, 3/2, 2/3, 1/4; 5/1, ... This order fills successively larger triangles if m/n is written at a point with coordinates (m, n). Every positive rational number will ultimately get counted, in fact repeatedly. We might now wonder whether *every* set is countable, but Georg Cantor showed, in about 1880, that the set of "real" numbers is not countable. A real number can be written as an infinite decimal; for example, $\pi = 3.1415926\ldots$ is a real number. We now show that the real numbers between 0 and 1 are uncountable by assuming that they *are* countable and then deducing a contradiction.

Imagine a sheet of squared paper having a top left-hand corner and extending infinitely to the right and downwards. Suppose the real decimals between 0 and 1 can be listed in the order $x_1, x_2, x_3, \ldots$. (We are not assuming, for example, that $x_1 < x_2 < x_3 < \ldots$ or that $x_1 > x_2 > \ldots$.) Let the digits of x_1 be written on the first row of the squared paper, the digits of x_2 on the second row, and so on. Now define a new decimal y whose nth digit (for each n) is not the same as the nth digit of x_n. Clearly y is not equal to x_n for any n. So there is a real number y not on the list and this contradicts our assumption that all the real numbers were on the list. Thus the real numbers are uncountable. (I have ignored the unimportant ambiguous notation for terminating decimals such as .19999 . . . .) This argument is called Cantor's *diagonal argument* and it shows that infinite sets are not all of the same "size". Some great mathematicians at the time called Cantor's work theology because they were unwilling to admit he was a genius.

The diagonal argument has since been used, in a very much more sophisticated manner, to prove remarkable results in the logic of mathematics. The first example was published by Kurt Gödel in 1931. He showed that no finite set of axioms about the integers could capture all their properties. In other words, given a finite set of axioms, and a precisely specified definition of what is meant by a proof, there are statements in the theory of numbers that are true but cannot be proved.



Courtesy Hutchinson.

Alan Turing, at the time of his election to the Fellowship of the Royal Society in 1951.

One might naively assume that the *meaning* of the truth of a mathematical statement is that it follows from the axioms. But this assumption can be seen to be dubious by means of the following simple example. Consider the conjecture that π contains arbitrarily long runs of 7s in its decimal expansion. For the sake of argument, suppose that this conjecture is true. Then, for each positive integer n, one could in principle prove that the decimal expansion contains runs of n consecutive 7s by straightforward computation. (When a mathematician says that something can be done "in principle" he usually knows that it is impracticable.) But it might not be possible to prove the conjecture itself because a proof must, by definition, be of finite length.

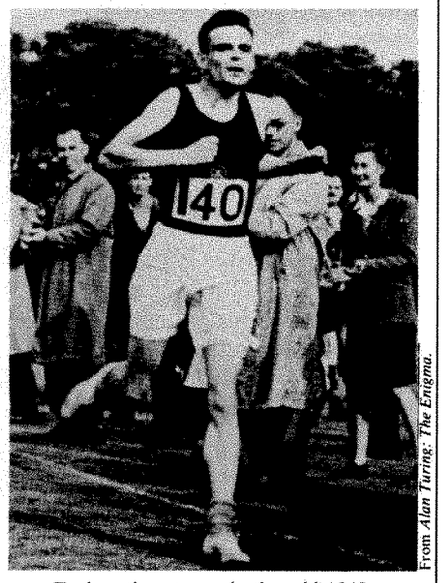
We are now ready to understand broadly what Turing discovered in 1935 when he was only 22. He first defined rigorously a "paper machine" capable of carrying out any conceivable calculation when suitably programmed, a concept now called a "Turing machine" by computer scientists. General-purpose electronic computers are approximately equivalent to Turing machines. He then defined a computable (real) number x as one for which a program (of finite length) could be written that would compute more and more digits of x. Since the number of possible programs is only countably infinite, and only some of them give computable numbers as output, it follows that the number of computable numbers is also only countably infinite. But Turing showed that there is no *explicit* way to do the counting. For suppose it is possible for a master program to have as its output a list of all the computable numbers, on squared paper, x_1 in row 1, x_2 in row 2, etc. Of course the computer would not list the whole of x_1 before starting on x_2, etc. It might, for example, print in order $x_{11}, x_{21}, x_{12}, x_{31}, x_{22}, x_{13}$, etc., where x_{ij} is the jth digit of the computable number x_i. (Compare the triangular process for "counting" the rational numbers.) But then one could define a *new* computable number by means of the diagonal process. So the alleged master program is not really a master program at all — and this completes the proof of Turing's result by *reductio ad absurdum*. From this result he was able to deduce that there is no automatic procedure, using only the rules of a given formal system, that can decide, for every mathematical statement, whether it is provable, unprovable or neither. In the book, Andrew Hodges provides a rigorous account of this work.

During the Second World War Turing worked on the cryptanalysis of the Enigma, a German cryptographic machine. He had an important idea for improving the "Bombe", which was the main cryptanalytic machine used for attacking the Enigma. The idea was based on the logical fact that from a contradiction anything can be proved, a fact that Wittgenstein thought was unimportant. It saved a vital factor of about 26 in the running time, and if it had not been for that the Allies might have lost the war. Turing also made important statistical contributions, but had little to do with the Colossus, a cryptanalytic machine which, unlike the Bombe, had much in common with later electronic computers.

Immediately after the War Turing worked on the design of the ACE (Automatic Computing Enigma) at the National Physical Laboratory (NPL), a post he took partly because he did not enjoy lecturing. The storage of the ACE was based on mercury delay lines; and its programming, as M.H.A. Newman said, was like catching mice just as they were entering a hole in the wall. Some machines with magnetic drums had the same disadvantage. Turing was not happy at NPL,

because he was not allowed to be both a mathematician and an engineer, and in 1947 he took a year's leave during which he wrote an influential philosophical paper on artificial intelligence. In this article he disposed of most of the arguments that are made against the possibility of the machine simulation of thought processes. In 1948 he moved to Manchester and produced the programming system for the Manchester University Computer, Mark II.

Turing was logical but emotional, excitable but kind, eccentric but natural, and intellectually and physically strong. He was a very good long-distance runner and



From *Alan Turing: The Enigma.*

Turing, the runner, in the mid-1940s.

might have run in the Olympic games, but developed a neurological complaint in a leg. After the war he admitted privately that he ran partly to work off libido and that he was a homosexual; fortunately MI5 did not know of his homosexuality during the war. In 1952 he published a seminal contribution to the mathematical theory of growth, but in the same year he was convicted for having a homosexual relationship with a 19-year-old man and was put on probation and on chemical therapy. In June 1954 Turing committed suicide.

In this biography Hodges treats most of these aspects of Turing's life, and many others, and attempts to trace the origins of his ideas in his early environment. The book is researched and written extraordinarily well; I found only a few minor inaccuracies in spite of having known over a hundred of the people mentioned by Hodges. It is a first-class contribution to history and an exemplary work of biography. □

I.J. Good is University Distinguished Professor of Statistics at the Virginia Polytechnic Institute and State University, Blacksburg, Virginia. A collection of his writings, Good Thinking: The Foundations of Probability and Its Applications, *was published last year by the University of Minnesota Press. During the Second World War, he was Turing's and later M.H.A. Newman's main statistical assistant in cryptanalytic work.*

Formats for language development

John McShane

Child's Talk: Learning to Use Language.
By Jerome Bruner.
W.W. Norton/Oxford University Press: 1983. Pp.144. Hbk $13.95, £11; pbk £5.95.

A CHILD must master a complex grammatical system in acquiring language. How he does so has been a central issue of debate and research for the past twenty-five years. Much of the discussion has concerned what sort of unique language-learning capacities human beings might or might not have. However that question is answered eventually, a child would not learn a language without being part of a community of other speakers.

Thus, any reasonably complete answer to the question of language acquisition must give an account of both the child's language-learning capacities and the environmental features that stimulate those capacities. Bruner's book is a summary of a decade of his work on the role of the environment in language acquisition. It is not a general review of research in this area; indeed, there is little mention of some standard issues such as the grammatical characteristics of parental talk to children. Instead, problems that have concerned Bruner during the past decade are examined, considered and summarized. The book provides a clear and illuminating introduction to these concerns but, inevitably perhaps, fails to satisfy one's desire for a thorough-going consideration of the positions of other theorists.

Chapter 2, which deals with the relations between language and non-language, is the clearest example of where more was needed. Bruner makes two points concerning the non-linguistic foundations of language. The first is that language structures have analogues in other areas of cognition. Thus, at least some of the structure of language might be derived non-uniquely. The trouble with this view is that it is notoriously difficult to move from pointing at plausible candidates from which to derive grammatical structures to demonstrating a causal link, as Bruner recognizes. Because he has not set out to write a detailed critical review, these complex issues are finessed. Bruner's other point is that a child is not simply learning grammatical structures in learning a language; he is also learning the conventions for the use of linguistic structures. He argues forcefully that many of these conventions are set-up by the child's social environment, independently of the child's grammatical abilities and usually well in advance of them. The mechanism by which this occurs is the heart of the book.

Bruner argues that adults create structured interactions with children and maintain the basic structure over many months while gradually handing over more and more control of the structure of the interaction to the child. Bruner calls these structures *formats*. Within a format a child gradually achieves an increasing degree of competence with the adult's aid and cajoling. As competence increases so the adult demands for competence increase. Formats for peekaboo games, reference and requesting are considered in some detail.

Not many researchers have followed Bruner's path of exploring the *interaction* of adult and child in detail and implicating the structure of the interaction in a causal model of development. To my mind this is a great pity. While this particular tack does not answer all questions about language development, it seems to be a more fruitful approach to the role of the environment than the more common technique of correlating features of parental speech with later features of the child's speech, in complete oblivion of the interactional context in which the speech occurred. The more dynamic approach of Bruner is both time-consuming and labour-intensive on the part of the researcher. This book makes a sound case for the investment required. □

John McShane is a Lecturer in the Department of Psychology at the London School of Economics and Political Science. He is author of Learning to Talk *(Cambridge University Press, 1980).*

Control by calcium

P.F. Baker

Intracellular Calcium: Its Universal Role as Regulator.
By Anthony K. Campbell.
Wiley: 1983. Pp.556. £36.75, $79.95.

THE importance of intracellular calcium as a widespread, if not universal, regulator of cell function is well recognized. "Control by calcium" exhibits three key features: maintenance of a very low cytosolic free Ca in the face of intracellular and extracellular pools of Ca at much higher concentrations; the presence of special voltage or receptor-controlled mechanisms for permitting Ca to enter the cytosol from one or more of these high Ca pools; and the existence within the cytosol of a special class of Ca-receptor proteins that serve to transduce alterations in free Ca to changes in enzyme activity and cell function. Despite this apparent simplicity, it is often difficult in practice to unravel the part played by calcium in a particular physiological process and much effort has been expended in trying to develop suitable experimental techniques. The success of this effort is

now reflected in an avalanche of publications in the primary literature.

When new material is appearing at such an alarming rate, to embark on a book can be a dangerous decision. Exceptions are books that aim to impose some sort of order on the apparent chaos of the primary literature and those that advance a novel framework within which the new discoveries can be set. Tony Campbell's *Intracellular Calcium* falls squarely into the first category.

After discussing the "Natural History of Calcium" and "Calcium Methodology", Campbell sets out to draw together the arguments for and against a role for Ca in a host of cellular processes with chapters on "Intracellular Ca and the Electrical Activity of Cells", "Calcium and Cell Movement", "Intracellular Ca and Intermediary Metabolism", "Endocytosis and Exocytosis", "The Reproduction and Development of Cells" and "The Pathology and Pharmacology of Intracellular Ca". Each of these often contains long accounts of basic cell biology as an essential introduction to discussion of the possible roles of calcium. Unfortunately, except where that role is already well known, these chapters often end rather inconclusively. They nevertheless certainly highlight many unsolved problems of cell biology in which calcium may well be involved.

Such a wide sweep has, however, posed obvious difficulties for the author and despite 556 pages the book falls somewhere between being a natural history of intracellular calcium and a critical discussion of those processes where involvement of calcium is still controversial. In consequence some important growth areas of real significance to "control by calcium" are under-represented and presented in a relatively uncritical fashion. Two obvious examples are phosphoinositol metabolism and the transport of calcium across membranes; but I suspect that most specialists will find fault with the presentation of their own particular neck of the calcium wood. It is a pity that the author did not seek more feedback before publication. In this way the most glaring errors might have been avoided and the author might also have been encouraged to reference all the most important observations with original papers. As it is, the policy is mixed: sometimes original papers are quoted, in other places reviews, but all too often interesting observations go incognito.

It is all too easy to find fault. This is a very personal account of the many roles of intracellular calcium that will be an obvious starting point for those entering the field. It will also, I feel sure, infect both the old hand and the new with some of Tony Campbell's great enthusiasm for his subject. □

P.F. Baker is Halliburton Professor of Physiology at King's College, University of London.

Look back to viral oncology

Robin Weiss

Oncogenic Viruses, 3rd Edn.
By Ludwik Gross.
Pergamon: 1983. Two volumes, pp.1,203. £100, $200.

LUDWIK Gross has influenced the development of experimental oncology in the post-War period through three distinct contributions. First, after many years of frustrating experimental work, in 1951 he showed that the thymic lymphoma of AKR mice could be transmitted to new-born C3H mice by cell-free filtrates; this discovery of murine leukaemia virus, following Bittner's demonstration of the milk factor causing mammary carcinoma in mice 15 years earlier, firmly established that viruses cause cancer in mice, and led to a resurgence of tumour virus research. Second, in 1953, Gross reported that salivary gland tumours (also induced by the thymoma extracts) were caused by a distinct agent, later named polyoma virus by Sarah Stewart, who independently discovered the virus in the same year. Finally, in 1961, Gross published the first book wholly devoted to tumour viruses.

Twenty years ago Gross's *Oncogenic Viruses* was the only comprehensive general text available. As such, the book was invaluable. A second enlarged edition was published in 1970 which included much new material, though it omitted discussion of some of the most interesting *in vitro* experimentation on oncogenic viruses and cell transformation established during the 1960s. Now Gross has produced a third edition, expanded to two volumes, and including some new chapters on oncogenic viruses of fish, "slow virus diseases" and iatrogenic transmission of human cancer.

I looked forward to reading this new edition in the hope of seeing the recent strides made in experimental oncology placed in perspective by the man who discovered two of the most intensively studied tumour viruses. I was deeply disappointed. Whilst Gross writes in his introduction that "all chapters . . . have been brought up-to-date in order to include the most important observations which have become available since the previous edition was prepared", this is a travesty of what follows. For instance, there is not a single citation of David Baltimore's work, either on reverse transcriptase (which is never mentioned), or on Abelson leukaemia virus (this important virus and its associated neoplasms are dismissed in a single paragraph).

Gross excludes any discussion of viral replication and genetics. This could be explained by his remark that "molecular biology is not discussed in this book". But while the author wisely did not aim to write a detailed molecular treatise, no modern text can afford to ignore the *results* of molecular biology in changing our conception of tumour virus biology, and indeed of cancer as a whole. It is difficult to see how any author can claim to present insights into viral oncogenesis without at least a nodding reference to "early" functions of papovaviruses or to retrovirus oncogenes.

By eschewing all data on viral and host genetics, whether obtained by classical or molecular methods, much of value in the detailed descriptions of early virus isolates is diminished. Such important topics as viral transmission, defectiveness, and host permissiveness and resistance are discussed in terms of concepts that were already outdated at the time of the second edition 13 years ago. For example Gross himself coined the terms "vertical" and "horizontal" transmission for tumour viruses in 1944, and the existence of hereditary infections is described at length in many chapters. Yet the crucial distinction between congenital infection and the Mendelian inheritance of integrated proviruses is not made until the final chapter, almost as a footnote. Classical papers, such as those by Payne and Chubb (*J. Gen. Virol.* **3**, 379; 1968) on the Mendelian inheritance of viral antigens in chickens and Bentvelzen and Daams (*J. Natl Cancer Inst.* **43**, 1025; 1969) on the chromosomal transmission of mammary tumour virus genomes in GR mice are not cited, let alone the wide body of knowledge on endogenous viral elements accrued in the 1970s which clarified the whole subject of retrovirus latency and activation. Gross devotes five chapters to murine leukaemogenesis without once mentioning xenotropic or recombinant viruses.

The considerable progress in the study of human oncogenic viruses since the last edition receives erratic treatment. With the burgeoning interest in human papilloma viruses and the isolation of new strains associated with particular neoplasms, it is astonishing to find the most recent reference dates from 1965. Hepatitis B viruses of humans and animals are summarized in one-and-a-half pages, and human T-cell leukaemia virus in one page plus an electron micrograph. There is, however, a whole chapter on Epstein–Barr virus, and a useful new chapter on "Inadvertent and Experimental Transmission of Human Cancer".

As an historical survey of viral oncology up to 1965, *Oncogenic Viruses* is unparalleled. But no reader or librarian should suppose that this new edition gives a contemporary account of viral oncology. As with Darwin's *Origin of Species*, I shall treasure the earlier editions, and regret that Gross has devoted much effort on a third edition which should not have been published. □

Robin Weiss is Director of the Institute of Cancer Research, London.

The foraminifera in time

Gerta Keller

Neogene Planktonic Foraminifera: A Phylogenetic Atlas.
By James P. Kennett and M.S. Srinivasan.
Hutchinson Ross: 1983. Pp.265. $36.50, £35.40.

A COMPREHENSIVE manual for the identification of Neogene planktonic foraminifera has been needed for some time. With this publication, James P. Kennett and M.S. Srinivasan have gone a long way to satisfying the need. The book contains high-quality scanning electron micrographs of 150 species which show the characteristic morphologies of type species as well as differences in surface ultrastructure. Each illustration is accompanied by a concise description of the species, and a note of its stratigraphic range and locality; a phylogeny based on morphological features is proposed for each lineage.

The book is especially noteworthy for the authors' representation of planktonic foraminifera by phylogenetic groups and for their erection of several new subgenera to distinguish evolutionary taxonomic lineages. This approach is both novel and useful for students, but unfortunately the subject is treated in a rather cursory manner. Apart from general comments in the introductory chapter there is little discussion of the proposed lineages and ancestor–descendant relationships, or of their controversial nature: ancestor–descendant relationships for many species are *not* well understood and, as noted by the authors, at present the use of phylogenetically defined subgenera is limited to themselves and one other worker.

There are a few minor, but important omissions of data on diachroneity of species ranges in the taxonomic descriptions. Species ranges (first and last appearances) are critical for the chronostratigraphic interpretation of sedimentary sequences. Therefore, care should be taken to note maximum species ranges (usually in the tropics) as well as known diachronous ranges of many species between tropical and temperate regions. The authors sometimes fail in this respect. For instance, *Globorotalia plesiotumida* (which defines the base of Zone N17) is diachronous even between the east and west equatorial Pacific, appearing at 9 Myr in the west and at 8 Myr in the east. For this reason, the first appearances of *Globigerinoides conglobatus* and *G. obliquus extremus* are more reliable in defining the base of Zone N17. Similarly, *Globoquadrina dehiscens* disappears in the tropics at 5 Myr, but at 10 Myr in temperate regions.

Some other species' ranges listed are also anomalous. For example, *Globorotalia birnagae* and *Globigerinoides bolli* are listed as first appearing in Zones N7 and N12 respectively. However, both species have been reported from the early Miocene Zone N4. *Globigerinoides bulloideus* which is reported as first appearing in the late Miocene Zone N17 actually evolves in the middle Miocene. Most of these differences probably stem from the fact that the authors' synthesis is based primarily on investigations of deep-sea sequences from the south-west Pacific which, contrary to their claim, are not representative of the world oceans. Students should be aware of such problems and consult recent reports of the Deep Sea Drilling Project and micropalaeontological journals as appropriate.

While I have dwelt on the shortcomings, *Neogene Planktonic Foraminifera* is a well-conceived book for which the authors are to be thanked. It is an extremely handy manual for species identification and biostratigraphic interpretation which will prove invaluable to research workers, and to teachers and their students. □

Gerta Keller is in the Branch of Paleontology and Stratigraphy of the US Geological Survey, Menlo Park, California.

In the beginning

A.G. Cairns-Smith

Earth's Earliest Biosphere: Its Origin and Evolution.
Edited by J. William Schopf.
Princeton University Press: 1983. Pp.543. Hbk $95, £88; pbk $42.50, £40.

THE Precambrian Paleobiology Research Group was a realized pipe dream of J.W. Schopf's. It consisted of 24 scientists of appropriately diverse callings brought together to work, at UCLA and elsewhere, between May 1979 and August 1980. They engaged in research, in the field and in the laboratory; they met frequently, they talked and they published more than a dozen papers. And now, as the main product of their efforts, there is this splendid book.

The book includes the practical results of the group's activities, but it is not merely a series of research papers — rather, it is more an advanced textbook. The chapters are mainly reviews, but in spite of there being 21 contributors are reasonably well integrated. This was all helped no doubt by the editor's active authorship in almost half of them.

Preston Cloud was invited to introduce the book with a perspective on what he calls the new paradigm of biogeologic history — a mid-twentieth-century move from a too devout Uniformitarianism. The Earth is seen as having been substantially different in its early days, with a change through its history from reducing to oxidizing conditions — a somewhat spasmodic change, though, with atmosphere, hydrosphere, biosphere and lithosphere evolving in concert.

A radical version of this new paradigm starts with the Oparin–Urey highly reducing atmosphere for the early Earth, a CH_4–NH_3–H_2O type. Under such an atmosphere the probiotic synthesis of organic molecules should have taken place easily. Sherwood Chang *et al.* discuss in detail the question of the character of the probiotic atmosphere, believing that a build-up of organic molecules on the primitive Earth was a necessary preliminary to the start of a biological evolution. The strongly reducing first atmosphere is now seen as only one possibility — and perhaps not the most likely. The difficulty in deciding this question comes from trying to determine sequences of events during the formation of the Earth itself, some 4.5 Gyr ago. It is now generally thought that the early atmosphere was derived mainly from outgassing of the Earth. The nature of this atmosphere would then have depended on how much metallic iron there had been near the surface of the Earth, and *that* would have depended on how and when the core of the Earth was formed. These matters are also discussed in a chapter by David J. Stevenson, and they form the starting point for a discussion of the evolution of the lithosphere by W. Gary Ernst. The core formed early on, it seems, and most of the outgassing was early as well.

So after a somewhat catastrophic start there is, after all, a more Uniformitarian picture of the early Earth, according to several of the authors here — but with some gradual changes superimposed, needless to say. James C.G. Walker *et al.* take such a view. By the time there was a stable surface — and certainly by 3.8 Gyr ago when the most ancient known sediments were formed — the Earth was not so vastly different a place from now. By that time, it seems, the atmosphere was basically CO_2–N_2–H_2O (whether or not it had ever been anything else) and evidently weathering and sedimentation processes were already in action. Walker and his colleagues make a plausible case for there having been much more carbon dioxide though — perhaps 100 times as much as now — and there is ample evidence for low or negligible oxygen levels.

Most people think that the appearance of significant amounts of oxygen in the atmosphere (very roughly 2 Gyr ago) was due to increasing photosynthetic activity by organisms. Jan Veizer suggests that organisms had perhaps been producing oxygen in quantity long beforehand and it was the gradual evolution of the lithosphere that brought about this atmospheric change: the Earth was cooling; reducing power derived from the mantle (e.g. Fe^{2+}) was being less vigorously injected into ocean waters and could no longer keep pace with the photosynthetic oxygen pro-

duction. . . . A neat inversion, this, of the more usual idea that evolutionary biology is the gradualist science and geological history the Uniformitarian one. Indeed ^{13}C studies on ancient sediments, reviewed by Shidlowski *et al.*, fit perfectly well with the notion that a biosphere of similar scale to today was already present 3.5, possibly 3.8 Gyr ago: there is a typical biological (autotrophic) signature in the isotope ratios of reduced and oxidized materials, although it must be said that such signatures can be forged by non-biological processes.

Chapters by Schopf, Malcolm R. Walter and Hans J. Hoffmann on Precambrian fossils serve to underline the stability of many biological forms. Not all that much seems to have been happening between 3.5 Gyr ago and towards the end of the Precambrian (0.57 Gyr ago) when truly multicellular forms of life came on the scene. The 3.5 Gyr date is of rocks from Western Australia that contain fossil stromatolites (microbial colonies) as well as fossils of filamentous bacteria similar to modern members of stromatolite communities. To be told that of 43 cases of pre-2.5-Gyr-old sets of "microfossil-like objects" there are only two that are well proven, is a little worrying — one wonders whether more work might cast doubt here too. Well, there is no absolute security in this field and the evidence seems pretty good to me. If it holds it is the nearest we have yet got to the origin of life (although still not very near if these are fossils of anything at all like modern prokaryotes).

Past sequences of events within the biosphere can perhaps be inferred from the ways in which present biochemical subsystems depend on each other. Howard Gest and Schopf see sugars as primary materials, with fermentation and then (non-oxygenic) photosynthesis having been achieved early on (by 3.5 Gyr ago on the basis of isotope and fossil evidence). J.M. Hayes makes a case for oxygen-producing photosynthesis having appeared by about 2.8 Gyr ago: a wide spread of carbon isotope ratios in reduced materials of that sort of age fits with the idea that around then both oxygen-rich and oxygen-poor sedimentary basins were common. Whatever the exact timing, the more general oxygenation of the environment is seen by David J. Chapman and Schopf to have left its mark in the structures of modern biochemical pathways which indicate a progression from our remoter ancestors, which could not put up with oxygen, through forms that could tolerate it, to those that needed oxygen to survive.

This is a good-looking book with plenty of data and models attractively presented in pictures, diagrams and tables. I can see it in studies, in laboratories and even on coffee tables. It should help to generate some new addicts for an untidy, unsafe but exciting field. □

A.G. Cairns-Smith is Reader in Chemistry at the University of Glasgow.

Astronomical city

David W. Hughes

The Story of Astronomy in Edinburgh.
By Hermann A. Brück.
Edinburgh University Press: 1983.
Pp.151. £8.50.

EDINBURGH is the home of a famous university and a Royal Observatory, and the history of astronomy in that city is a tale of the intricate symbiotic relationship between the two establishments. Both are old. Nineteen-eighty-three saw the 400th anniversary of the University and 1972 the 150th anniversary of the Royal Observatory, so it is a fitting time to delve into the development of astronomy in the city. Between the years of 1957 and 1975 Hermann A. Brück held "by far the most attractive astronomical post in the United Kingdom", that of being jointly Astronomer Royal for Scotland and Regius Professor of Astronomy at the University of Edinburgh. He has written a delightful book which will appeal to a wide variety of readers.

The story divides easily into three parts, the first centring around Calton Hill for which William Playfair designed an observatory modelled on the Greek Temple of the Winds. This replaced the Old Observatory on the same hill, which had been used by the Astronomical Institution of Edinburgh, a society founded in 1811, and the first British society whose activities were entirely devoted to the pursuit of astronomy. Their aim was to establish an observatory which could be used both for scientific research and for popular observation. In 1785 Robert Blair was appointed as the first professor of astronomy and this was followed in 1822 by King George IV being "graciously pleased to permit the Observatory on Calton Hill to be styled The Royal Observatory". In 1834 the Astronomical Institution transferred the use of the Observatory to the University on the condition that the Government of the day appointed an Observer who would be titled Astronomer Royal for Scotland and would also occupy the Chair of Astronomy at the University of Edinburgh. Thomas Henderson was appointed and was followed by Charles Piazzi Smyth who dominated Edinburgh astronomy between 1846 and 1888.

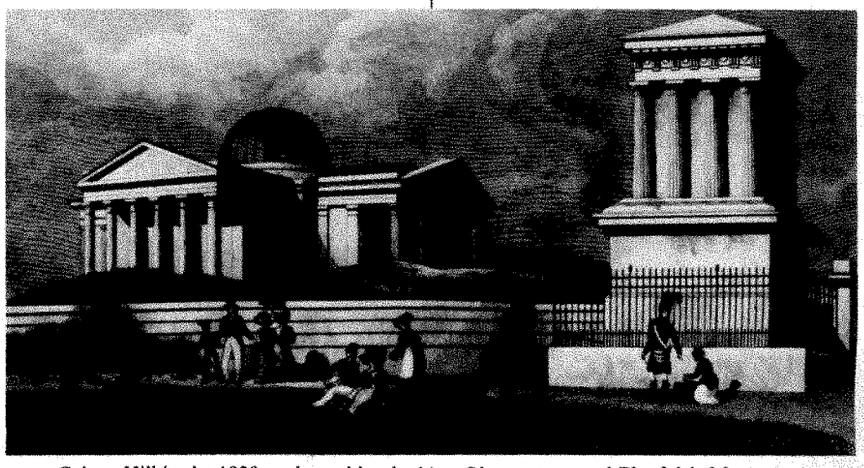
Smyth was one of the most fascinating personalities in the scientific world during the Victorian era. He founded "mountain astronomy", realizing that height improved the effectiveness and precision of astronomical observations, immersed himself in pyramidology and ignored his university duties as there were "generally no students at all for so untoward, despised and poverty-stricken a subject in Scotland".

Things obviously had to change and Ralph Copeland, Smyth's successor, witnessed the building of a new observatory at Blackford Hill to the south of Edinburgh and the transfer to that observatory of the Dunecht instruments which previously belonged to the 26th Earl of Crawford. Spectroscopic astrophysics flourished and under the successive leadership of Frank Dyson, Ralph Sampson and W.M.H. Greaves the observatory developed spectrophotometry and high-precision photographic photometry.

In 1957 Hermann Brück took over and in his 18 years supervised a great expansion of the observatory, the numbers of staff growing from less than ten on his arrival to well over a hundred on his retirement. Research in Edinburgh led to the development of autoguider systems, photoelectric exposure control for spectroscopes, ultraviolet observatories in space, satellite tracking and the GALAXY and COSMOS machines which can automatically scan photographic plates and measure the positions and brightness of stars and galaxies.

Brück writes that his "years on Blackford Hill have been extremely happy ones", a happiness which echoes through his story. There can be few better advertisements for astronomy, or for Edinburgh as a place to pursue the subject. □

David W. Hughes is a Lecturer in Physics and Astronomy at the University of Sheffield.



Calton Hill in the 1820s, adorned by the New Observatory and Playfair's Monument.

Teleki to Leakey

Andrew Hill

Koobi Fora: Researches into Geology, Palaeontology, and Human Origins. Volume 2 The Fossil Ungulates: Proboscidea, Perissodactyla, and Suidae.
Edited by J.M. Harris.
Oxford University Press: 1983. Pp.320. £45, $95.

IT WAS less than a hundred years ago that Europeans first looked upon Lake Turkana, when Count Samuel Teleki von Szek and his fellow traveller Ludwig von Hohnel came across it on 6 March 1888. "An entirely new world was spread before our astonished eyes", wrote von Hohnel on seeing the huge expanse of water in the deserts of northern Kenya.

After Teleki few Westerners visited the lake. Sir Vivian Fuchs led an expedition there in the 1930s, and in 1943 Louis Leakey was told of the existence of fossils by a District Commissioner, but was denied access at that time. It was left to his son Richard to rediscover the palaeontological potential of the area in 1967. He began the Koobi Fora Research Project in 1968 and it still continues. While it may be hyperbolic to suggest an entirely new world has been spread before our astonished eyes as a result, certainly the research of the Project has changed our view of palaeoanthropology in significant ways.

In 1968 only three specimens of early hominid were known from Kenya. The Koobi Fora expedition has changed that, for a great deal of material has come from Lake Turkana, most of it discovered by an expert group of Kenyan fossil prospectors led by Kamoya Kimeu. Now the list of hominid fossils exceeds 150 for Koobi Fora alone, and the project has spawned as many publications. This monograph, edited and largely written by John Harris, is the most recent of them. It is dedicated to the memory of the late Sonia Cole, the technical editor of this and the first Koobi Fora volume, who in many ways made notable contributions to palaeoanthropology in Africa.

Aside from the sheer abundance of hominid specimens, what are the main achievements of the Project? Koobi Fora is one of three large sites in eastern Africa that document the radiation of hominids between two and one million years ago. Olduvai in Tanzania provided the first evidence, an assortment of specimens comprising two new taxa, *Australopithecus boisei* and the originally more controversial *Homo habilis*. It also produced the earliest complete assemblages of lithic culture. Work near the Omo River in southern Ethiopia resulted in important collections of vertebrate fossils, and a series of volcanic tuffs through the succession made it possible to establish a reliable temporal framework by potassium–argon dating. Although numerically abundant, the hominid finds were not spectacular, as sedimentary conditions in that area favoured mainly the preservation of isolated teeth.

Koobi Fora provided more complete specimens. Amongst them are good examples of *Australopithecus boisei*, enabling us to appreciate the high degree of sexual dimorphism and range of variation in this interesting creature. There are specimens that appear to confirm the reality of *Homo habilis*, and yet others that probably represent a third species. This might explain some of the earlier interpretative problems within the Olduvai sample. Another step was the recognition at Koobi Fora of the great antiquity in Africa of *Homo erectus*. This is the species generally thought to be immediately ancestral to our own, and is also the first hominid found outside Africa. Most specimens from the Far East and Europe, though, are more recent than one million years, whereas some from Koobi Fora are half-a-million years older. This makes it possible that even *Homo erectus* originated in Africa rather than elsewhere.

A theory being pushed with some vigour until a few years ago was that once hominids and material culture were established there was room ecologically for only one species. The finding of *Homo erectus* at this level, where it was contemporary with at least *Australopithecus boisei*, successfully demolished that notion. Hominid post-cranial bones were also found, resulting in a better understanding of locomotion in these early groups.

A variety of other research has been conducted under the auspices of the expedition. Some of this is general and basic, for example that on evolutionary theory using the excellent record of fossil freshwater gastropods. Other work, such as the investigations of the geology, palaeoecology and taphonomy of recent and fossil bone assemblages, is essential to understanding the environmental and time context of the hominids. Some deals fairly directly with hominid activities. Glynn Isaac, who with Richard Leakey is the Project's co-director and co-editor of this Koobi Fora monograph series, has led a team of archaeologists which has described two successive patterns of early stone tool technology that comprise distinctive facies within the Oldowan Industrial Complex. The team has also done much valuable work on principles of site interpretation.

Although the stone tools and the bits and pieces of old human skeletons form the glamorous treasures of palaeoanthropology, over 6,000 fossils of a wide range of other vertebrates have also been collected. These span the period from four to one million years ago, and are intrinsically important for what they tell us about the origins and evolution of certain taxonomic groups world wide, and also for their contribution to understanding the environments and ecology in which the hominids evolved. John Harris is the coordinator of palaeontology in the Project, and the present volume is the first of two dealing with the fossil ungulates. It is not designed for casual reading. Five of the seven chapters are detailed taxonomic treatments of the Deinotheriidae, Elephantidae (by Michel Beden), Rhinocerotidae, Equidae (by Vera Eisenmann), and Suidae, with complete listings and field locations of specimens. An introductory chapter gives the history of exploration and stratigraphical interpretation; a concluding one discusses correlation with other sites, biozonation and environmental change.

Correlation is one of the crucial questions to which the palaeontological information relates. The initial radiometric dating at Koobi Fora led to the impression that the artefacts there, and the fossil material belonging to the genus *Homo*, were the oldest in the world. Palaeoanthropologists traditionally have a proprietary attitude to their finds, and if they can possess the oldest they are in a very attractive position. It is attractive for publicity and therefore funding if nothing else, and so is a position they are understandably loath to give up. Such resistance was shown at Koobi Fora. Evidence was accumulating from the fauna which made clear an apparent time disjunction between there and other sites, particularly the Omo, only a few tens of kilometres distant. A number of ingenious explanations were devised to account for this disparity, before fresh dating, and more detailed work on the quite complicated stratigraphy, showed that earlier correlations were simply wrong. The hominids and artefacts are comparable in age to the oldest of their kind found elsewhere in East Africa, such as those from Olduvai. While slightly tarnishing the tinsel, this does little to reduce the area's scientific appeal.

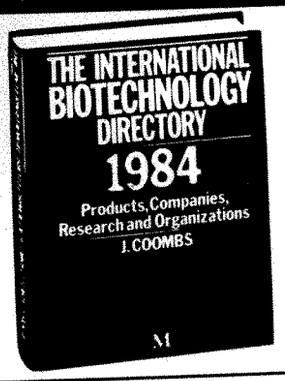
Palaeontology is more than a description of biological events that just happen to have taken place in the past, and more than a simple narrative of phylogenetic histories. This present volume is an important work, and should in no sense be seen as a mere epitaph to these departed beasts. Rather it provides the basic data which, when taken with comparable information from other sites, could answer fundamental biological questions in evolution and ecology that neontology's perspective, limited as it is to extant creatures and communities, is too narrow to solve. □

Andrew Hill is a Research Fellow at Harvard University.

● *Pluto's Republic*, Sir Peter Medawar's foray into "the intellectual underworld", has recently been published in paperback. The book first appeared in 1982, and in his review (*Nature* **300**, 118; 1982) Stuart Sutherland wrote of Medawar's "Johnsonian thump of common sense". Publisher of the paperback is Oxford University Press; price is £4.95.

EXAMINE ON FREE APPROVAL WITHOUT COMMITMENT

TWO NEW reliable, up-to-date and complete biotechnology information resources.



Detailed information on every aspect of biotechnology worldwide

An indispensable guide to:

★ Products, services and research
★ Companies ★ Information sources.
★ Government and international agencies

THE INTERNATIONAL BIOTECHNOLOGY DIRECTORY 1984 —

- is thoroughly international
- features a unique BUYER'S GUIDE to products, research and services
- covers both the old and new biotechnologies

The Directory tells you:

★ Who your competition is, and what they are doing.
★ Who are potential customers and suppliers.
★ Where to buy equipment, chemicals and services.
★ Where culture collections are located and what cultures they hold.
★ Who imports/exports biotechnology products in over 20 countries.
★ Which government departments are associated with biotechnology.

All compiled by the eminent biotechnologist Dr. Jim Coombs and now available to you on **FREE APPROVAL.**

Macmillan Paperback 320pp **September 1983**
ISBN 0 333 36178 4 **£40.00/$80.00**

The ONLY guide to the selection of cost effective biotechnology information sources

★ covers both old and new technologies
★ covers technical and commercial literature
★ covers difficult and hard to trace sources
★ includes over 20 at-a-glance evaluation tables
★ provides an Index of publishers and their addresses

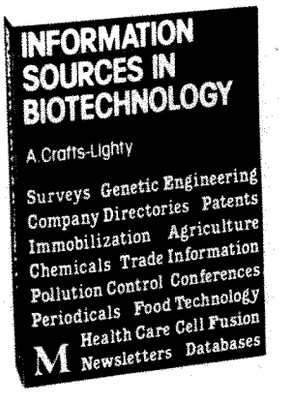
Dr. Crafts-Lighty, Head of Information and Library Services at Celltech Limited, systematically and critically reviews all the important information sources:

• books • conferences • trade periodicals • research periodicals • computer databases • patent • market surveys • directories • organisations • secondary sources

Macmillan Hardcover 400pp **November 1983**
ISBN 0 333 35140 1 **£50.00/$100.00**

Send no money now.
Examine on FREE APPROVAL

You can take these new books for 30 days examination without commitment. If you are not completely satisfied, just return the book(s) in good condition within 30 days and we will cancel your invoice.



Globe

Globe Book Services Limited.
Brunel Road, Houndmills,
Basingstoke, Hants RG21 2XS, England.
Telephone: Basingstoke (0256) 26222
A DIVISION OF MACMILLAN PUBLISHERS LTD

The Nature Press,
15 E 26 Street, New York, N.Y. 10010.
Telephone: 212-532-4811

FREE APPROVAL ORDER FORM

To: Ian Jacobs, Globe Book Services Ltd., FREEPOST, Brunel Road, Houndmills, Basingstoke, Hants RG21 2BR, England.
The Nature Press, 15 E 26 Street, New York. N.Y. 10010. Credit card callers call toll — free 800-221-2123 (or in New York: 212-532-4811)

Please send me on 30 days FREE APPROVAL _____ copies of *The International Biotechnology Directory 1984* at £50.00/$100.00 each, and/or _____ copies of *Information Sources in Biotechnology* at £40.00/$80.00 each.

If I am not entirely satisfied, I may return any book in good condition within 30 days and you will cancel my invoice.

Postage and Packing
UK subscribers £2.00 (Overseas £3.00)
US Subscribers $1.50

Tick preferred method of payment

☐ Bill me. I need send no money now.
☐ I enclose cheque for £/$ _____
☐ Charge my credit card on publication £/$ _____
☐ Visa ☐ Access/Mastercharge
☐ Am. Express ☐ Diners

Card No. _____
Expiry Date _____
Signature _____
(Orders cannot be accepted without a signature)

Name _____ (PLEASE PRINT)
Address _____
_____
Postcode/Zip Code _____

Books will be delivered to you on publication.

UK Subscribers POST TODAY — NO STAMP NEEDED N2

At last . . . a rapid-publication monthly for astronomers!

Astronomy Express

Editor: Michael Hoskin *University of Cambridge*

We are pleased to announce the launch of this exciting new current-awareness journal in July this year. Volume 1 of *Astronomy Express* will be published as six monthly issues. Two volumes of six parts each will be published from 1985.

Astronomy Express aims to

★ publish up-to-the-minute information on significant new findings in astronomy, astrophysics and planetary sciences
★ feature news of current developments and trends as well as full-length research papers
★ utilise streamlined submission and refereeing procedures to ensure *speed of publication*
★ use quality paper to allow good reproduction of astronomical photographs
★ make no page charges to contributors (who will also receive a free supply of offprints)
★ offer special subscription rates to individuals – all prices will include rapid delivery by air mail or air speeded delivery

to: Aileen Askwith, Journals Publicity Controller at the Press. North American enquiries should be sent to Harry Florentine in our New York branch.

Please send me

Publicity leaflet and information on the 1984/1985 privelege subscription offer ☐
Specimen gridsheet ☐
Complimentary copy of the first issue ☐

Astronomy Express

name.....
address.....
.....
.....

CAMBRIDGE UNIVERSITY PRESS
The Edinburgh Building, Shaftesbury Road, Cambridge CB2 2RU — 32 East 57th Street, New York, NY 10022

Circle No.12 on Reader Service Card.



IUPHAR 9TH INTERNATIONAL CONGRESS OF PHARMACOLOGY

29 JULY – 3 AUGUST 1984

LOCATION

Barbican Centre for Arts and Conferences, City of London

SCIENTIFIC PROGRAMME

The scientific programme will extend over five days and consist of symposia, invited lectures, pharmacological debates, an educational meeting and free communications, the latter as both oral and poster sessions. The symposia will consist of three simultaneous sessions morning and afternoon and each day there will be four invited lectures. A new feature of the International Conference is the pharmacological debates. In these the intention is to select topics on which there are clear differences in evidence or its interpretation and have the issue debated between protagonists of each view with sufficient opportunity for the audience to participate. In the educational meeting it is hoped to concentrate on the place of various forms of programmed learning in Pharmacology and the instrumentation available. Finally, there will be free communications and to the fullest extent possible authors will be allowed to select whether to give these as oral communications or as a poster.

PROCEEDINGS

The Congress Proceedings are being published by Macmillan Press and may be ordered in advance on the registration form or at the Congress.

SOCIAL EVENTS

A full social programme is being arranged for delegates and accompanying guests. Details are given in the advance programme.

ACCOMMODATION

Accommodation has been reserved at advantageous rates in a number of hotels in various price categories and in student halls of residence. All accommodation is within easy travelling distance of the Barbican Centre.

DATES TO REMEMBER

1 May 1984	Deadline for college accommodation reservations.
18 May 1984	Deadline for receipt of registration at the standard fee (£150). There is a late fee after this date of a further £40. Deadline for receipt of papers for Proceedings volumes.

CONGRESS SECRETARIAT

Advance programmes, registration forms and abstract forms can be obtained from the Congress Secretariat:–

Congress Associates ICP
34 Stanford Road
London W8 5PZ
Telephone: 01 937 3163

Mathematics

Banach Bundles, Banach Modules and Automorphisms of C*- Algebras. Research Notes in Mathematics, Vol. 92. By M.J. DUPRÉ and R.M. GILLETTE. *Pitman: 1983. Pp.111. Pbk ISBN 0-273-08626-X. Np.*

Birkhoff Interpolation. Encyclopedia of Mathematics and Its Applications, Vol. 19. *Addison-Wesley: 1983. Pp.237. ISBN 0-201-13518-3. $32.50.*

Contributions to Nonlinear Partial Differential Equations. Research Notes in Mathematics, Vol. 89. By C. BARDOS *et al. Pitman: 1983. Pp.284. Pbk ISBN 0-273-08595-6. Np.*

Encyclopedia of Statistical Sciences, Vol. 3. S. KOTZ and N.L. JOHNSON (eds). *Wiley: 1983. Pp.722. ISBN 0-471-05549-2. £75.50, $105.75.*

The Method of Differential Approximation. By Y.I. SHOKIN. *Springer-Verlag: 1983. Pp.296. ISBN 3-540-12225-7/0-387-12225-7. DM 96, $41.40.*

Nonlinear Partial Differential Equations and Their Applications: Collège de France Seminar, Vol. 5. Research Notes in Mathematics, Vol. 93. By H. BREZIS and J.L. LIONS. *Pitman: 1983. Pp.370. Pbk ISBN 0-273-08620-0. Np.*

Similarity Solutions of Nonlinear Partial Differential Equations. Research Notes in Mathematics, Vol. 88. By L. DRESNER. *Pitman: 1983. Pp.124. Pbk ISBN 0-273-08621-9. Np.*

Space Science

Active Experiments in Space. Proceedings of an International Symposium held May 1983, Austria. *European Space Agency: 1983. Pp.375. Pbk FF.140.*

The Astronomical Almanac for the Year 1984. *HMSO: 1983. Np.*

Diffuse Matter in Galaxies. J. AUDOUZE *et al.* (eds). *Reidel: 1983. Pp.262. ISBN 90-277-1626-9. Np.*

Early Evolution of the Universe and Its Present Structure. G.O. ABELL and G.C. ARINI (eds). *Reidel: 1983. Pp.536. Hbk ISBN 90-277-1653-6; pbk ISBN 90-277-1662-5. Hbk Dfl. 160, $64; pbk Dfl. 85, $34.*

Glimpsing an Invisible Universe: The Emergence of X-Ray Astronomy. By R.F. HIRSH. *Cambridge University Press: 1983. Pp.186. ISBN 0-521-25121-4. £20, $39.50.*

Guidance and Control 1983, Vol. 51: Advances in the Astronautical Sciences. E.J. BAUMAN and Z.W. EMSLEY (eds). *American Astronautical Society: 1983. Pp.480. Hbk ISBN 0-87703-182-7; pbk ISBN 0-87703-183-5. Hbk $60; pbk $50.*

The Near-Earth and Interplanetary Plasma, Vol. 1: General Properties and Fundamental Theory. By Ya. L. AL'PERT. *Cambridge University Press: 1983. Pp.299. ISBN 0-521-24364-5. £35, $69.50.*

The Near-Earth and Interplanetary Plasma, Vol. 2: Plasma Flow, Plasma Waves and Oscillations. By Ya. L. AL'PERT. *Cambridge University Press: 1983. Pp.259. ISBN 0-521-24601-6. £30, $59.50.*

Observational Basis for Velocity Fields in Stellar Atmosphere. R. STALIO (ed.). *Observatorio Astronomico, Trieste: 1983. Pp.477. Np.*

Software Engineering. Proceedings of ESA/ESTEC Seminar held October 1983, The Netherlands. *European Space Agency: 1983. Pp.275. Pbk FF.140.*

Solar and Stellar Magnetic Fields: Origins and Coronal Effects. Symposium No. 102. J.O. STENFLO (ed.). *Reidel: 1983. Pp.564. Hbk ISBN 90-277-1619-6; pbk ISBN 90-277-1621-8. Np.*

Spacecraft Vibration Testing Using Multi-Axis Hydraulic Vibration Systems. Proceedings of a Workshop held May 1983, The Netherlands. *European Space Agency: 1983. Pp.173. Pbk FF.90.*

Physics

Advances in Laser Spectroscopy. NATO Advanced Science Institutes Series, Vol. 95. F.T. ARECCHI, F. STRUMIA and H. WALTHER (eds). *Plenum: 1983. Pp.506. ISBN 0-306-41355-8. $69.50.*

Advances in Superconductivity. NATO Advanced Science Institute Series, Vol. 100. B. DEAVER and J. RUVALDS (eds). *Plenum: 1983. Pp.529. ISBN 0-306-41388-4. $75.*

Amorphous Magnetism and Metallic Magnetic Materials-Digest: A Survey of the Literature with a Complete Bibliography. By A.R. FERCHMIN and S. KOBE. *Elsevier Biomedical/North-Holland: 1983. Pp.354. ISBN 0-444-86532-2. $65.50, Dfl.165.*

Concise Encyclopedia of Solid State Physics. R.G. LERNER and G.L. TRIGG 6eds). *Addison-Wesley: 1983. Pp.311. Hbk ISBN 0-201-14204-X; pbk ISBN 0-201-14205-8. Hbk $39.50; pbk $22.95.*

Elastic Media with Microstructure II: Three-Dimensional Models. Springer Series in Solid-State Sciences, Vol. 44. By I.A. KUNIN. *Springer-Verlag: 1983. Pp.272. ISBN 3-540-12078-5/0-387-12078-5. DM 98, $38.90.*

The Elements of Mechanics. by G. GALLAVOTTI. *Springer-Verlag: 1983. Pp.575. ISBN 0-387-11753-9/3-540-11753-9. DM 109, $44.60.*

A First Course in Fluid Dynamics. By A.R. PATERSON. *Cambridge University Press: 1983. Pp.528. Hbk ISBN 0-521-25416-7; pbk ISBN 0-521-27424-9. Hbk £30, $59.50; pbk £12.50, $24.95.*

Fourth Nordic Meeting on Nuclear Physics held August 1982, Fuglsø. Physica Scripta, Vol. T5. J. BONDORF And G. HAGEMANN (eds). *Royal Swedish Academy of Sciences: 1983. Pp.223. Np.*

The General Stokes' Theorem. By H. GRUNSKY. *Pitman: 1983. Pp.113. ISBN 0-273-08510-7. £18.95.*

Hyperfine Interactions of Radioactive Nuclei. Topics in Current Physics, Vol. 31. J. CHRISTIANSEN (ed.). *Springer-Verlag: 1983. Pp.366. ISBN 3-540-12110-2/0-387-12110-2. DM 84, $36.20.*

Introduction to the Fourier Transform and Pseudo-Differential Operators. by B.E. PETERSEN. *Pitman: 1983. Pp.356. ISBN 0-273-08600-6. £36.*

Ion Implantation: Equipment and Techniques. Springer Series in Electrophysics, Vol. 11. Proceedings of the 4th International Conference held September 1982, FRG. H. RYSSEL and H. GLAWISCHNIG (eds). *Springer-Verlag: 1983. Pp.556. ISBN 3-540-12491-8/0-387-12491-8. DM 90, $38.80.*

Nonlinear Methods of Spectral Analysis. Topics in Applied Physics, Vol. 34. S. HAYKIN (ed.). *Springer-Verlag: 1983. Pp.263. Pbk ISBN 3-540-123865/0-387-12386-5. DM 58, $23.10.*

Nordic Conference on Surface Science held August 1982, Tampere. Physica Scripta, Vol. T4. M. PESSA and R. NIEMINEN (eds). *Royal Swedish Academy of Sciences: 1983. Pp.210. Np.*

Point Defects in Semiconductors II: Experimental Aspects. Springer Series in Solid-State Sciences, Vol. 35. By J. BOURGOIN and M. LANNOO. *Springer-Verlag: 1983. Pp.295. ISBN 3-540-11515-3/0-387-11515-3. DM 82, $35.40.*

Process and Device Simulation for MOS-VLSI Circuits. P. ANTOGNETTI *et al.* (eds). *Martinus Nijhoff: 1983. Pp.632. ISBN 90-247-2689-1. Dfl. 180, $78.50.*

Production and Physics of Highly Charged Ions. Proceedings of an International Symposium held June 1982, Stockholm. Physica Scripta, Vol. T3. L. LLLJEBY (ed.). *Royal Swedish Academy of Sciences: 1983. Pp.256. Np.*

Quantum Chromodynamics: An Introduction to the Theory of Quarks and Gluons. By F.J. YNDURAIN. *Springer-Verlag: 1983. Pp.227. ISBN 0-387-11752-0/3-540-11752-0. DM 78, $31.*

The Quest for Quarks. By B. McCUSKER. *Cambridge University Press: 1983. Pp.160. ISBN 0-521-24850-7. £7.95, $14.95.*

Statistical Physics and the Atomic Theory of Matter From Boyle and Newton to Landau and Onsager. By S.G. BRUSH. *Princeton University Press: 1983. Pp.356. Hbk ISBN 0-691-08325-8. Hbk £38.70; pbk £12.70.*

Statistical Physics I: Equilibrium Statistical Mechanics. Springer Series in Solid-State Sciences, Vol. 30. By M. TODA, R. KUBO and N. SAITO. *Springer-Verlag: 1983. Pp.249. ISBN 3-540-11460-2/0-387-114690-2. DM 82, $33.90.*

Techniques and Concepts of High-Energy Physics II. NATO Advanced Science Institute Series, Vol. 99. T. FERBEL (ed.). *Plenum: 1983. Pp.358. ISBN 0-306-41385-X. $49.50.*

Topics in Ocean Physics. Proceedings of the International School of Physics Course held July 1980, Villa Monastero. A.R. OSBORNE and P.M. RIZZOLI (eds). *Elsevier Biomedical/North-Holland: 1983. Pp.550. ISBN 0-444-86160-2. $98, Dfl. 255.*

Chemistry

Advanced Organic Chemistry, 2nd Edn. Part B: Reactions and Synthesis. By F.A. CAREY and R.J. SUNDBERG. *Plenum: 1983. Pp.650. Hbk ISBN 0-306-41088-5; pbk ISBN 0-306-41199-7. Hbk $59.50; pbk $16.95.*

Chemical Thermodynamics. By P.A. ROCK. *Oxford University Press/University Science Books: 1983. Pp.548. ISBN 0-19-855712-5/0-935702-12-1. £22.50.*

Chemists. The Biographical Dictionary of Scientists. D. ABBOTT (ed.). *Blond Educational: 1983. Pp.203. ISBN 0-584-70000-8. £10.95.*

Derivatives of Hydrazine and Other Hydronitrogens Having N–N Bonds. By P.A.S. SMITH. *Benjamin/Cummings: 1983. Pp.335. ISBN 0-8053-8902-4. $49.95.*

Design 1982. The Institution of Chemical Engineers Symposium Series No. 76. *Pergamon: 1983. Pp.381. ISBN 0-08-028773-5. £31.50, $63.*

Molecular Ions: Spectroscopy, Structure and Chemistry. T.A. MILLER and V.E. BONDYBEY (eds). *Elsevier Biomedical/North-Holland: 1983. Pp.278. ISBN 0-444-86717-1. $48, Dfl. 125.*

Organic Chemistry in Colour. by P.F. GORDON and P. GREGORY. *Springer-Verlag: 1983. Pp.322. ISBN 3-540-11748-2/0-387-11748-2. DM 178, $70.70.*

Problems in Quantum Chemistry. By P. JØRGENSEN and J. ODDERSHEDE. *Addison-Wesley: 1983. Pp.286. ISBN 0-201-05486-8. $26.95.*

Progress in Crystal Growth and Characterization, Vol. 5. B.R. PAMPLIN (ed.). *Pergamon: 1983. Pp.420. ISBN 0-08-031011-7. £78, $141.*

Progress in Reaction Kinetics, Vol. 11. K.R. JENNINGS and R.B. CUNDALL (eds). *Pergamon: 1983. Pp.274. ISBN 0-08-030040-5. £60, $103.*

Progress in Solid State Chemistry, Vol. 14. G.M. ROSENBLATT And W.L. WORRELL (eds). *Pergamon: 1983. Pp.312. ISBN 0-08-030998-4. £66, $120.*

Steric Effects in Drug Design. Topics in Current Chemistry, Vol. 114. M. CHARTON and I. MOTOC (eds). *Springer-Verlag: 1983. Pp.161. ISBN 3-540-12398-9/0-387-12398-9. DM 76, $30.20.*

Computer Science

Advances in Computers, Vol. 22. M.C. YOVITS (ed.). *Academic: 1983. Pp.377. ISBN 0-12-012122-0. $60.*

Basic Microcomputing and Biostatistics: How to Program and Use Your Microcomputer for Data Analysis in the Physical and Life Sciences, including Medicine. By D.W. ROGERS. *Humana: 1983. Pp.304. ISBN 0-89603-015-6. $39.50 (US), $49.50 (elsewhere).*

Biodata Handling with Microcomputers. By R.B. BARLOW. *Elsevier Biomedical/North-Holland: 1983. Pp.261. Pbk ISBN 0-444-80539-7. $30, £18.*

Microcomputers in Plain English. By B.D. STRONG. *David & Charles: 1983. Pp.112. ISBN 0-7153-8509-7. £5.95.*

Earth Sciences

Air–Sea Exchange of Gases and Particles. P.S. LISS and W.G.N. SLINN (eds). *Reidel: 1983. Pp.561. ISBN 90-277-1610-2. Dfl. 160, $69.50.*

Changing Climate: Report of the Carbon Dioxide Assessment Committee. *National Academy Press: 1983. Pp.496. Pbk ISBN 0-309-03425-6. $29.50.*

Chemical Oceanography, Vol. 8. J.P. RILEY and R. CHESTER (eds). *Academic: 1983. Pp.398. ISBN 0-12-588608-X. £45, $75.*

Coastal Oceanography. NATO Conference Series, Vol. 11. H.G. GADE, A. EDWARDS and H. SVENDSEN (eds). *Plenum: 1983. Pp.582. ISBN 0-306-41374-4. $79.50.*

Continental Basalts and Mantle Xenoliths. C.J. HAWKESWORTH and M.J. NORRY (eds). *Shiva, Cheshire: 1983. Pp.272. Hbk ISBN 0-906812-33-X; pbk ISBN 0-906812-34-8. Hbk £25; pbk £12.50.*

Dictionary of Petrology. By S.I. TOMKEIEFF. *Wiley: 1983. Pp.680. ISBn 0-471-10159-1. £49.50, $97.50.*

Geochemistry of Sedimentary Ore Deposits. By J.B. MAYNARD. *Springer-Verlag: 1983. Pp.305. ISBN 0-387-90783-1/3-540-90783-1. DM 69, $27.40.*

International Minerals: A National Perspective. A.F. AGNEW (ed.). *Westview: 1983. Pp.164. ISBN 0-86531-622-8. Np.*

Large-Scale Dynamical Processes in the Atmosphere. B. HOSKINS and R. PEARCE (eds). *Academic: 1983. Pp.390. ISBN 0-12-356680-0. £33, $54.50.*

Mega-Geomorphology. By R. GARDNER and H. SCOGING. *Oxford University Press: 1983. Pp.240. ISBN 0-19-823244-6. £20.*

Migmatites, Melting and Metamorphism. Proceedings of the Geochemical Group of the Mineralogical Society. M.P.ATHERTON and C.D. GRIBBLE (eds). *Shiva, Cheshire: 1983. Pp.326. Hbk ISBN 0-906812-26-7; pbk ISBN 0-906812-25-9. Hbk £25; pbk £12.50.*

The Mountains of Northeastern Tasmania: A Study of Alpine Geomorphology. By N. CAINE. *A.A. Balkema: 1983. Pp.200. ISBN 90-6191-289-X. £15.50.*

Noble Gas Geochemistry. By M. OZIMA and F.A. PODOSEK. *Cambridge University Press: 1983. Pp.367. ISBN 0-521-23939-7. £40, $79.50.*

Optimal Seismic Deconvolution: An Estimation-Based Approach. By J.M. MENDEL. *Academic: 1983. Pp.254. ISBN 0-12-490780-6. $37.50.*

Palaeomagnetism: Principles and Applications in Geology, Geophysics and Archaeology. By D.H. TARLING. *Chapman & Hall: 1983. Pp.379. Hbk ISBN 0-412-23920-5; pbk ISBN 0-412-25100-0. Hbk £25; pbk np.*

Technology

Advances in Nuclear Science and Technology, Vol. 15. J. LEWINS and M. BECKER (eds). *Plenum: 1983. Pp.406. ISBN 0-306-41392-2. $57.50.*

Hydrogen: Energy Vector of the Future. *Graham & Trotman: 1983. Pp.230. ISBN 0-86010-451-6. £22, $41.*

Modern Chlor-Alkali Technology, Vol. 2. C. JACKSON (ed.). *Ellis Horwood/Halsted: 1983. Pp.389. ISBN 0-85312-525-2/0-470-27471-9. £35.*

Remote Sensing Applications for Environmental Studies. Proceedings of the EARSeL/ESA Symposium held April 1983, Brussels. *European Space Agency: 1983. Pp.291. Pbk FF.170.*

Thin Film Device Applications. By K.L. CHOPRA and I. KAUR. *Plenum: 1983. Pp.300. ISBN 0-306-41297-7. $42.50.*

VLSI Electronics Microstructure Science, Vol. 6: Materials and Process Characterization. N.G. EINSPRUCH and G.B. LARRABEE (eds). *Academic: 1983. Pp.601. ISBN 0-12-234106-6. $79.*

Applied Biological Sciences

Advances in Cancer Research, Vol. 39. G. KLEIN and S. WEINHOUSE (eds). *Academic: 1983. Pp.343. ISBN 0-12-006639-4. $46.*

Analytical Profiles of Drug Substances, Vol. 12. K. FLOREY (ed.). *Academic: 1983. Pp.735. ISBN 0-12-260812-7. $47.*

Biofeedback and Family Practice Medicine. *W.H. RICKLES et al. (eds). Plenum: 1983. Pp.244. ISBN 0-306-41386-8. $29.50.*

Biological Aspects of Alzheimer's Disease. Banbury Report 15. R. KATZMAN (ed.). *Cold Spring Harbor Laboratory: 1983. Pp.375. ISBN 0-87969-213-8. $55 (US), $66 (elsewhere).*

Biotechnology, Chemical Feedstocks and Energy Utilization. By D.F. GIBBS and M.E. GREENHALGH. *Frances Pinter: 1983. Pp.116+ appendix. ISBN 0-86187-346-7. £16.50.*

Chemical Mutagens: Principles and Methods of Their Detection, Vol. 8. F.J. de SERRES (ed.). *Plenum: 1983. Pp.386. ISBN 0-306-41336-1. $45.*

Clinical and Biological Aspects of Peripheral Nerve Diseases. Neurology and Neurobiology, Vol. 4. Proceedings of the Symposium held September 1982, Italy. L. BATTISTIN, G.A. HASHIM and A. LAJTHA 6eds). *Alan R. Liss: 1983. Pp.402. ISBN 0-8451-2703-9. £37.*

Compartmental Modeling and Tracer Kinetics. Lecture Notes in Biomathematics, Vol. 50. By D.H. ANDERSON. *Springer-Verlag: 1983. Pp.302. Pbk ISBN 3-540-12303-2/0-387-12303-2. DM 44, $17.50.*

Computer-Aided Electromyography. Progress in Clinical Neurophysiology, Vol. 10. J.E. DESMEDT (ed.). *Karger: 1983. Pp.334. ISBN 3-8055-3748-4. SwFr. 165, DM 198, $99.*

Culture of Animal Cells: A Manual of Basic Technique. By R.I. FRESHNEY. *Alan R. Liss: 1983. Pp.310. ISBN 0-8451-0223-0. £38.*

Diabetes, Obesity and Hyperlipidemias II. G. CREPALDI, P.J. LEFÈBVRE and D.J. GALTON (eds). *Academic: 1983. Pp.552. ISBN 0-12-195480-3. £25, $42.*

The Drug Handbook 1983–4. By P. TURNER and G.N. VOLANS. *Macmillan Press, London: 1983. Pp.168. Pbk ISBN 0-333-34974-1. £4.95.*

Ecological Aspects of Used-Water Treatment, Vol. 2: Biological Activities and Treatment Processes. C.R. CURDS and H.A. HAWKES (eds). *Academic: 1983. Pp.308. ISBN 0-12-199502-X. £32, $55.*

Female Stress Incontinence. Contributions to Gynecology and Obstetrics, Vol. 10. U. ULMSTEN (ed.). *Karger: 1983. Pp.72. ISBN 3-8055-3665-8. SwFr. 55, DM 66, $33.*

Food Chains to Biotechnology. Selected Topics in Biology. By J.B. LAND and R.B. LAND. *Nelson: 1983. Pp.208. Pbk ISBN 0-17-448088-1. £4.50.*

Food Microbiology: Advances and Prospects. The Society for Applied Bacteriology Symposium Series No. 11. T.A. ROBERTS And F.A. SKINNER (eds). *Academic: 1983. Pp.416. ISBN 0-12-589670-0. £29.95, $49.95.*

Sleep/Wake Disorders: Natural History, Epidemiology, and Long-Term Evolution. G. GUILLEMINAULT and E. LUGARESI (eds). *Raven: 1983. Pp.302. ISBN 0-89004-906-8. $58.50.*

Techniques in Immunocytochemistry, Vol.2. G.R. BULLOCK and P. PETRUSZ (eds). *Academic: 1983. Pp.300. ISBN 0-12-140402-1. £25, $41.50.*

Ultrasound Interactions in Biology and Medicine. R. MILLNER, E. ROSENFELD and U. COBET (eds). *Plenum: 1983. Pp.216. ISBN 0-306-41367-1. $37.50.*

Upgrading Waste for Feeds and Food. By D.A. LEDWARD, A.J. TAYLOR and R.A. LAWRIE. *Butterworths: 1983. Pp.321. ISBN 0-408-10837-1. £34.*

Urologic Cancer: Chemotherapeutic Principles and Management. Recent Results in Cancer Research, Vol.85. F.M. TORTI (ed.). *Springer-Verlag: 1983. Pp.151. ISBN 3-540-12163-3/0-387-12163-3. DM 98, $42.30.*

Psychology

Advances in Clinical Child Psychology, Vol.6. B.B. LAHEY and A.E. KAZDIN (eds). *Plenum: 1983. Pp.328. ISBN 0-306-41330-2. $39.50.*

CNS Receptors: From Molecular Pharmacology to Behaviour. Advances in Biochemical Psychopharmacology, Vol.37. P. MANDEL and F.V. DeFEUDIS (eds). *Raven: 1983. Pp.528. ISBN 0-89004-827-4. $60.*

Current Issues in Clinical Psychology, Vol.1. E. KARAS (ed.). *Plenum: 1983. Pp.301. ISBN 0-306-41362-0. $42.50.*

The Psychology of Cognition, *2nd Edn.* By G. COHEN. *Academic: 1983. Pp.277. Hbk 0-12-178760-5; pbk ISBN 0-12-178762-1. Hbk np; pbk £8.40, $16.*

Research on the Viral Hypothesis of Mental Disorders. Advances in Biological Psychiatry, Vol.12. P.V. MOROZOV (ed.). *Karger: 1983. Pp.175. Pbk ISBN 3-8055-3706-9. SwFr. 91, DM 109, $54.50.*

History of Science

L'esprit des Protéines: Historie et Philosophie Biochimiques. By C. DEBRU. *Hermann, Paris: 1983. Pp.368. Pbk ISBN 2-7056-5952-8. FF 96.*

Geometry and Algebra in Ancient Civilizations. By B.L. VAN DER WAERDEN. *Springer-Verlag: 1983. Pp.223. ISBN 3-540-12159-5/0-387-12159-5. DM 78, $32.30.*

History of Staining, 3rd Edn. By G. CLARK and F.H. KASTEN. *Williams & Wilkins: 1983. Pp.304. ISBN 0-683-01705-5. £22.25.*

Raymond Arthur Dart: A Pictorial Profile. By F. WHEELHOUSE. *Transpareon Press, Sydney: 1983. Pp.113. ISBN 0-908021-046. Np.*

Anthropology

Ancient Sedimentary Environments and the Habitats of Living Organisms. By J-C. GALL. *Springer-Verlag: 1983. Pp.220. ISBN 3-540-12137-4/0-387-12137-4. DM 60, $24.80.*

Koobi Fora, Vol.2: The Fossil Ungulates. J.M. HARRIS (ed.). *Oxford University Press: 1983. Pp.321. ISBN 0-19-857398-7. £45.*

Man, A Geomorphological Agent: An Introduction to Anthropic Geomorphology. By D. NIR. *Reidel: 1983. Pp.165. ISBN 90-277-1401-0. Np.*

Quaternary Coastlines and Marine Archaeology: Towards the Prehistory of Land Bridges and Continental Shelves. P.M. MASTERS and N.C. FLEMMING (eds). *Academic: 1983. Pp.660. ISBN 0-12-479250-2. £25, $42.*

The Search for our Beginning: An Enquiry, Based on Meteorite Research, into the Origin of our Planet and id Life. By R. HUTCHISON. *Oxford University Press/British Museum (Natural History): 1983. Pp.164. ISBN 0-19-858505-5/0-190520435-5/0-565-00851-X. £7.95.*

The Shanidar Neandertals. By E. TRINKAUS. *Academic: 1983. Pp.502. ISBN 0-12-700550-1. $47.50.*

General

Acid Rain: A Review of the Phenomenon in the EEC and Europe. *Graham & Trotman: 1983. Pp.159. ISBN 0-86010-501-6. £12.50.*

Advanced Synergetics: Instability Hierarchies of Self-Organizing Systems and Devices. Springer Series in Synergetics, Vol.20. By H. HAKEN. *Springer-Verlag: 1983. Pp.356. ISBN 3-540-12162-5/0-387-12162-5. DM 98, $38.90.*

Apples: A Guide to the Identification of International Varieties. By J. BULTITUDE. *Macmillan, London: 1983. Pp.323. ISBN 0-333-34971-7. £27.50.*

The Arid Lands: Their Use and Abuse. By R.L. HEATHCOTE. *Longman: 1983. Pp.323. Pbk ISBN 0-582-30048-7. £7.95.*

Betrayers of the Truth. By W. BROAD and N. WADE. *Simon & Schuster: 1983. Pp.256. Pbk ISBN 0-671-49549-6. $6.95.*

Beyond Mind. By W.E.R. MONS. *Rider, London: 1983. Pp.255. ISBN 0-09-153550-6. £6.95.*

Biomedical Institutions, Biomedical Funding, and Public Policy. H.H. FUDENBERG (ed.). *Plenum: 1983. Pp.212. ISBN 0-306-41231-4. $29.50.*

Birdwatch Around Scotland. By W.R. MITCHELL. *Robert Hale: 1983. Pp.175. ISBN 0-7090-1248-9. £9.95.*

Care of the Wild: Family First Aid for All Wild Creatures. By W.J. JORDAN and J. HUGHES. *Rawson Associates: 1983. Pp.223. Hbk ISBN 0-89256-226-9; pbk ISBN 0-89256-240-4. Hbk $13.95; pbk $8.95.*

Chemistry and World Food Supplies: The New Frontiers. Chemrawn II. L.W. SHEMILT (ed.). *Pergamon: 1983. Pp.664. Hbk ISBN 0-08-029243-7; pbk ISBN 0-08-029242-9. Hbk £75, $135; pbk £42, $75.*

The Classification of Finite Simple Groups, Vol.1: Groups of Noncharacteristic 2 Type. By D. GORENSTEIN. *Plenum: 1983. Pp.487. ISBN 0-306-41305-1. $59.50.*

Cosmic Crystals. By R. BONEWITZ. *Turnstone Press: 1983. Pp.192. Pbk ISBN 0-85500-205-0. £3.95.*

Country Man on the Moors. By J.C. ATKINSON. *Oxford University Press: 1983. Pp.160. Pbk ISBN 0-19-281414-1. £2.50.*

The Culture of Technology. By A. PACEY. *Basil Blackwell: 1983. Pp.210. ISBN 0-631-13236-8. £15.*

The Dangers of New Weapon Systems. W. GUTTERIDE and T. TAYLOR (eds). *Macmillan Press, London: 1983. Pp.241. ISBN 0-333-35018-9. £25.*

Dark-Time World: Watching and Photographing Wildlife at Night. By E. BARTLETT. *Robert Hale: 1983. Pp.176. ISBN 0-7090-0996-8. £9.50.*

Dear Lord Rothschild: Birds, Butterflies and History. By M. ROTHSCHILD. *Hutchinson: 1983. Pp.398. ISBN 0-09-153740-1. £14.95.*

Decentralizing Electricity Production. H.J. BROWN (ed.). *Yale University Press: 1983. Pp.259. ISBN 0-300-02569-6. £27.*

The Demon in the Aether: The Story of James Clerk Maxwell. By M. GOLDMAN. *Paul Harris, Edinburgh: 1983. Pp.224. ISBN 0-86228-026-5. £18.*

Dictionary of Scientific and Technical Terms, 3rd Edn. S.P. PARKER (ed.). *McGraw-Hill: 1983. Pp.1,846. ISBN 0-07-045269-5. $70.*

Energy Storage, Compression and Switching, Vol.2. V. NARDI, H. SAHLIN and W.H. BOSTICK (eds). *Plenum: 1983. Pp.1,067. ISBN 0-306-41014-1. $125.*

NATURE CLASSIFIED

LONDON OFFICE Keith Walesby 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024)
AMERICAN OFFICES NEW YORK Miss Gene Fein 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900
SAN FRANCISCO JJHS (415) 392 6794 ●**PASADENA** JJHS (213) 796 9200
CHICAGO Didier & Broderick (312) 498 4520 ● **BOSTON** CEL Assoc (617) 848 9306
CANADIAN OFFICE TORONTO Peter Drake 17 Pine Crescent, Toronto, Ontario M4E 1L1 (416) 690 2423

RATES UK — Rest of world — £11.00 per centimetre. Minimum 3 cm. £2 is charged for the re-direction of replies to advertisements with a box number.

CONDITIONS. The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL AND POSTGRADUATE RESEARCH ASSISTANTS

required for MRC-funded posts to work on various projects on protein engineering and DNA replication under the supervision of Professor A R Fersht, FRS. One postdoctoral position is available immediately, the other two to start in September 1984. Work will involve protein chemistry and enzyme kinetics, gene cloning and mutation and oligodeoxynucleotide synthesis.

Salary scale: £8,376 — £10,611 for postdoctoral position and £7,496 — £9,716 for postgraduate, inclusive of London Allowance.

Applications (full curriculum vitae and names of two referees) to be sent to Professor A R Fersht, Department of Chemistry, Imperial College, London SW7 2AY. (1228)A

UNIVERSITY OF CALIFORNIA
San Diego

DEPARTMENT OF BIOLOGY

TENURE-TRACK FACULTY

position available July 1, 1984. In the general areas of cell and/or developmental biology. Salary and rank will be commensurate with qualifications and experience. The appointee must have had post-doctoral training and is expected to conduct a vigorous research program. Excellence in teaching and in the pursuit of an independent research program are the criteria to be met.

Applicants should send curriculum vitae, a list of publications, and a brief statement of research interests, by March 30, 1984 to: Ms Joan Turner, Department of Biology B-022, University of California, San Diego, La Jolla, California 92093.

The University of California at San Diego is an equal opportunity/affirmative action employer. (NW306)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF MOLECULAR BIOLOGY

POST-DOCTORAL RESEARCH FELLOW

Applications are invited for a post-doctoral research position to investigate the molecular structure and function of a novel transposable sequence found in wide host-range IncP plasmids. Experience in molecular genetics or plasmid DNA manipulations is desirable. The appointment is available from 1st March 1984 for one year and one month. Salary will be on Research Range 1A.

Applicants should send a cv, references and a letter describing their research interests to Prof N S Willets, Department of Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR.

Please quote reference No 5147. (1200)A

DURHAM UNIVERSITY SENIOR RESEARCH ASSISTANT IN SYNCHROTRON RADIATION RESEARCH

Applications are invited for the post of Senior Research Assistant tenable in the Physics Department for two years from early 1984. The successful applicant will work, under the supervision of Dr B K Tanner, on an SERC supported project to develop X-ray diffraction techniques for the characterization of strains in III-V semiconductors. Part of the work will be carried out on the Synchrotron Radiation Source at Daresbury Laboratory.

Initial salary in the range of £7630 — £8530 on National Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by 2 March 1984 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (1217)A

Imperial Chemical Industries PLC
Agricultural Division: Billingham: Cleveland
Biochemical Group

Biotechnologists

ICI Agricultural Division has three vacancies within its Biochemical Group of the Research Department.

We require top class scientists to make major inputs to the fields of: fermentation technology, microbiology, enzymology, protein chemistry, separation and enzyme immobilisation technology.

In particular we are looking for:

- a Fermentation Scientist with wide experience of fermentation processes and technology
- a Separation Scientist with experience in protein and polymer separation, enzyme purification and immobilisation.
- a Microbiologist to lead a small team engaged in strain improvement programmes for the microbial production of enzymes and metabolites.

The Division's Research Department has an excellent record of scientific innovation backed by strong commercial and engineering resources. This combination has placed ICI in the forefront of the developments in Biotechnology.

Applicants should possess a good honours degree in the appropriate discipline with a PhD demonstrating original thinking and commensurate technical skills. Post Doctoral or industrial/academic experience whilst desirable is not essential, but more mature candidates with this background are encouraged to apply.

Remuneration will be commensurate with qualifications and experience. The Company operates house purchase, profit sharing and contributory pension schemes and offers financial assistance towards removal expenses.

Applications giving details of age, qualifications and experience should be sent as soon as possible to:-

Mr M A J W Pegg, Personnel Department, Imperial Chemical Industries PLC, Agricultural Division, PO Box No 1, Billingham, Cleveland, TS23 1LB.

(1248)A



INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS

(ICRISAT)

ICRISAT

near Hyderabad, India needs

PROGRAM LEADER (Millet Improvement)

To provide scientific and administrative leadership to the program of Millet Improvement at ICRISAT Center under the general supervision of the Director of Research. He will formulate and implement research programs that will meet the program goals set by the Institute. The Millet Improvement Program currently has subprograms in Breeding, Pathology, Physiology, and Microbiology involving 14 scientists. The Program Leader will coordinate research with other programs at ICRISAT Center, and between research at headquarters and in other countries. He will participate in the overall guidance and management of the Institute, in its global activities, and in fostering appropriate institutional interrelationships. *Qualifications:* Doctorate in an appropriate discipline with an excellent academic record, proven ability to lead a team of scientists and contribute to interdisciplinary research programs, and ability to speak and write English well are essential. Substantial experience in plant breeding, at least ten years post-doctorate research experience, and ability to speak French are desirable.

Salary for the position is at principal staff level, according to qualifications and experience, with attractive fringe benefits. Work would be centered at ICRISAT Center (1394 ha) near Hyderabad, India, where excellent scientific and technical support is available. Hyderabad has international airline connections and good living conditions, recreational facilities, and medical and educational facilities (for children to age 10-12 years).

Send résumé and names of three referees by 31 March, 1984, to Director General, ICRISAT Patancheru PO 502 324, Andhra Pradesh, India. (W753)A

PROTEIN BIOCHEMIST

A senior biochemist is required to join a team working on scrapie and the related unconventional slow infections of the CNS. The appointee will be expected to develop and lead an innovative research programme to characterise (a) the infectious unit of scrapie (b) a gene product controlling the disease (c) cerebral amyloid and (d) scrapie-associated fibrils.

Applicants should have a good honours degree in chemistry or biochemistry and a PhD or the equivalent. A considerable proven research record in protein biochemistry is essential, proferably with experience in the analysis of hydrophobic proteins. The appointment will be as Principal Scientific Officer with salary in the range £11,343 – £14,931.

Applications giving full curriculum vitae and names of two referees to The Secretary, AFRC MRC Neuropathogenesis Unit, West Mains Rd., Edinburgh EH9 3JF. (1176)A

BIOTECHNOLOGISTS!

Are you reading Bio/Technology, the International Journal for Industrial Biology?

For a free sample copy of this new monthly Journal from the Publishers of *Nature*, please write or phone:

Mirta Soto
Bio/Technology
15 East 26th Street
New York 10010
USA
Tel: (212) 689 5900

Felicity Parker
Bio/Technology
Houndmills Industrial Estate
Brunel Road, Basingstoke
Hampshire, UK
Tel.: (0256) 29242

TRANSGENE

As a consequence of continued growth and expansion, and of a recent move into new, larger premises, TRANSGENE, a leading genetic engineering company based in STRASBOURG, FRANCE, has immediate openings in the following positions.

– SENIOR MOLECULAR BIOLOGIST

with extensive experience in eukaryotic cell vectors and in the expression of foreign proteins in cultured cells.

– BACTERIOLOGIST

with research experience in the physiology and genetic manipulation of microorganisms of industrial importance (the streptococci, lactobacilli, streptomyces . . .).

– MOLECULAR BIOLOGIST

with 2-3 years practical experience of cDNA cloning techniques.

– PROTEIN CHEMIST

with practical experience in the synthesis of peptides.

TRANSGENE is an independent recombinant DNA research and development company, backed by a group of major French industrial and financial organizations.

Interested candidates are invited to submit, in confidence, a full curriculum vitae, a list of publications, and the names and addresses of three referees to:

Dr. J. P. LECOCQ
Scientific Director
11 rue de Molsheim
67000 STRASBOURG
FRANCE
Tel: (88) 22.24.90.

(W756)A

RESEARCH ASSOCIATE IN STRUCTURAL BIOCHEMISTRY AND MASS SPECTROMETRY

The Department of Pharmaceutical Chemistry and the NIH National Mass Spectrometry Resource at UCSF have a long-term staff position at the Adjunct Assistant or Adjunct Associate Professor level available immediately with a salary range from $23,000 – $38,000. We are seeking an individual with experience in elucidation of structures of peptides and proteins including hormones, peptide mapping of cloned gene products, covalently modified proteins, antigenic determinants and/or substrate receptor complexes. The person must have experience in peptide and protein chemistry, photoaffinity labeling and sequencing by biochemical as well as mass spectrometric techniques. A strong candidate with appropriate isolation, degradation, and structural characterization credentials and a strong interest in learning the present advanced techniques of mass spectrometry will also be considered. The successful applicant will be expected to develop a strong core and collaborative research program, devote a portion of his/her effort to interdisciplinary training and service projects, and contribute to graduate teaching.

Applicants should sumbit curriculum vitae, a brief description of their proposed research and teaching interests, copies of their reprints and manuscripts, and three letters of recommendation to Prof. A. L. Burlingame, Dept. of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143. The University is an Equal Opportunity/Affirmative Action employer. Women and minorities are encouraged to apply. (NW307)A

Synthetic Organic Chemists For Drug Discovery

In the middle of 1984, as part of a major expansion programme, we shall be opening an additional 70,000 sq ft of laboratory and office space at our research centre at Sandwich, Kent. This will enable us to increase our commitment to human drug research, especially in the cardiovascular area.

We are looking for:

A Project Leader

to take charge of one of our cardiovascular projects.

Our need is for a synthetic organic chemist with 6/10 years' post-doctoral experience, mainly in the drug industry. He/she will probably be leading a small team already and seeking to broaden his/her responsibilities as a scientist. Experience in the cardiovascular area would be preferable but not essential.

Three Post-Doctoral Chemists

with experience in synthetic organic chemistry coupled with a desire to discover drugs. Close collaboration with biological colleagues is essential in our multi-disciplinary project teams.

Our need here is for one chemist with 3/5 years' research experience in the pharmaceutical or agricultural/veterinary industry and two chemists who are completing 2/3 years' post-doctoral research.

Graduate/GRSC/LRSC Chemists

We need several young, energetic chemists experienced in modern methods to synthesise novel molecules for biological evaluation.

Pfizer

Our laboratories are modern and well equipped with up-to-date instrumentation and support services. All our chemists have ready access to the Research Division computers and molecular graphic techniques are used regularly to aid the design of biologically active molecules.

If you have the necessary qualifications and experience and want to further your career in drug discovery please send your cv to: **D. W. Sells, Personnel Manager, Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ.**

If you have applied for a similar position with us in the past two years your previous application will be considered automatically; there is no need to reapply. All applications will be treated in strict confidence.

(1216)A

1979

Reproductive Biologist

Experienced embryologist with Ph.D. required to manage busy laboratory and devoted to both service and research of human infertility.

Facilities include a successful IVF programme (16 delivered infants to date) gamete and embryo cryopreservation, techniques for the study and treatment of oligospermia, the induction and intensive monitoring of ovulation and active research into the causes and treatment of early pregnancy wastage.

The laboratory has a nucleus staff of 3 biologists and 2 technologists and is linked to a large private Infertility Clinic with research undertaken in affiliation with the University of Western Australia. The successful applicant is expected to manage the laboratory, supervise research projects and liaise with the clinical staff to optimise patient management.

Salary negotiable in keeping with applicant's experience and expectations.

Apply by 31st March 1984 with detailed curriculum vitae to:
Dr. J.L. Yovich, Director, PIVET Laboratory, 442 Barker Road, Subiaco, Western Australia. Tel: 09-382-1677.

(W761)A

IMMUNO

AG FÜR CHEMISCH-MEDIZINISCHE PRODUKTE

Microbiologist/ Biochemist

Immuno AG is seeking a scientist for its Research and Development Laboratories, Research Center Orth near Vienna, to work on specific, goal-oriented biomedical research projects. The candidate must have a Ph. D. or equivalent degree as well as practical experience in at least one of the following areas: microbial antigens, microbial immunology, host defense mechanisms, and immunity to infectious diseases. Candidate must be able to work with existing multidisciplinary groups. Research facilities and working facilities are excellent. Please send curriculum vitae, list of publications, and names of 2 referees to:

IMMUNO AG Rudolf Lukschanderl, Recruiting Officer
Industriestrasse 67
A-1220 Vienna, Austria

(W750)A

IMMUNO

SABBATICAL LEAVE IN SPANISH LABORATORIES

In order to promote the interaction between the Spanish Scientific Community and scientists working outside Spain, the CAICYT (Spanish National Council for Research & Development) is launching a program encouraging scientists to spend their Sabbatical leave in Spanish Laboratories. The maximum length of stay is one year and the Program will be in operation from 1st September 1984 to 31st July 1986. The visiting scientists will receive a salary that will be awarded on the basis of qualifications and of the percentage of their current salary that is maintained by their home institution. Also some funds for expendable material in the receiving institution will be provided.

Scientists willing to take part in this Program are requested to contact the Spanish laboratory in which they intend to stay. The CAICYT will also help, on request, to establish a connection between Scientists eventually interested in the Program and those Spanish laboratories working in their area of interest. These contacts should result in a joint proposal that must be submitted to CAICYT before 1st June 1984.

Full details of the data that will be required are available either from the Spanish institutions or from CAICYT. The selected candidates will be notified not later than 1st July 1984.

All information concerning this advertisment should be requested from *Dr. Marisa Pacios, Caicyt, Edificio Torre Rioja, calle Rosario Pino, 14-16, Madrid 20, Spain. Tel: (34)-1-4501063.* (W752)A

PREMIER'S DEPARTMENT
THE AUSTRALIAN MUSEUM

SCIENTIFIC OFFICER

Curator of Birds or Mammals, (one position) Position No: AUS 83/11. Wage: A$17,110 range A$37,751. Subject to certain conditions, successful applicants may be eligible for reclassification as research scientist/senior research scientist/principal research scientist, with progression to $A39,051.

Qualification: Essential — University degree in zoology with research experience in an appropriate field. Applicants should have some knowledge of modern museum curatorial practice. Research interests should be orientated towards work which involves the use of the collections, with research on systematics (especially at supraspecific levels), evolutionary biology and biogeography preferred. A wide knowledge of mammalian or avian biology is essential and is more important than a thorough knowledge of Australian mammals or Birds. Duties: To curate the mammal or bird collections and to be responsible for the answering of public and professional scientific enquiries. To participate in the museum's display and educational programmes in the appointee's field of study, and to carry out an active programme of research in some field of Australian mammalogy or ornithology. Conditons: Subject to certain conditions, the successful applicant will be eligible for: payment of fares to Sydney, Financial assistance towards cost of removal expenses, and Financial assistance towards initial accommodation expenses.

Enquiries: Telephone (02) 339-8111 (Dr. Cogger). Applications are to be sent to the Staff Clerk, The Australian Museum, 6-8 College Street, Sydney 2001, by 10 April, 1984. (W751)A

THE UNIVERSITY OF CALGARY

Department of Chemistry Graduate Studies

Chemistry: Analytical, Inorganic, Organic, Physical and Theoretical Chemistry, and Applied Chemistry in projects dealing with Energy Conversion, Oil Sands Recovery, Sulphur Chemistry, and Synthetic Membrane Technology.

Programs leading to PhD and MSc degrees; Excellent laboratory, instrument, and library facilities; Minimum stipend of approximately $10,000 p.a. for Teaching and Research Assistantships; Maximum stipend of approximately $13,000 p.a. for outstanding students holding Scholarship combined with part-time Teaching Assistantships; Program fees remitted for full-time teaching appointments; travel allowance available.

For further information and descriptive brochures write to the following, indicating field of interest:

Chairman of Graduate Studies
Department of Chemistry
The University of Calgary
Calgary, Alberta T2N 1N4
Canada (NW192)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PHYSIOLOGY

UNIVERSITY LECTURER

Applications are invited for a University Lectureship, the appointment to commence on 1 October 1984. Candidates should hold a Ph.D. degree in some area of physiology, or a Medical or Veterinary qualification together with some experience of physiological research.

The appointment will be for three years, with the possibility of reappointment to the retiring age.

The pensionable scale of stipends, for persons not ordinarily resident in college, is £9,875 a year, rising by eleven annual increments to £15,085. There is no grade of Senior Lecturer.

Further information about the duties and conditions of appointment may be obtained from the Secretary of the Appointments Committee for the Faculty of Biology 'B', 19 Trumpington Street, Cambridge, CB2 1QA, to whom applications (12 copies), including a curriculum vitae, list of publications and the names of not more than three referees, should be sent so as to reach him not later than 1 April 1984. A candidate resident overseas may submit only one copy of an application. (1229)A

APPLICATIONS are invited for the post of a Temporary Research Technician Grade 5 to work on enzyme activity in field crops. The work is based at the University farm at Bramham where accommodation may be available. The appointment is for 10 months starting 1st March or as soon as possible thereafter at a salary of £6,279 pa. Applicants should have HNC or a Degree and relevant experience. Apply to Professor J Elston, Department of Plant Sciences, Agricultural Sciences Building, Leeds University LS2 9JT. (1223)A

PURDUE UNIVERSITY

LABORATORY OF RENEWABLE RESOURCES ENGINEERING

POSDOCTORAL POSITION AVAILABLE IMMEDIATELY

Applicants should have background in Microbiology, Molecular Genetics and/or Protein Chemistry. Experience in recombinant DNA, DNA-sequencing, and protein purification, particularly the latter is desired; nevertheless, interest and enthusiasm are equally important. Stipend is competitive.

Send curriculum vitae, transcripts, description of current research interests and objectives, and three letters of recommendation to: Dr N W Y Ho, Molecular Genetics Group, Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN 47907. Purdue is an equal opportunity/affirmative action employer. (NW297)A

UNIVERSITY CHEMICAL Laboratory, Cambridge. Applications are invited for a position as a post-doctoral assistant (SERC-funded in the IA salary scale, £8,080-£8,530) to work with Dr Ian Fleming on stereocontrolled organic synthesis using silicon-containing compounds. Appointment will be for up to 2 years starting 1 Oct 1984. Curriculum vitae and the names of 2 referees should be sent to Dr Ian Fleming, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. (1203)A

THE KENNEDY INSTITUTE

POST DOCTORAL RESEARCH FELLOW

Applications are invited for a Cellular or Molecular Biologist to join an active group led by Dr T E Hardingham and Professor H Muir FRS investigating the molecular basis of articular joint function. Previous experience investigating phenotypic expression in primary cell culture or mRNA isolation, in vitro translation and development of cDNA probes would be an advantage. The post is available immediately and is funded by the MRC until November 1986. Starting salary up to £10,161 (including London allowance) depending on age and experience.

Applications, together with the names of two referees, should be sent to the General Secretary, Mathilda and Terence Kennedy Institute of Rheumatology, 6 Bute Gardens, London W6 7DW. (1231)A

Allelix INC

POST-DOCTORAL RESEARCH Positions in MOLECULAR BIOLOGY

Allelix Inc., Canada's first major biotechnology company, has a number of post-doctoral positions available within the Molecular Biology Division. Applications are particularly invited from young scientists with experience in yeast molecular biology/genetics who will be available in the near future, but molecular biologists of all backgrounds are encouraged to apply, as emphasis will be placed on scientific ability rather than field of experience.

Allelix provides the opportunity to learn from and interact with experienced staff scientists in addition to the immediate project leader, in a well-equipped modern laboratory with strong links to local universities. Salaries benefits are at fully competitive levels.

Interested candidates are asked to send complete résumés, including three references and quoting file number 0061, to:

Dr. R. W. Davies
Allelix Inc.
6850 Goreway Drive
Mississagua, Ontario
L4V 1P1

(NW291)A

SANDOZ, a world leader in the development of ethical pharmaceuticals, has an opening in Preclinical Research for a well-qualified

Pharmacologist

to work on the development of new drugs for the treatment of degenerative diseases of the CNS, particularly senile dementia.

The successful candidate will be responsible for designing and supervising *in vivo* and *in vitro* experiments to elucidate the central and peripheral pharmacological profiles of newly synthesized compounds.

Applicants for this permanent post should have relevant post-doctoral experience in the use of classical and modern pharmacological techniques, and should be keen to work as a member of a multidisciplinary team comprising medicinal chemists, biochemical and behavioural pharmacologists, and electrophysiologists. The candidate should desire to pursue a research career, and will be expected to initiate and design scientific projects.

Applications, including a detailed curriculum vitae, should be addressed to:

Sandoz LTD.,
The Personnel Department, Ref. 8415
CH-4002 Basle, Switzerland (W757)A

SANDOZ

WANTED a young scientist with expertise in immunology or virology for a tenure track position with guaranteed funding for five years and a joint appointment in the Departments of Opthalmology and Microbiology at the College of Physicians & Surgeons of Columbia University. Candidate will be expected to develop an independent vision oriented research program. Interested candidates should send their curriculum vitae and names of three references to either: Peter Gouras, MD, Director of Research, Department of Opthalmology or Harold Ginsberg, MD, Chairman, Department of Microbiology, College of Physicians & Surgeons, Columbia University, 630 W 168 St, New York, NY 10032. (NW292)A

UNIVERSITY OF OXFORD IN ASSOCIATION WITH WORCESTER COLLEGE

UNIVERSITY LECTURERSHIP IN PHARMACOLOGY

Applications are invited for the above post. Stipend according to age on the scale £7,190 to £15,085.

The successful candidate may be offered a stipendiary Tutorial Fellowship by Worcester College. Details from: Sir William Paton, Department of Pharmacology, South Parks Road, Oxford OX1 3QT, to whom applications (nine typed copies; two for overseas applicants) with the names of three referees should be sent by 30 April 1984. (1215)A

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE POSTDOCTORAL RESEARCH FELLOW

Available immediately. Applications are invited for a 2-3 year postdoctoral research appointment in molecular biology/protein chemistry to investigate structure/function of oncogenes, growth factors, and transforming proteins.

Apply with curriculum vitae and two references to Dr Thomas F Deuel, Division of Hematology, Washington University School of Medicine, 216 S Kingshighway, St Louis, MO 63110. (NW271)A

UNIVERSITY OF UTAH

DEPARTMENT OF BIOENGINEERING

Applications from candidates with an earned doctorate are invited for one/two tenure track faculty positions, assistant to full professor level. The appointee(s) will teach courses in bioengineering and will be expected to conduct an outstanding independent program of research. Joint appointments with other departments possible.

Send curriculum vitae and three recent publications to: Prof Richard A Normann, Department of Bioengineering, University of Utah, Salt Lake City, Utah, 84112. Application deadline April 15, 1984. An affirmative action/equal opportunity employer. (NW294)A

ARMAGH OBSERVATORY

Armagh, N. Ireland

RESEARCH ASTRONOMER

Applications are invited for the post of Research Astronomer tenable from 1st June 1984. Salary will be at an appropriate point on the University Lecturer's scale £7,190 — £14,125 p.a. according to qualifications and experience. Applicants should send a detailed curriculum vitae and the names of three referees before 31 March 1984 to The Secretary, Armagh Observatory, Armagh BT61 9DG, N. Ireland from whom further particulars may be obtained. (1235)A

THE UNIVERSITY OF SHEFFIELD

WEST RIDING CHAIR OF MICROBIOLOGY

Applications are invited for the above Chair, which fell vacant on the appointment of Professor J R Quale, FRS to the Vice-Chancellorship of the University of Bath.

The Chair will be tenable from 1 October 1984. Salary in the range for professorial appointments (average £20,300: minimum £17,275).

Particulars from the Registrar and Secretary (Staffing), The University, Sheffield S10 2TN to whom applications (one copy), quoting the names of three referees, should be sent by 5 March 1984.

Quote ref: R32/G. (1134)A

POSTDOCTORAL Position. Available immediately, to study gene expression and recombination in mammalian cells using a bovine papilloma virus vector. Involves gene cloning, cell transformation, and somatic cell genetics. Experience in nucleic acid biochemistry desirable. Send CV and names of references to: Dr William T McAllister, Department of Microbiology, University of Medicine and Dentistry of New Jersey — Rutgers Medical School, PO Box 101, Piscataway, New Jersey 08854. An Equal Opportunity/Affirmative Action Employer. (NW289)A

MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH DIVISION OF PARASITOLOGY

A postdoctoral protein chemist is required for a WHO funded fellowship for a period of up to three years studying surface antigens of schistosomes. Experience in protein purification, peptide analysis and microsequencing is essential.

The salary will be in the range £8,530 - £10,250 per annum plus £1,186 London allowance, with superannuation provision.

Applications should include a curriculum vitae and the names of two referees and should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA, before 16th March 1984, quoting ref: P/5/SRM. (1210)A

Post Doctoral Scientists Biochemistry/Biochemical Pharmacology

SmithKline Beckman, a leader in pharmaceutical research, is seeking Post Doctoral Scientists to work with Dr. Philip Schein in an environment designed to foster creative, innovative research and to promote personal and professional growth.

We consider post doctoral studies to be of major importance in an individual's career and encourage publication of findings in leading journals and a full interaction with the scientific community.

Our postdoctoral appointments are of 1 year duration and with mutual agreement, renewable for a 2nd year. The successful candidates will join a multidisciplinary team whose goal is to understand the biochemical mechanism of anticancer agent cytotoxicity and tissue-specific selectivity. We are particularly interested in seeking individuals who can make a significant contribution to one of the following current programs:

- structure-activity analyses of sites of alkylating agent binding and repair in transcriptionally active chromatin and chromatin substructure.
- define methods of modulating the structure and function of chromatin.
- determination of sites within chromatin which are critical for alkylating agent cytotoxicity.
- identification of anticancer agents that have specificity for neoplastic tissue.

Candidates are required to have a Ph.D. in Biochemistry and/or Biochemical Pharmacology with 0-3 years post doctoral experience.

Preference will be given to candidates having a strong background in biochemistry/ biochemical pharmacology and relevant publications in major scientific journals.

We offer excellent compensation and benefits and an attractive relocation policy. For confidential consideration, send your CV and names of three references to: Mr. Walter B. Flagg, **SMITHKLINE BECKMAN CORPORATION,** 1596 Spring Garden Street, Philadelphia, 19101. We are an equal opportunity employer, M/F/H/V.

(NW290)A

SK&F

A SmithKline Beckman Company

UNIVERSITY OF KONSTANZ
Federal Republic of Germany

RESEARCH FELLOW ELECTROPHYSIOLOGY

Applications are invited for the above position to work on the electrophysiology of active ion transport in cell membranes.

Applications, with curriculum vitae, giving details of experience and the names of two referees should be sent to Prof. Dr. P. Läuger, Department of Biology, University of Konstanz, post-office-box 5560, D - 7750 Konstanz / Germany.

(W759)A

PUBLIC HEALTH LABORATORY SERVICE BOARD
PHLS Centre for Applied Microbiology and Research
MICROBIAL TECHNOLOGY LABORATORY

A Post-graduate Biochemist is required to evaluate novel support matrices for the large scale affinity chromatography of proteins. This post is sponsored by the Engineering Sciences Division of AERE Harwell under the auspices of the BIOSEP project and is available for one year in the first instance. Applicants should have a PhD or an equivalent research qualification. Experience in the purification of proteins on a larger than normal laboratory scale and some understanding of the engineering aspects of large scale chromatography is desirable.

Appointments, subject to qualification and experience will be made on the post-probationary basic grade scale, £6,520 – £8,349. NHS terms and conditions will apply.

Applications with full curriculum vitae and the names and addresses fo two professional or academic referees should be sent to: Mrs. M. Bushby, Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG. Candidates seeking further information should contact Dr. M. Scawen on Idmiston (0908) 610391 extension 220.

Closing date for applications will be 2 March 1984. (1236)A

STATE UNIVERSITY OF NEW YORK
POSTDOCTORAL BIOPHYSICS AND PHYSIOLOGY

Intracellular recording and patch clamping. Contact K N Leibovic, Department of Biophysics, Suny at Buffalo, 14214. (716) 831-3249 or 836-5837. (NW287)A

CENTRAL BIRMINGHAM HEALTH AUTHORITY
Birmingham Maternity Hospital
BASIC GRADE CYTOGENETICIST

Applications are invited for a Basic Grade Cytogeneticist. The Cytogenetics Laboratory is situated in the Infant Development Unit at the above Hospital. This Laboratory provides a comprehensive service for the Birmingham area and a large part of the West Midlands Region. Applicants should hold a 1st or 2nd Class Science Honours Degree. The salary scale is £6,277 – £7,953 per annum.

Arrangements to visit the Laboratory may be made with Mrs S Bowser-Riley, Principal Cytogeneticist.

For an application form and job description, please telephone the Personnel Department on 021-472 1377 ext 118.

Closing date: Thursday 1st March 1984. (1206)A

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG
MRC/UNIVERSITY CIRCULATION RESEARCH UNIT
ASSISTANT RESEARCH OFFICER

Applications are invited from suitably qualified persons, regardless of sex, race, colour or national origin for appointment to the post of Assistant Research Officer in the University Circulation Research Unit.

The Assistant Research officer will be expected to participate in an on-going programme on the control of coronary blood flow in health and disease, as well as the biochemistry of autonomic receptors in the cardiovascular system. Applicants should have at least a BSc Honours degree with a background in physiology, biology, zoology biochemistry or veterinary science.

The salary attached to this post wil be within the range R9,075 – R14,21' per annum. A pensionable allowanc of 12% is also payable. The initia notch will be determined by th qualifications and experience of th successful applicant.

Intending applicants should obtair the information sheet relating to thi post from the Secretary, Soutl African Universities Office Chichester House, 278 High Holborn London WC1V 7HE, England o from the Assistant Registra (Academic Staffing) University of th Witwatersrand, 1 Jan Smuts Avenue Johannesburg 2001 South Africa wit whom applications should be lodge not later than 31 March 1984.

(W754)A

MRC CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow,
Middx HA1 3OJ
TECHNICIAN

A vacancy exists in the division o immunochemical genetics for a tech nician with a degree, HNC o equivalent qualification in chemistr or biochemistry.

The work of the division i concerned with the molecular basi and genetic regulation o carbohydrate antigens as exemplifie by certain blood group antigens. Th successful applicant will be require to isolate oligosaccharides fron natural sources and to undertak chemical systheses of disaccharide and nucleotide sugars by establishe procedures. Previous experience i carbohydrate chemistry would be a advantage.

Salary will be on the scale £6,087 – £8,343 (Inclusive of Londo Weighting) depending on age an experience.

Applications forms and furthe details may be obtained by applyin in writing to Miss J Cottam Personnel Section quoting re 134/2/3649.

Closing date for applications 9th March 1984. (1208)A

Ethical Pharmaceuticals

Director of Research

S E England — Not less than £30,000 + car etc

My client is a profitable, sizeable and autonomous subsidiary of one of the world's most prestigious drug companies. In the UK it is engaged in the research, manufacture and marketing of a wide range of ethical pharmaceuticals.

It now wishes to appoint a new Director of Research, who will earn and use the support of a young, energetic and interested Board and prove worthy of appointment to it. Reporting to the Managing Director, the Director of Research will have full responsibility for the considerable Research Function, its enviable facilities and more than 200 staff.

The highest academic research record is vital; skills in leadership, management and development of the research teams must combine with a wholehearted and effective input to this multinational's research operations worldwide for the discovery of drugs that are medically valuable and commercially viable.

If you are interested in the very real challenge and rewards (salary will not be a limiting factor) of this job, write or phone me, Mrs. Indira Brown, Corporate Resourcing Group Ltd., 6 Westminster Palace Gardens, Artillery Row, London SW1P 1RL. Telephone: 01-222 5555. As an independent consultant, I shall treat your enquiry with complete discretion.

Corporate Resourcing Group

Management Consultants. Executive Search

Part of Berndtson International

BRUSSELS·COPENHAGEN·FRANKFURT·GENEVA·LONDON·MADRID·NEW YORK·PARIS

(1249)A

UNIVERSITY OF UTAH

DEPARTMENT OF MECHANICAL AND INDUSTRIAL ENGINEERING

CHAIRPERSON

Applicants with outstanding research accomplishments and a genuine interest in the future of Mechanical and Industrial Engineering are urged to apply. A complete vita/resume, list of publications, at least three technical references, and a letter outlining personal academic and research goals should be directed to Dr David Pershing, Chairman, M&IE Search, 310 Park Bldg, University of Utah, Salt Lake City, Utah 84112. The University of Utah is an equal opportunity employer. (NW295)A

POSTDOCTORAL RESEARCH ASSOCIATE

A postdoctoral position is available immediately to investigate messenger ribonucleoproteins, especially their role in translation and in attachment of polyribosomes to the cytoskeleton. The work will utilize UV crosslinking and other advanced techniques, including antibodies and recombinant DNA. Candidates should have a recent PhD in molecular or cellular biology, or in biochemistry.

Send curriculum vitae and three letters of recommendation to: Dr Jay R Greenberg, c/o Personnel Office, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545.

An Equal Opportunity/Affirmative Action Employer. (NW288)A

MEDICAL RESEARCH COUNCIL

RADIOBIOLOGY UNIT, HARWELL

SHORT TERM SCIENTIFIC APPOINTMENT

Applications are invited for a short term three year post-doctoral appointment to the Council's non-clinical scientific staff, to join a group studying the problems associated with the inhalation of radioactive particles. The work will include quantitative autoradiographic studies of lung tissue. Applicants with backgrounds in biological or physical sciences will be considered; computing experience would be useful. (1220)A

QUEEN MARY COLLEGE

University of London

LECTURESHIP IN APPLIED MATHEMATICS

Applications are invited for this Lectureship available from 1 October 1984. Preference will be given to candidates whose research interests are in classical general relativity and/or theoretical astronomy. The successful applicant will be expected to teach mathematics both to specialist students and to other students in the Faculties of Science, Engineering and Social Studies.

Salary on scale £7,190 — £14,125 pa plus £1,186 London Allowance.

Application forms and further details obtainable from The College Secretary, Queen Mary College, Mile End Road, London E1 4NS, to be returned by 12 March. (1238)A

About us: BOEHRINGER MANNHEIM belongs to the foremost names of the research pharmaceutical industry. Every 5th employee of ours is active in research and development.

In order to further expand our applied orientated research in the field of animal cell culture, we are seeking a Ph.D. graduate

Cell Biologist

To be located in our Biochemical Research Centre in Tutzing in the Starnberg Lake, just south of Munich and in an area of exceeding natural beauty.

We expect our new co-worker to provide a substancial background experience in this scientific discipline and also to undertake management duties.

The primary tasks will be to optimise the production characteristics of cells, to further develope appropriate fermenters and to design suitable conditions for scale up processes.

We would like to explain all further details in a personal discussion with you. We request your letter of application in preparation for such a meeting. Citizen of european countries are prefered.

(W758)A

mannheim boehringer diagnostica therapeutica

Boehringer Mannheim GmbH
Biochemica Werk Tutzing
Personalabteilung
Bahnhofstraße 9–15, 8132 Tutzing
West Germany

UNIVERSITY OF ARIZONA

ASSISTANT/ASSOCIATE PROFESSOR

Microbiologist/Immunologist

Applications are being invited for several tenure track positions in the recently reorganized Department of Microbiology and Immunology at the Assistant or Associate Professor level, to begin summer-fall semester, 1984. Duties include teaching undergraduate students, medical students, and graduate students, and conducting an independent research program. Research interests should be within the broad area of microbiology/immunology. Individuals with a strong background in immunology, pathogenics, bacterial physiology, and virology are particularly being sought. The successful candidate must have a doctoral degree and a record of excellence and productivity in scholarly work. Postdoctoral experience is preferred.

Qualified applicants should send a résumé and arrange to have three letters of recommendation sent to Professor William Meinke, Search Committee Chairman, Department of Microbiology and Immunology, College of Medicine, University of Arizona, Health Sciences Center, Tucson, Arizona 85724. Complete applications should be received by April 1, 1984. The University of Arizona is an Equal Opportunity/Affirmative Action Employer. (NW279)A

THE MEDICAL COLLEGE of ST. BARTHOLOMEW'S HOSPITAL (University of London) and ST. MARK'S HOSPITAL for DISEASES OF THE RECTUM AND COLON (The City & Hackney Health Authority)

SENIOR LECTURER

(HONORARY CONSULTANT) IN HISTOPATHOLOGY

Applications are invited for the post of whole-time Senior Lecturer (Honorary Consultant) in Histopathology at St. Mark's Hospital for Diseases of the Rectum and Colon and in the Medical College of St. Bartholomew's Hospital.

This newly estabished post is wholly funded by the Imperial Cancer Research Fund and is tenable for a period of ten years in the first instance. The successful applicant will work in the ICRF Colo-rectal Cancer Unit at St. Mark's Hospital. Clinical duties in histopathology will be largely confined to furtherance of the ICRF colo-rectal cancer studies. Some undergraduate and postgraduate teaching will be undertaken at St. Mark's and St. Bartholomew's Hospital Medical College.

The salary will be on the Senior Lecturer/Consultant scale.

Candidates are invited to discuss the post with Dr B. Morson at St. Mark's Hospital (01-253 1050), with Prof G. Slavin at St. Bartholomew's Hospital (01-600 9000 ext 2865) and with the Dean of the Medical College (01-606 7404). Copies of the job description can be obtained by telephoning 01-606 7404.

Applications, in the form of a curriculum vitae (10 copies please — overseas applicants 1 copy) should be sent to the Dean of the Medical College, West Smithfield, London EC1A 7BE, together with the names of three referees, by Monday 16th March 1984. (1197)A

POSTDOCTORAL Position Available to Study (1) the nucleic acid-protein interaction, (2) the mechanism and regulation of gene expression in both prokaryotic and eukaryotic systems, using biophysical, biochemical or recombinant DNA techniques. Applicant should send curriculum vitae and three letters of recommendation to Professor Cheng-Wen Wu, Department of Pharmacological Sciences, SUNY Stony Brook, Stony Brook, NY 11794. SUNY Stony Brook is an equal opportunity/affirmative action employer. AK No 21-84. (NW303)A

JOHN INNES INSTITUTE

DEPARTMENT OF APPLIED GENETICS

HIGHER SCIENTIFIC OFFICER

required to assess genetic variation for photosynthesis in Pisum sativum and to relate this to other physiological and biochemical projects aimed at improving the dried pea crop.

The post, funded by the Perry Foundation, will be initially for one year, but with the expectation of an extension for a further two years.

Applicants should possess a First or Upper Second Class honours degree plus at least two years relevant post qualifying experience and have a working knowledge of techniques for determining net CO_2 exchange. A PhD would be preferred.

The salary on the Higher Scientific Officer scale will be in the scale £7149 — £9561. Non-contributory superannuation.

Applications, quoting reference AG/155, giving full particulars and experience and the names of two referees, should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich, NR4 7UH, not later than 9 March 1984. (1219)A

MRC CELL BIOPHYSICS UNIT

Applications are invited for a three-year, short-term

POSTDOCTORAL RESEARCH POSITION

available from 1 October 1984, to work on the molecular biology of dedifferentiation and morphogenesis in amphibian limb regeneration. This position is part of a collaboration between Dr Jeremy Brockes and Dr Roger Patient involving the analysis of gene expression in relation to these phenomena. Recent studies have led to the development of monoclonal antibodies as markers for blastemal and differentiated cell types both in vivo and in vitro; further studies will also involve the application of nucleic acid methodology. Experience in recombinant DNA techniques would be an advantage.

The salary will be on the scale £8,376 — £11,896 inclusive per annum; MRC superannuation scheme.

Applications with the names and addresses of two referees should be sent by 29 February 1984 to Mr D M Drummie, MRC Cell Biophysics Unit, King's College London, 26-29 Drury Lane, London WC2B 5RL; telephone 01-836 8851. (1159)A

ROTHAMSTED EXPERIMENTAL STATION
Harpenden, Herts, AL5 2JQ

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER — MOLECULAR BIOLOGIST

Applications are invited for a Scientific Officer/Higher Scientific Officer to join a team in the Biochemistry Department involved in the isolation and transfer of plant genes between crop plant species. The work will involve the characterisation of cDNA and genomic clones and ultimately the study of the expression of transferred genes. The person appointed is likely to be a graduate in Biochemistry or a related subject and with practical experience in some aspects of recombinant DNA technology. The work is funded by the EEC and involves collaborative work with other European laboratories. The appointment is from May 1st, or as soon as possible thereafter, for a period up to March 1986.

Qualifications: First or upper second class honours degree in Biochemistry or related subject with at least two years relevant post-qualifying experience for HSO.

Appointment in grade of Scientific Officer (£5,682 — £7,765) or Higher Scientific Officer (£7,149 — £9,561). Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref: 548 by 9th March 1984. Further details on request. (1243)A

University of Oxford Sir William Dunn School of Pathology

MEMBRANE BIOCHEMIST/ MOLECULAR BIOLOGIST

Grade IA Salary £7190–£11,615

Applications are invited for a postdoctoral research position, funded by a programme grant from the Medical Research Council. The research concerns the characterisation of macrophage plasma membrane antigens with a view to further studies on gene expression in macrophages. Experience in membrane biochemistry, immunochemistry and/or molecular genetics is essential. The appointment, starting as soon as possible after 1 June 1984, is likely to be for five years. Applications, including curriculum vitae and the names and addresses of two referees, should be sent to Dr. S. Gordon, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE (1234)A

POST DOCTORAL research position available, industry funded, to study the energy metabolism of anaerobic bacteria. Experience in bioenergetics, membrane transport and/or anaerobic organisms desirable. Please send your curriculum vitae and names of three references to Dr Eva R Kashket, Department of Microbiology, Boston University School of Medicine, 80 E Concord St, Boston, MA 02118, US. An affirmative action/equal opportunity employer. (NW299)A

THE UNIVERSITY OF LEEDS

PROCTER DEPARTMENT OF FOOD SCIENCE

LECTURER

Applications are invited from Biochemists or Chemists for the above post. The successful applicant will be required to develop an interest in meat science and in modern developments in the processing of animal products.

Salary on the scale for Lecturers (£7,190 — £14,125) according to age, qualifications and experience.

Informal enquiries should be made to Professor David S Robinson, telephone (0532) 431751, ext 540.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference no 72/17, to whom applications should be addressed. Closing date for applications 8 March 1984. (1242)A

ATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

MICROBIOLOGY DEPARTMENT

A RESEARCH SCIENTIST

required to devise and develop new apid methods for estimating the bacriological quality of milk, milk roducts and other foods. The urther development and automation f the direct epifluorescent filtration echnique (DEFT) is envisaged. dditionally, the application of adioimmune, ELISA, and allied echniques for food analysis will be xplored.

Appointment will be as Scientific Officer (£5,682 — £7,765) or Higher Scientific Officer (£7,149 — £9,561). Non-contributory superannuation.

Candidates should have a First or Upper Second Class Honours degree in biological or physical sciences with at least two years' relevant postqualifying experience for appointment as HSO.

Applications to the Secretary, NIRD Shinfield, Reading RG2 9AT.

Please quote reference 83/19 (1205)A

UNIVERSITY OF GÖTTINGEN

DEPARTMENT OF HUMAN GENETICS

POSTDOCTORAL RESEARCH FELLOW

Applications are invited from persons with a background in recombinant DNA research to work on human genome mapping and the molecular diagnosis of genetic disease. The appointment will be initially for 20 months, from May 1st, 1984, with the possibility of renewal. Knowledge of German is not essential.

Applications including CV and names of two referees should be sent to Dr Jörg Schmidtke, Institut für Humangenetik der Universität, Nikolausberger Weg 5a, D-3400 Göttingen, FRG. (W749)A

UNIVERSITY OF NEW BRUNSWICK

DEPARTMENT OF BIOLOGY

Fredericton Campus requires a

GENETICIST

with expertise in Botany or Plant Science with a main responsibility to teach genetics and to carry out a vigorous research program. The candidate must have a PhD degree and proven ability in research and teaching.

Assistant Professor, 2-year term appointment possibly leading to tenure track, to start July 1, 1984, subject to budgetary approval.

Apply, giving full c/v, a letter indicating the nature of a proposed research program and names of three academic referees to: Dr M D B Burt, Chairman, Department of Biology, University of New Brunswick, Bag Service 45111, Fredericton, NB E3B 6E1.

In accordance with the Canadian Immigration requirements, this advertisement is directed to Canadian Citizens and permanent residents. (NW286)A

WELSH NATIONAL SCHOOL OF MEDICINE

(University of Wales)

DEPARTMENT OF SURGERY

POST-DOCTORAL RESEARCH OFFICER IN CELL BIOLOGY

is required to work on a 3-year project to establish the roles of intracellular regulators in oxygen radical production by phagocytes and their importance in inflammatory diseases. Candidates should have a first degree in a relevant discipline and recently have obtained or submitted a thesis for a PhD. Previous research experience in cell separation, cell culture and fluorescence microscope techniques would be advantageous though not essential. Starting salary up to £7,190 per annum.

Further particulars (quoting ref No S19/2/67) are available from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN to whom application in the form of a curriculum vitae with the names and addresses of two referees should be submitted by 19th March, 1984. (1213)A

UNIVERSITY OF ABERDEEN

CHAIR OF CHEMISTRY

Applications are invited from candidates with a proven record in any branch of chemistry for the Chair of Chemistry which will become vacant on 1 October 1984 following the retirement of Professor P Meares.

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 23 March 1984. (1221)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF PURE AND APPLIED BIOLOGY

POSTDOCTORAL SCIENTIST

required for a three year World Health Organisation-funded project which is part of a new group on the immunology of nematode infections and involves an antigenic analysis of filarial parasites, combining monoclonal antibody production with a range of immunochemical techniques for immunodiagnostic development. There will also be an opportunity for field work in endemic areas. Post available from May 1984, although a later starting date will be considered for suitable applicants. Salary on scale £7,190 — £11,615 plus £1,186 London Allowance.

Further details from Dr R M Maizels, Department of Pure and Applied Biology, Imperial College, London SW7 2BB (01-589 5111 ext 2252) to whom applications should be submitted by 23 March 1984. (1237)A

---

nature

the weekly international journal of science

seeks an unusual sub-editor

to help 500,000 readers keep up with science

Essential qualifications:
Literacy — Zeal — Meticulousness — Tact

Also desirable, **two or more** of the following:
Two years experience as a sub-editor
Wide general knowledge of science
Scientific or technical degree
Work experience in a professional environment
First-hand experience of research

Duties include negotiation with scientist authors of acceptable and intelligible forms of words and preparation of manuscripts for the press. Opportunities for other journalistic work not excluded. Salary by negotiation. Age, Sex, etc. immaterial. Applications from exceptional part-timers considered.

Replies by 8 March with full *curriculum vitae* to the Editor, *Nature*, Macmillan Journals Limited, 4 Little Essex Street, London WC2A 3LF. (1233)A

BIOCHEMIST

The Department of Biochemistry, at the Faculty of Medicine, invites applications for a full-time Faculty Position to commence by the summer of 1984. Appointment is at the Assistant Professor level. Preference will be given to candidates with programmatic expertise in molecular and/or cellular biology with particular reference to mammalian systems; but outstanding candidates in other biochemistry subspecialities will be given full consideration. Post doctoral training and potential for an independent career as an experimental scientist is a prerequisite. An interest in developing expertise and a desire to become an effective educator are desirable qualities.

The position is open to both women and men. In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. For full consideration, send a concise description of projected research goals and interests, a list of a minimum of 4 individuals for letters of reference, a curriculum vitae and any additional information to:
Dr. J. N. Kanfer, Department of Biochemistry, Faculty of Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3. (NW300)A

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF MICROBIOLOGY

GENE EXPRESSION AND MANIPULATION

Applications are invited for a Postdoctoral Research Assistant financed by the SERC for work on the expression and in vitro mutagenesis of the genes encoding the respiratory succinate dehydrogenase of E coli. Tenable from 1 May 1984 or later, for 17 months.

Initial salary £7,190 — £8,975 a year, Range IA. Experience in molecular genetics or protein biochemistry desirable, but not essential.

Further information, by letter or telephone (0742 — 78555 ext 4406) from Professor J R Guest, Department of Microbiology, The University, Sheffield S10 2TN, to whom applications, including curriculum vitae and the names of two referees, should be sent by 5 March 1984. Quote ref: R36/G (1241)A

The Queen's University of Belfast

POSTDOCTORAL OR GRADUATE RESEARCH ASSISTANT

Department of Agriculture and Food Chemistry

Applications are invited from graduates with either a doctorate or a good honours degreee for a two-year research assistantship funded by the Livestock Marketing Commission for Northern Ireland to investigate the physico-chemical properties of mechanically-deboned beef. The work will include a study of the rheological properties of mechanically-deboned beef in comparison with normal meat systems and an investigation of the functional properties of mechanically-deboned beef to clearly identify its potential for use in food products. Applicants should preferably have experience in the area of meat science or food engineering.

Commencing salary is in the range of £6312 – £8082 per annum, initial placing depending on experience and qualifications.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, Northern Ireland, BT7 1NN. Closing date: 16th March 1984. (Please quote Ref. 84/N). (1218)A.

CHAIRPERSON
DEPARTMENT OF CELL AND TUMOR BIOLOGY

As a comprehensive cancer center involved in basic, clinical, and educational programs in cancer, Roswell Park Memorial Institute requests nominations or applications for the above position which involves leadership of a major research program in the molecular and cellular biology of cancer and which is presently staffed by sixty individuals, including thirty scientists.

Qualified candidates should minimally posses a PhD or MD degree and have a distinguished record in research on molecular and cellular aspects for cancer and have demonstrated capacities for scientific administration. Preference will be given to individuals with a record of collaborative studies with clinical colleagues on the molecular biology of human cancers.

Applications by qualified women and minority candidates are welcomed. Nominations and applications should be sent to: Dr. Timothy E. O'Connor, Associate Director for Scientific Affairs, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY, 14263.

An Affirmative Action, Equal Opportunity Employer.

(NW298)A

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

DEPARTMENT OF PHYSIOLOGY

SENIOR LECTURER/ LECTURER

Applications are invited from suitably qualified persons, regardless of sex, race, colour or national origin, for appointment to the above posts in the Department of Physiology. Applicants should hold a higher science degree or a medical or veterinary qualification.

Successful applicants will be expected to participate in the teaching and research programmes of the Department. The major research interests of the Department of Physiology include: cardiovascular physiology, neurophysiology, thermoregulation, biomechanics/ energetics, mathematical biology, and computer simulation.

The Department offers outstanding opportunities for research. In the case of registered medical practitioners arrangements can be made for a remunerated part-time attachment to a clinical department for the purpose of patient care and research.

The salary will be in the range: Senior Lecturer: R22,533 — R28,185 per annum; Lecturer: R14,880 — R25,080 per annum.

A pensionable allowance of 12% is also payable.

The initial salary notch will be determined according to the qualifications and experience of the successful applicants. Benefits include an annual bonus, medical aid, a housing subsidy for those eligible and substantial removal aid.

Intending applicants should obtain the information sheet relating to this post from the Secretary, South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE, England or from the Assistant Registrar (Academic Staffing) University of the Witwatersrand, 1 Jan Smuts Avenue, Johannesburg 2001 South Africa with whom applications should be lodged not later than 31 March 1984.

(W755)A

THE UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENISTRY

CLINICAL GENETICS

A vacancy has arisen in the above department for a Research Associate to work on cytogenetic aspect of mental retardation.

Salary — Research Associate 1B £6310 — £8530 (plus superannuation) for two years.

Further information from Assistant Registrar, The Medical School, Birmingham B15 2TJ to whom applications (three copies) must be sent by 9th March 1984. Quote ref RA/CG/TW. (1247)A

UNIVERSITY COLLEGE DUBLIN
DEPARTMENT OF PHARMACOLOGY

Applications are invited for the following posts which are funded by University College Dublin and the E.E.C. Research and Development Programme in the field of Environment. The project is headed by Dr C. Regan and will investigate the effects of lead toxicity on neuronal cell surface functions believed to be involved in brain development.

RESEARCH ASSISTANT

The successful candidate will be expected to have a good basic degree in a biological science. Experience in tissue culture is advantageous but not essential. Salary will be in the range of IR£4,500 — 5,000 per annum.

POSTDOCTORAL RESEARCH ASSOCIATE

Applicants should possess a PhD degree (or shortly expect to receive one) and have some experience in protein chemistry. Salary will be in the range of IR£7,500 — 8,000 per annum.

Applications, including curriculum vitae and the names of two referees, should be sent as soon as possible to Dr C Regan, Department of Pharmacology, University College, Fosters Avenue, Blackrock, Co Dublin, Ireland. Tel (01) 693244 ext 557. (1239)A

Southampton THE UNIVERSITY

DEPARTMENT OF OCEANOGRAPHY
RESEARCH ASSISTANT PHYSICAL OCEANOGRAPHY/ REMOTE SENSING

A good honours graduate in physical science is required very soon to fill a post in connection with a M.O.D. research contract using satellite data to study the dynamics of the N. Atlantic Ocean. Salary range IB (from £6,310 — £8,530). Enrolment of a research degree encouraged.

Applications with full cv and names of two referees to Dr I S Robinson, Department of Oceanography, University of Southampton, SO9 5NH, UK from whom further information is available.

(1225)A

CHAIRMAN

DEPARTMENT OF ANATOMY

Applications are invited for the position of Professor and Chairman, Department of Anatomy, Schools of Medicine and Dentistry, University of Alabama in Birmingham. We are seeking candidates with research and teaching experience, leadership qualities, and administrative skills. This department has teaching responsibilities for graduate students in anatomy, medical, dental and other students.

Please submit applications and a curriculum vitae to: Antonietta D Hyche, Administrative Officer, University of Alabama School of Medicine, Dean's Office — MEB 306, Birmingham, Alabama 35294. An Equal Opportunity/Affirmative Action Employer. (NW263)A

THE SCHOOL OF PHARMACY UNIVERSITY OF LONDON

DEPARTMENT OF PHARMACOLOGY

LECTURESHIP

Applications are invited from suitably qualified candidates for the above post. The successful applicant will be required to pursue research on fundamental mechanisms of drug action and to contribute substantially to the teaching of pharmacology and/or related subjects to students undertaking the University of London BPharm and BSc Toxicology and Pharmacology degrees.

Salary within the Lecturer range — £7,190 — £14,125 pa + LA £1,186 pa.

Enquiries to Professor D A Brown, Department of Pharmacology, The School of Pharmacy (Tel: 01-837 7651 ext 31).

Applications, including names of two referees, to: Personnel Officer, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, by 9 March 1984. (1226)A

University College of Swansea

Chair of Mechanical Engineering and Headship of Department

Applications are invited for the Chair and Headship of the Department of Mechanical Engineering from October 1, 1984.

Further particulars may be obtained from the undersigned, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom applications (15 copies) should be sent by *March 16, 1984*.

V.J. Carney
Registrar
(1198)A

UNIVERSITY OF CHICAGO

ASSISTANT OR ASSOCIAT PROFESSOR IN THE RESEARCH ASSOCIATE TRACK

to assume major responsibility in cellular electrophysiology laborato studying arrhythmogenesis and t actions of antiarrhythmic drug Experience in voltage clampin cable analysis, ion sensitive micr electrodes, and computer pr gramming preferable. Excelle scientific environment.

Renewable term appointment wi full fringe benefits. Immedia availability.

Please send curriculum vita representative reprints, and names three references to: Morton Arnsdorf, MD, Chief, Section Cardiology, University of Chica Hospitals, Box 423, 950 E 59 Street, Chicago, Ill 60637.

(NW305)A

---

nature classified

LONDON OFFICE
Keith Walesby
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

NEW YORK OFFICE
Miss Gene Fein
15 East 26 Street
New York, NY 10010
Tel: (212) 689 5900

TORONTO OFFICE
Peter Drake Associates
32 Front Street West
Suite 200, Toronto,
Ontario M5J 1C5
Tel: (416) 364 1623

CONFERENCES and COURSES

JAPANESE BIOTECHNOLOGY STUDY TOUR

An in-depth series of site visits, conferences and seminars with some of Japan's leading industrial scientists and executives in biotechnology.

This exclusive study tour is designed to give western scientists and executives an opportunity to become acquainted with the Japanese marketing and research capacity. The mission will be led by Christopher G. Edwards, Editor in Chief of BIO/TECHNOLOGY, and organized by the Technology Transfer Institute. Visits will be conducted throughout Japan from March 31-April 15.

For further information, contact: Technology Transfer Institute — New York, One Penn Plaza, Suite 1411, 250 W. 34th St., New York, N.Y. 10119. 212-947-2648 telex 420057TTIUI. Attention: Mr. Hashizume. (NW174)C

4TH CONFERENCE OF EUROPEAN SOCIETY FOR COMPARATIVE SKIN BIOLOGY

In Grenoble from 3-6 July 1984. Details from Professor P. Sengel, Laboratoire de Zoologie, Université scientifique et medicale de Grenoble, BP N°68, 38402 Saint Martin D'Heres, France. (1212)C

EMBO Course on

RECOMBINANT DNA TECHNIQUES

September 17–29, 1984

National Research Foundation, Athens, Greece

The course will provide training in the basic techniques of molecular cloning. It will be suitable for participants at an advanced predoctoral or post-doctoral level who wish to utilize recombinant DNA techniques in their research. Basic nucleic acid and microbiological techniques will be taught. No previous knowledge of cloning technology will be assumed. Participants will prepare and screen cDNA and genomic clone banks and characterize the isolated recombinants. A lecture programme will cover areas which cannot be included in the practical course and will include invited speakers. The course language will be English. Applications should include a short curriculum vitae, statement of research interests and a letter of recommendation. The registration fee is 5.000 Dr. Upon motivated request some fellowships for board and lodging will be granted.

The closing date for applications is April 30, 1984

Those accepted will be notified not later than June 15, 1984. Applications should be sent to: G.J. Dimitriadis, Biological Research Center, National Hellenic Research Foundation, 48 Vas. Con/nou Av., Athens 11635, Greece (W762)C

Royal Postgraduate Medical School

(University of London)

MSc Degree Course

in Experimental Pathology (Toxicology)

Applications are invited for the above, one-year, full-time course which is being offered from 1 October 1984.

Applicants should possess a first degree in medicine, veterinary medicine or an appropriate science subject.

It is hoped that a number of Medical Research Council Studentships will be available.

Further details and application forms are available from:
School Office (MSc)
Royal Postgraduate Medical School
Hammersmith Hospital
Du Cane Road
London W12 0HS
Telephone: 01-743 2030 ext 351

Please note that the closing date for receipt of applications will be: 11 May 1984. (1195)C

MOLECULAR BIOLOGY FOR IMMUNOLOGISTS A BRITISH SOCIETY OF IMMUNOLOTY

SUMMER SCHOOL Edinburgh July 2nd-4th 1984

This is an introductory lecture course aimed at individuals with little or no experience in the molecular cloning field. The lectures will cover the basic steps included in molecular cloning and will illustrate how recently developed cloning techniques can be used to study both basic immunological systems and in the diagnosis and treatment of disease.

SPEAKERS INCLUDE:

Dr F. Baralle, Oxford.
Professor S. Cohen, London.
Dr D. Cosman, Seattle, U.S.A.
Dr W. Cushley, Glasgow.
Dr S. Emtage, Slough.
Dr F. Grosveld, London.
Dr T. Harris, Slough.
Dr N. Hastie, Edinburgh.
Dr C. Hentschel, Slough.
Dr A. Kingsman, Oxford.
Dr A. Mellor, London.
Professor R. Old, Warwick.
Dr M. Owen, London.
Dr K. Reid, Oxford.
Dr J. Williams, London.
Prof. R. Williamson, London.

Further details of this programme and registration forms can be obtained by writing to Dr Keith James, Education Secretary, British Society for Immunology, Department of Surgery, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG.

Closing date for applications — 31st May 1984. (1244)C

Please mention

nature

when replying to these advertisements

STUDENTSHIPS

THE OPEN UNIVERSITY

RESEARCH STUDENTSHIPS IN SCIENCE

The Faculty of Science at the Open University invites applications for full-time research studentships, tenable from 1st October, 1984 at Milton Keynes and the Oxford Research Unit. A wide variety of research topics is available within the following Disciplines and research groups:

BIOLOGY including BRAIN RESEARCH GROUP

CHEMISTRY

EARTH SCIENCES including PETROGENESIS RESEARCH GROUP

PHYSICS including OXFORD RESEARCH UNIT

Successful applicants will be awarded either an Open University or Research Council research studentship. Applicants should normally have (or expect to gain in 1984) a good honours degree.

For further particulars and application forms please send a POSTCARD to the Higher Degrees Office S/3, The Open University, PO Box 49, Walton Hall, Milton Keynes MK7 6AD or telephone Milton Keynes (0908) 653806.

PLEASE SPECIFY THE AREA(S) IN WHICH YOU ARE INTERESTED AND QUOTE REFERENCE S/3.

Applications should be submitted as soon as possible.

(1227)F

UNIVERSITY OF NOTTINGHAM MRC INSTITUTE OF HEARING RESEARCH POSTGRADUATE RESEARCH

Applications are invited for MRC Studentship(s) for research in auditory physiology and anatomy leading to a higher degree at the MRC Institute of Hearing Research. Current research emphasises development of structure and function in hereditary deafness, and mechanisms of drug interaction which damage hearing. Students(s) have the opportunity to learn a variety of electrophysiological techniques, scanning and transmission electron microscopy, and computer programming.

The successful candidate(s) will have (or expect to obtain during 1984) at least an upper second class honours degree in any biological subject.

Applications are also invited from those with degrees in any appropriate subject and interested in human experimental work on hearing, covering perception, speech, ergonomics and social factors in disability and service take-up.

For further information, please write to: Dr G Bock, MRC Institute of Hearing Research, University of Nottingham, University Park, Nottingham NG7 2RD. (1209)F

UNIVERSITY OF DUNDEE

THE DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACOLOGY (DUNDEE)

Applications are invited for a

THREE-YEAR ORGANON-SUPPORTED RESEARCH STUDENTSHIP

to work on a project under the supervision of Dr J J Lambert using radioligand binding, patch clamp and other electrophysiological techniques to investigate the action of calcium channel blockers on isolated cardiac myocytes.

Applicants should hold at least an upper second class Honours degree in physiology, pharmacology, biophysics, electronics or related disciplines. Financial conditions are similar to those of an SERC studentship.

For further information please contact Dr J J Lambert, Department of Pharmacology and Clinical Pharmacology, University Medical School, Ninewells Hospital, Dundee, Scotland as soon as possible (Tel Dundee 60111 ext 2527/2161). Applications (including the names of two referees) should be sent to Dr Lambert by 15th March 1984.

(1196)F

UNIVERSITY OF EDINBURGH

DEPARTMENT OF BOTANY

in conjunction with

UNILEVER RESEARCH

COLWORTH LABORATORY

PLANT BIOCHEMISTRY

A case studentship has been awarded for studies on the role of cytoplasmic calcium and membrane-bound protein kinase in controlling the division cycle of cultured plant cells and protoplasts.

Appointment is for 3 years with registration for M.Phil/Ph.D degree. Undergraduates (or graduates) in Biochemistry/Plant Biochemistry or Biology/Botany with a strong physiological or biochemical bias may send applications (c.v. + 2 referees) to Dr. A. Trewavas, Botany Department, University of Edinburgh, Edinburgh EH9 3JH or Dr. W. Hughes, Unilever Research, Colworth House, Sharnbrook, Bedford. (1232)F

UNIVERSITY OF OXFORD

INORGANIC CHEMISTRY LABORATORY

CASE RESEARCH STUDENTSHIPS

Applications are invited for an SERC CASE Studentship with BP Chemicals Ltd as the co-operating industrial body. The studentship is intended to lead to a DPhil degree.

The research project is entitled 'Activation of Carbon Dioxide in Low-temperature Matrices' and preference will be given to applicants having interests and background experience in the methods of vibrational spectroscopy and/or synthetic inorganic or organometallic chemistry.

Applications, with the names of two referees, should be sent to Dr A J Downs, Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QR. (1240)F

AWARDS

THE UNIVERSITY OF OXFORD GRADUATE AWARDS AT ST. CATHERINE'S COLLEGE

The College proposes to award five Graduate Scholarships of £200 per annum, tenable for two years in the first instance, for men or women commencing a research degree at Oxford in October 1984 in any subject. The awards are intended for those who have taken first degrees at universities within the UK and European Community (except Oxford).

The College also offers a Graduate Scholarship of £1,500 per annum, for two years, and five Bursaries of £200 intended for graduates who are NOT citizens of a European Member State.

Further details may be obtained, before 30 April 1984, from the Senior Tutor, St. Catherine's College, Oxford OX1 3UJ. (1222)N

FELLOWSHIPS

THE ROYAL SOCIETY OF EDINBURGH

ROBERT CORMACK BEQUEST FELLOWSHIP

Applications are invited from 1st Class University Honours graduates for a Robert Cormack Bequest Fellowship in astronomy or subjects directly related thereto, tenable from October 1st, 1984.

Further details and application forms, which should be returned no later than March 31st, 1984, may be obtained from the Executive Secretary, The Royal Society of Edinburgh, 22/24 George Street, Edinburgh EH2 2PQ. (1204)E

NEWNHAM COLLEGE CAMBRIDGE VISITING RESEARCH FELLOWSHIP IN THE SCIENCES

Applications are invited fro academically qualified women wh wish to pursue research Cambridge. The Fellowship, tenab at Newnham College, is for a perio of at most eleven months (between 1 October and 31st August) and may b awarded either for 1984-85 or fo 1985-86.

The purpose of the Fellowship is t enable visitors to the University particularly those on sabbatic leave, to take part in the life of th College while undertaking their ow work. No stipend is offered, bu holders of the Fellowship a provided with a study in College an are entitled to meals without charg at the High Table. If require further living accommodation ma also be provided at a charge to b agreed with the College.

Further information may b obtained from the College Secretar Newnham College CB3 9DF. Th closing date for applications is 31 March 1984. (1207)E

A POSTDOCTORAL Fellowship available in protein crystallograph related to active sites and regulato mechanisms, including enzym modified at the DNA level by recor binant techniques. Applicants fro the area of small molecule cryst lography will also be considere Applicants may write to Pr William N Lipscomb, Gibb Chemical Laboratory, Harvard Un versity, Cambridge, MA 0213 USA. Harvard University is Affirmative Action/Equal Oppo tunity Employer. (NW301)E

THE UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL FELLOWSHIP

Applications are invited for a po doctoral research position (CR funded) to work on an ongoi project directed toward clarifying t relationship between Tyrosin specific phosphorylation of the cyt skeletal protein vinculin, and t morphology of cells transformed Rous sarcoma virus (RSV).

Methods involved will include c culture (transformation of prima chick cells with RSV), immun chemical and immunofluorescen techniques.

Applicants should have a PhD Biochemistry and experience in so area of Tumour Biology is desirab

Salary £7,190 to £8,975.

The position is availat immediately, although the starti date is negotiable, and will be fo years.

Applications (along with t names of two referees) should be s to Dr D R Critchley, Department Biochemistry, University Leicester, University Road, Leices LE1 7RN. (1202)E

THE UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral research position (MRC-funded) to work on the interaction between tetanus toxin and neural tissue. The project will include studies on (1) the "receptor" for tetanus toxin (2) the biochemical and ultrastructural features of the internalization process and (3) structure function relationships within the toxin molecule itself.

Techniques involved will be in part similar to those previously developed to study the interaction of cholera toxin with target tissues (J Biol Chem 256 (1981) 8724), but will also involve the extensive use of monoclonal antibodies.

Applicants should have a PhD in Biochemistry or related discipline, and preference may be given to those with experience in cell culture and immunochemical techniques, and with an interest in membranes.

Salary: £7,190 to £8,080.

The position is immediately available although the starting date is negotiable, and will be for a maximum of 3 years.

Applications (along with the names of two referees) should be sent to Dr D R Critchley, Department of Biochemistry, University of Leicester, University Road, Leicester LE1 7RN. (1201)E

UNIVERSITY OF GLASGOW

DEPARTMENT OF GENETICS

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a Post-doctoral Research Fellow to work with Dr R G Sutcliffe on a three year MRC-supported project that is aimed at the cloning and analysis of the gene for human placental alkaline phosphatase (PLAP). This is a tissue specific member of a presumptive multigene family of alkaline phosphatases, which exhibits a notable degree of polymorphism and is reactivated in certain tumours. In addition, our recent work suggests that PLAP exists in soluble and membrane-associated forms, which are structurally distant.

Prior experience in recombinant DNA methodology is essential.

The starting salary will be within Range 1A of the salary scale for Research and Analogous staff (£8,530 — £9,425 plus superannuation), with an early starting date.

Applications (citing two referees) and enquiries should be to: Roger Sutcliffe, Department of Genetics, University of Glasgow, Glasgow G11 5JS (041-339 8855 ext 7108).

In reply please quote Ref No 5198M. (1211)E

UNIVERSITY OF WARWICK

Applications are invited for the following research posts in the Department of Chemistry and Molecular Sciences:

(a) Postdoctoral Research Fellowship (Physical Chemistry/Physics) To investigate the potential of Langmuir-Blodgett Films, coated on electronic devices, as sensors for chemicals. Applicants should have background in either physical chemistry or physics and should have an interest in both Langmuir-Blodgett films and in solid-state devices. The post is for two years. Initial salary up to £8,530 p.a. on the Research Range IA scale £7,190 — £11,615 p.a.

(b) Postdoctoral Fellowship (Membrane Biochemistry). To study the structure and properties of olfactory sensory membranes. Applicants should have a strong background in membrane biochemistry or enzymes and an interest in receptor proteins. The post is for two years. Initial salary up to £8,080 p.a. on the Research Range IA scale £7,190 — £11,615 p.a.

(c) Research Assistant (Biochemistry). Graduate research associate to study the biochemical aspects of flavour perception. The work will involve both binding studies and chemical modification methods. Applicants should have a good honours degree in biochemistry or biological chemistry. Initial salary up to £7,190 p.a. on the Research Range IB scale £6,310 — £8,530 p.a.

Applicants should state for which post they wish to be considered. Applications (no forms) should include a full curriculum vitae and the names of two referees to Dr G H Dodd, Department of Chemistry and Molecular Sciences, University of Warwick, Coventry CV4 7AL to whom informal enquiries may be made. (tel. 0203-24011-extn. 2234). (1246)E

UNIVERSITY OF KARLSRUHE

POSTDOCTORAL FELLOWSHIP

Applications of molecular biologists are invited to join the bacteriological research group working on bioenergetics of anaerobic respiration. Individuals are expected to have experience in molecular cloning and recombinant DNA techniques. Microbiologists or biochemists with corresponding experience will also be given strong consideration.

The position is available immediately for at least two years, to be renewed after first year. Salary is equivalent to German Government Employees (BAT IIA).

Send application with full cv, research experience, and three references to Prof Dr W Zumft, Lehrstuhl Fuer Mikrobiologie, Universitaet Karlsruhe, Kaiserstr 12, D-7500 Karlsruhe 1, West Germany (Closing Date April 1). (W760)E

MYRON A. BANTRELL POSTDOCTORAL FELLOWSHIPS FOR SCIENTIFIC AND ENGINEERING RESEARCH

The California Institute of Technology, Massachusetts Institute of Technology, and The Weizmann Institute of Science (Israel) will award a number of Bantrell Fellowships for postdoctoral study for the academic year 1984-85, beginning September 1, 1984.

The Fellowship will be granted for one year and will be extended for an additional year upon recommendation of the host institution. In recognition of outstanding academic achievements, the Fellowship may be extended, to those Fellows who have been abroad, for a third year of study in the Fellow's home country (Israeli citizens shall be eligible for such a third year fellowship only at The Weizmann Institute), thus enabling the Fellow to select a position in research or industry.

According to the local practice at the host institution, this Fellowship may include a generous stipend for the Fellow, a travel allowance (one round trip for the Fellow) to the host institution, travel to scientific conferences, and health and life insurance. The program is restricted to citizens of Israel and the United States. US citizens must be less than 30 years of age, and preference will be given to Israeli citizens.

The Bantrell Fellowship Program has been established to offer scientists the best possible opportunity to develop their talents. Fellowships will be granted in the following fields:

California Institute of Technology: Biology, Chemical Catalysis, Earthquake Seismology, Microscience of Materials (Applied Physics), and Theoretical Physics, Astrophysics, and Optical Astronomy.

Massachusetts Institute of Technology: Computer Logic, Energy Conversion, Mechanics of Fluids, Molecular Biology of Higher Organisms, Neurosciences, and Surface Science.

The Weizmann Institute of Science: Chemistry, Life Sciences, Physics, and the Study and Development of National Resources of Israel.

Application and materials in support of any nomination should include a curriculum vitae, bibliography of publications, preprints of manuscripts not yet published, a letter of nomination from the dean or chair in the candidate's university, and two letters of recommendation from faculty who are familiar with the candidate's research. Notification will be mailed by June 30, 1984.

Applications should be sent to the appropriate institution and should arrive by April, 1984. Mail to:

Provost's Office,
California Institute of Technology,
Pasadena, California 91125.

Kenneth A. Smith,
Associate Provost and Vice President for Research,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139.

Professor Shmuel Shaltiel,
Dean, Feinberg Graduate School,
The Weizmann Institute of Science,
Rehovot, Israel 76100.

(NW302)E

GRANTS and SCHOLARSHIPS

THE NUFFIELD FOUNDATION

OLIVER BIRD FUND for RESEARCH INTO RHEUMATISM

Applications are invited for research grants for work directed to the better understanding, and treatment, of rheumatic disease. The Oliver Bird Fund, from which over £150,000 was spent on research grants during 1983, is administered by the Trustees of the Nuffield Foundation on the advice of an expert committee whose chairman is Dr D.A.J. Tyrell, CBE, FRC. Grants may be made only for research in UK institutions.

Further details of how to apply are available from the Nuffield Foundation. The closing date for applications is 31st March 1984.

Applications will be considered at a meeting in May, 1984.

The Director
Nuffield Foundation, Nuffield Lodge
Regents's Park, London NW1 4RS

(1230)H

THE ROYAL SOCIETY

Travel and Subsistence Grants from the Parliamentary Grant-in-Aid

Notice is hereby given that the following closing dates will apply in respect of applications for travel and subsistence grants: 1 March, 1 June, 1 October and 1 December 1984. Grants are awarded only in respect of short scientific visits overseas of up to 13 days (generally for Conference attendance), and applications should be submitted on forms to be obtained from the Executive Secretary, (Ref BdeV), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, (01-839 5561, ext 222). Grants are only available to scientists of at least PhD status who are ordinarily resident in the UK and are not in the employ of governmental or research council institutions.

For certain major conferences there may be special closing dates and those who may wish to apply should inquire as far in advance as possible, and in requesting forms should indicate the meeting they wish to attend.

Applications in respect of longer term visits overseas can be considered under other schemes administered by the Society, and those wishing to apply should inquire well in advance of travel indicating the approximate duration of the visit, and the country to be visited. (1199)H

CONGRESS

SECOND INTERNATIONAL CONGRESS ON COMPUTERS IN SCIENCE

CALL FOR PAPERS

The Second Conference on Computers in Science, sponsored by Science Magazine, in conjunction with Scherago Associates, will be held in Washington DC. from 28 October – 1 November 1984. The conference will emphasize the use of the workstation for the scientist. There will be invited talks in a number of areas including Biology Workstations, Computer Aided Molecular Design, Workstation Hardware, Artificial Intelligence, Databases, Laboratory Automation and Robotics, and Management of the Electronic Laboratory. In addition there will be submitted poster talks, workshops in a number of areas and a large vendor exhibition. Prospective authors should send for an abstract form, registration and hotel information to Ed Ruffing, Scherago Associates, 1515 Broadway, New York, New York 10036 (212) 730-1050. Completed abstracts should be sent to the conference chairman, Dr. Stephen R. Heller, EPA, PM-218, Washington DC 20460 (202) 382-2424.

Circle No.17 on Reader Service Card.

WORKSHOP

Ninth International Herpesvirus Workshop, August 24-29, 1984
Seattle, Washington

Organizers: Dr Denise Galloway, Fred Hutchinson Cancer Research Center, Seattle: Dr Jack Stevens, University of California, Los Angeles

For information contact: Ms Tamara Moats, Conferences and Institutes, University of Washington DW-50, Seattle, WA 98195 (206) 543 5280. (NW8)V

MISCELLANEOUS

RESEARCH VESSEL FOR ECONOMICAL WORLD WIDE CHARTER

A 55'6" × 19' crabber/seiner design of Ed Monk with a range of 6,000 miles at eight knots. Party of six, experienced crew, extensive electronics including SATNAV.

Spice Island Traders, One Boston Place, Boston, MA 02108 (617) 723-1955. (NW750)J

ACCOMMODATION

AMERICAN going on sabbatical year seeking 2-3 bedroom flat with central heating, washer and dryer for family beginning 1 August, 1984 in Swiss Cottage-Hampstead-Golders Green-Finchley area. Possibility of exchange for 3 bedroom house in Philadelphia suburb (Wynnewood). Contact Prof M M Civan (Physiology Dept, U of Pennsylvania, Richards Bldg/G4, Phila, PA 19104, USA). (NW293)S

ASSISTANTSHIPS

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSICS

RESEARCH ASSISTANTSHIPS IN FIBROUS BIOPOLYMER RESEARCH

Applications are invited for thr posts to undertake structural studi in fibrous biopolymers. The resear will be supported by the Agricultur and Food Research Council's Li Group Scheme, on the basis of colaboration between tl University's Physics Department a the Council's Meat Researc Institute. This project is part of ongoing research programn concerned with structure a morphology of fibrous biopolyme using X-ray diffraction, electr microscopy, Fourier transform infr red spectroscopy and biochemi analysis and characterisation.

Prospective candidates shou have a PhD degree and experience biophysics, biochemistry or materi science. The posts are tenat immediately and renewable for up three years. Salary range £8,530 £9,425 pa plus superannuation.

Applications by letter, wi curriculum vitae and the names two referees should be sent Professor E D T Atkins, H H Wi Physics Laboratory, Tynd Avenue, University of Bristol, Brist BS8 1TL, as soon as possible but later than February 29th, 1984. (1214)P

POSITIONS WANTED

APPOINTMENTS WANTE advertise your qualities and qual cations through the most influen science weekly in the world a special reduced cost of *25p a wo* (Personal Box Numbers £1). Advertisements must be pre-paid sent to: Nature Classified (Ap Wtd), 4 Little Essex Street, Lond WC2R 3LF. (890)

PROTEIN — and Immunochemi PhD, experienced in purificatic modification, chemical and immur logical characterization prokaryotic and eukaryotic protei seeks research/developme position. Fluent in German a English. Box NW304 c/o Nature, East 26 Street, New York NY 100 (NW304)

BIOLOGIST, EXPERIENCE agri-chemicals, arid-lands a desertification work, several ye teaching and ecological research North Africa. Seeks suitable post or overseas. Contact: P O'Bri MSC, MI BIOL (UK), Woodlands, Kerry Pike, Cou Cork, Ireland. Phone 021 45012. (1245)

Typesetting: J.W. Arrowsmith Ltd, Bristol and Outline Graphics Ltd. London EC4Y 0HP. Printing: Kingsdale Press Ltd, Reading, Berks, in association with Godfrey Lang Ltd. London WC1R 4EB and The William Byrd Press Inc., Richmond, Virginia, USA Published by MACMILLAN JOURNALS LTD, 4 Little Essex Street, London WC2R 3LF. — 16 February 1984.



nature is keeping ahead. Are you being left behind?

New research results, the very latest international science news, key papers by the world's leading scientists and speed of publication ensure *Nature* remains one of the foremost weekly scientific journals in the world.

But if you're tied in to a circulation list at work, all this vital weekly information will undoubtedly reach you late, often incomplete and sometimes not at all.

With your own personal copy, you solve the problem. Every week you'll have all the latest research results and science news in your hands within days of publication:

News reports from throughout the world of science and technology.

A **News and Views** section, where new ideas are analysed and discussed by leading researchers.

Articles and Letters in which key investigators disclose their work for the first time.

Book Reviews – Eminent figures offer expert judgments on a wide variety of recent publications.

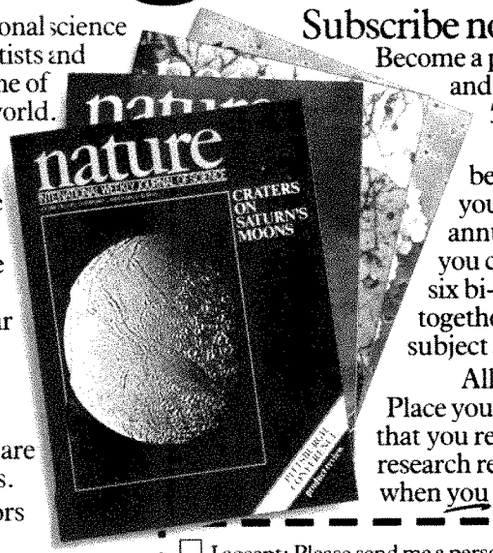
Special Issues providing an in-depth treatment of topics of importance.

Product Reviews – Detailed descriptions of laboratory equipment newly available.

Classified Advertisements for a wide variety of positions all over the world.

You will no longer be dependent upon other people passing down the 'list' copy, and because you will automatically receive every issue, you won't risk missing a single copy through absence from work. Moreover, you keep all this key information for permanent reference.

Through your door every week



Subscribe now – at Half Price

Become a personal subscriber now and you will receive a discount of 50% off the full rate.

All 51 weekly issues will be delivered to the address of your choice. You can start your annual subscription with any issue you choose. And you will receive six bi-monthly author indexes, together with the annual index of subject and author.

All this for just 94p a week.

Place your order now and make certain that you receive the scientific news and research results crucial to your work when you want them.

☐ I accept: Please send me a personal subscription to *Nature* for one year (51 issues) at the special half-price rate of £49.00.

☐ I enclose my personal cheque for £49.00 made payable to *Nature*. (Note: Institutional cheques cannot be accepted at this special rate).

☐ Please charge to my credit card account.
Access/American Express/Visa/Diners Club.

Account No._____

Card Expiry Date_____

Signature_____

Name_____

Address_____

_____

_____

Date_____ 7B18

Please return this form to:
Circulation Manager
Nature
Brunel Road, Houndmills
Basingstoke
Hants RG21 2XS

This offer applies to UK and North America only. Please allow approximately 4 weeks for delivery of the first issue in your subscription year.

nature

Circle No.90 on Reader Service Card.

Melts the sample - freezes the reading



It's as easy as that when you use Gallenkamp's new Melting Point Apparatus. Housed in a stylish case it will make melting point determinations easier, quicker and more accurate.

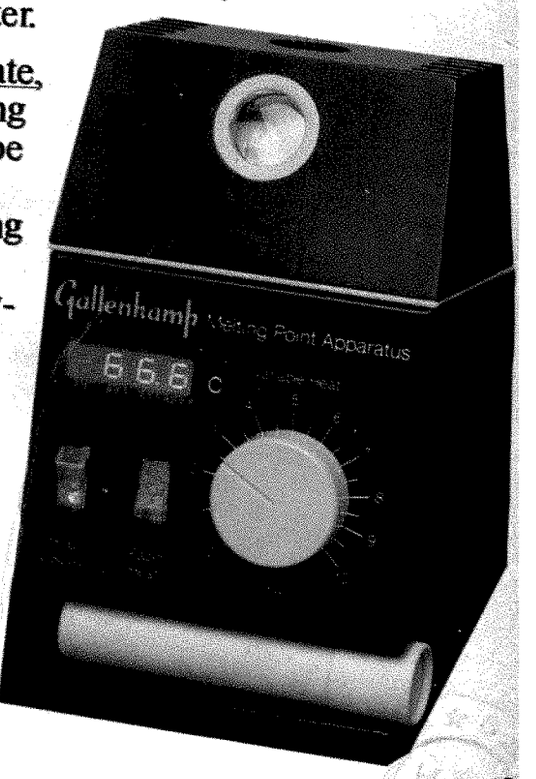
Easier, because the operator can concentrate entirely on the melting sample – no need to keep half an eye on the thermometer.

Quicker, because the platinum resistance sensor responds more quickly to temperature change than a mercury-in-glass thermometer.

More accurate, because the melting temperature can be immediately 'frozen' by pressing the hold button as soon as melting is observed – ordinary mercury-in-glass thermometers can go on rising and errors can be made.

Isn't it time you threw away your old mercury thermometer?

For further details, please write to, or telephone Gallenkamp.



Gallenkamp

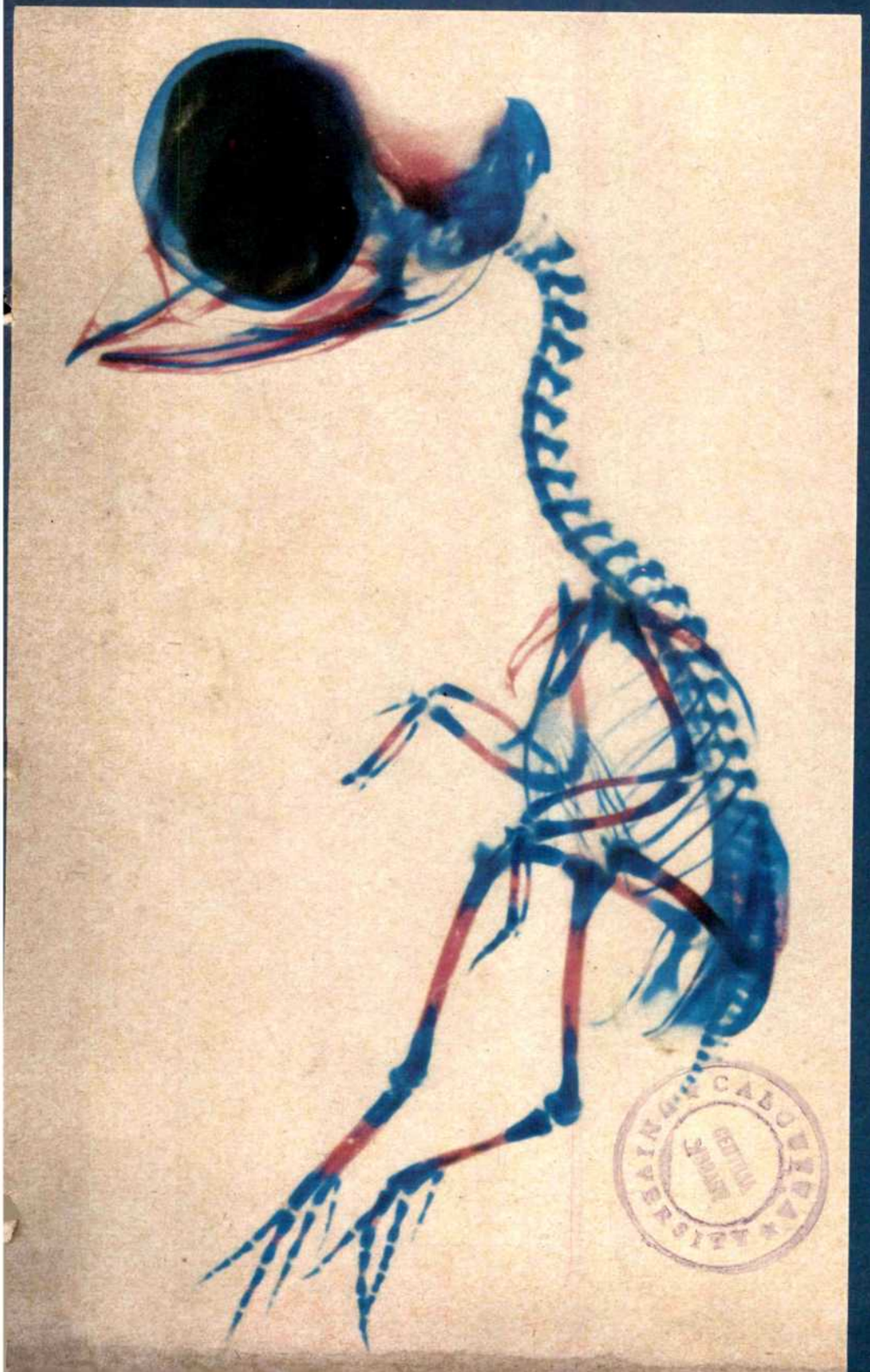
PO Box 290, Technico House, Christopher Street, London EC2P 2ER Telephone 01-247 3211 Telex 886041
Fisons plc Scientific Equipment Division

Circle No.09 on Reader Service Card.

nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

Vol 307 No 5953 23-29 February 1984 £1.80 $4.50



OSTRICHES PRIMITIVE NOT DEGENERATE

MICROBIOLOGY
product review

Only with the new LKB Horizontal Electrophoresis System do you also get...

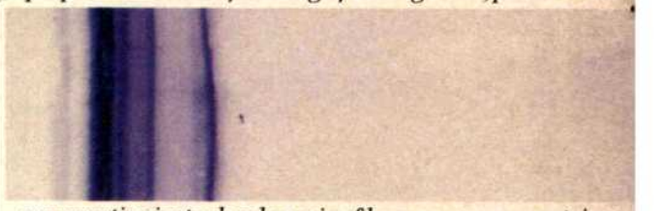
A choice of 3 preparative methods

Preparative electrofocusing in Immobiline gels is the newest, most efficient way of separating proteins in quantities up to 1 gram. LKB Immobilines combine greatly improved resolution with sample recovery rates of up to 98%. And the absence of soluble carrier ampholytes means that high resolution preparative electrofocusing of low molecular weight substances is now fully practicable.

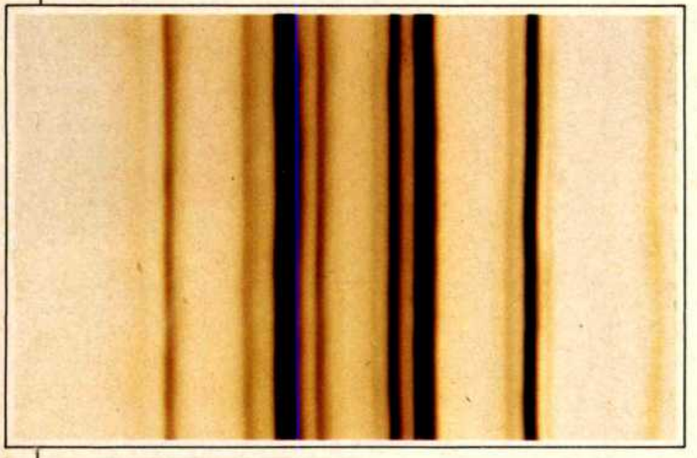
Preparative electrofocusing in granular gels is a more traditional method using Ampholine carrier ampholytes, and is also catered for by the LKB Horizontal Electrophoresis System. The enlarged cooling plate of the new Multiphor II doubles the sample capacity, while the movable electrodes give increased flexibility.



– preparative electrofocusing of hemoglobin, pH 6.0–8.5



–preparative isotachophoresis of human serum proteins



Preparative isotachophoresis is the third choice offered by the LKB Horizontal Electrophoresis System, and is a low-cost alternative giving good resolution for separations based on protein mobility. The twin integral buffer reservoirs permit gels to be run either across or along the length of the cooling plate.

◀ *– preparative electrofocusing of hemoglobin using Immobiline, pH 6.5 to 8.5*

This is one in a series of advertisements describing the unrivalled versatility of the LKB Horizontal Electrophoresis System. For further information about preparative methods, and the many other applications of the system, send today for our brochure.

THE LKB SYSTEM

Only LKB can offer the research scientist a complete, integrated system in which each component works with and complements all the others. Based on the new and improved Multiphor II, the system also includes a 5KV power supply, thermostatic circulator, laser densitometer and data evaluation software.

In addition to the instrumentation, LKB provides a range of 18 major kits plus a complete programme of high quality chemicals, supplies and accessories, backed by the full worldwide support of the market leader in electrophoresis and electrofocusing.

LKB – the electrophoresis experts.



LKB BROMMA

Head office:
LKB-Produkter AB,
Box 305, S-16126 Bromma, Sweden.
Tel. 08-98 00 40, telex 10492

Main US sales office:
LKB Instruments, Inc.
9319 Gaither Road, Gaithersburg, Maryland 20877.
Tel. 301-963-3200, telex 909870 (dom.), 64634 (intern.)

UK sales office:
LKB Instruments Ltd.,
232 Addington Road, S. Croydon, Surrey CR2 8YD.
England.
Tel. 01-657 8822, telex 264414

Other sales offices in:
Antwerp, Athens (for Middle East), Bangkok, Copenhagen, Lucerne, Moscow, Munich, Paris, Rome, Turku, Vienna, Zoetermeer

Circle No.43 on Reader Service Card.

DESIGNED FOR A MAJOR ADVANCE IN PEPTIDE SYNTHESIS



THE CRB PEPSYNTHESISER

The first peptide synthesiser incorporating the simplicity and high efficiency of the 'Fmoc-POLYAMIDE' solid phase method with the speed, economy and ease of operation of a CONTINUOUS FLOW SYSTEM.

- Low cost
- Exceptionally mild reaction conditions
- High product yield
- Simple, rapid procedure
- Continuous UV monitoring of reaction progress
- High product purity

The Fmoc-Polyamide Method as developed by Dr. R.C. Sheppard and his group replaces the customary polystyrene resin with a polar polyamide resin. This resin is freely permeated by polar solvents such as dimethylformamide which is a particularly good reaction medium for peptide synthesis. The use of Fmoc amino acids and t-butyl protecting groups avoids the repetitive and vigorous acid treatments required in conventional synthesis and considerably reduces the accumulation of byproducts.

The Continuous Flow System for removal of excess reactants through a column is inherently more efficient than batchwise washing which is used in conventional peptide synthesisers. our continuous flow system has been designed especially for use with 'PEPSYN K'. This polyamide resin is held within the pores of an inert inorganic support which allows the rapid diffusion of reactants without generation of high back pressure.

The Continuous UV Monitoring of both the amino acid addition and the deprotection steps provides reassuring evidence that the synthesis is proceeding normally.

Pepsyn Reagents are provided as a complete range of high purity products prepared specially to ensure maximum success with the versatile Fmoc-Polyamide method. They include Fmoc amino acids and derivatised resins for the synthesis of free peptides, peptide amides and side chain protected peptides.

Technical Support is based on highly successful and extensive use of the Fmoc-Polyamide method in our own laboratories.

For further details please contact:

Head Office and Laboratories.
Cambridge Research Biochemicals Ltd
Button End Industrial Estate,
Harston, Cambridgeshire
CB2 5NX ENGLAND
Tel: Cambridge (0223) 871674
Telex: 817694 CRBLTD G

U.S.A.
Cambridge Research Biochemicals Ltd.
P.O. Box 58, 1887 Park Street,
Atlantic Beach, New York
11509, U.S.A.
Tel: (516) 239 3831
Telex: 971398

CRB
CAMBRIDGE
RESEARCH
BIOCHEMICALS

Circle No.57 on Reader Service Card.

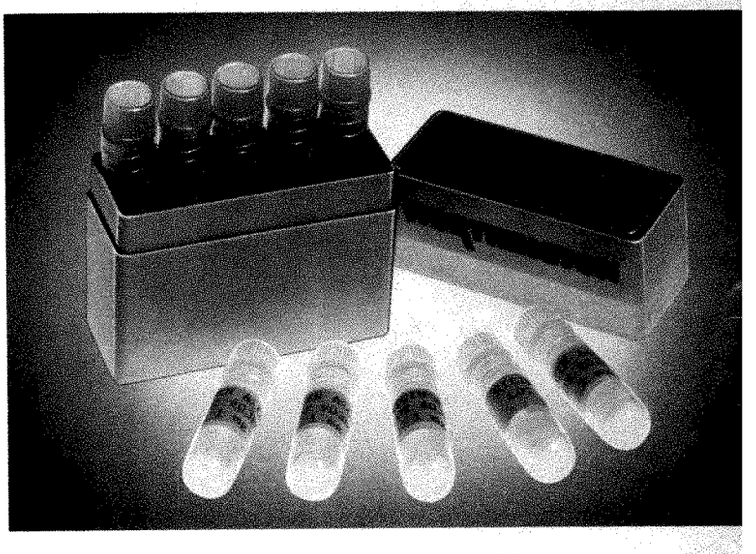
Now safe for laboratory use!
WORTHINGTON
PURIFIED RICIN-A CHAIN
for targeted cytotoxicity

GREATLY REDUCED TOXICITY

- Free of B chain mediator of cell entry
- 10,000 times less toxic to whole cells than complete Ricin
- Non-toxic laboratory handling

SUITABLE FOR THERAPEUTIC PRODUCT DEVELOPMENT

- Highly purified for use in whole animal injection studies
- Can be conjugated to monoclonal antibodies, hormones or other ligands to target cytotoxic effect
- Projected human LD_{50} of 1 to 3 grams based on animal studies



STABLE FOR LABORATORY USE

- Shipped in solution
- Remains soluble while stored frozen

NOW EVEN MORE EFFECTIVE WITH THE INTRODUCTION OF RICIN-B CHAIN (THE NATURAL MEDIATOR OF RICIN-A CELL ENTRY)

CALL TOLL FREE (800) 523-7620
IN PA. (800) 662-2440
OR COMPLETE COUPON BELOW

☐ Please send me ordering information for Worthington Purified Ricin-A and Ricin-B Chains

Please notify me of other new Worthington products in these areas of research use:

☐ Tissue Culture/ Cell Biology
☐ Molecular Biology
☐ Protein Purification Structure
☐ Cell Surface
☐ Immunology
☐ Others _____

Name _____ Title _____

Company/Institution _____

Department _____

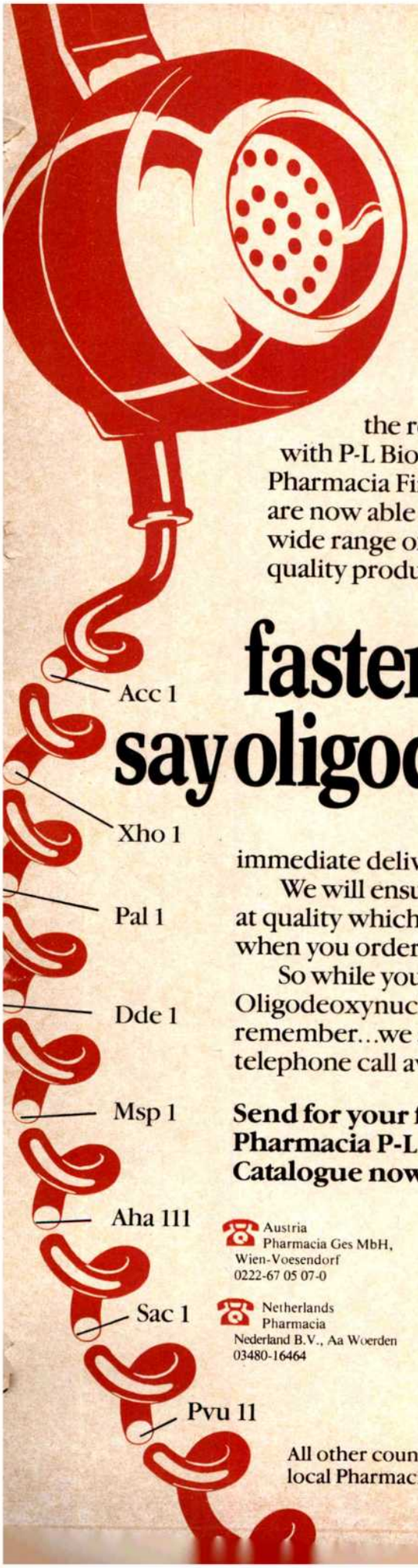
Address _____

City _____ State _____ Zip _____

Tel. No. _____

COOPER Biomedica
Scientific Divisio
One Technology Cour
Malvern, Pa. 1935

Circle No.46 on Reader Service Card.



Now order your research biochemicals...

As a result of the recent merger with P-L Biochemicals, Pharmacia Fine Chemicals are now able to bring you a wide range of over 2,200 high quality products for molecular biology and biochemical research.

And all you have to do to order any of our speciality biochemicals is to contact your local Pharmacia office who are holding large stocks ready for

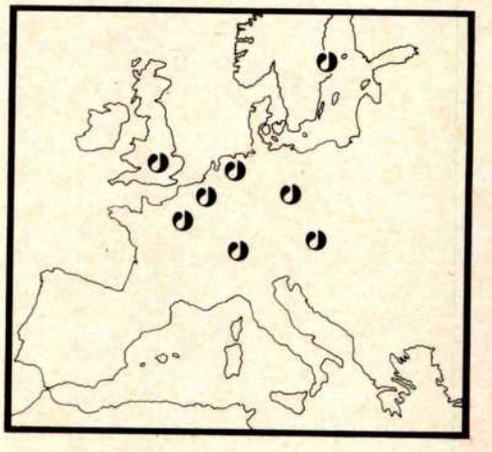
faster than you can say oligodeoxynucleotides

immediate delivery.

We will ensure a fast delivery at quality which you expect when you order from Pharmacia.

So while you are trying to say Oligodeoxynucleotides, remember...we are only a telephone call away.

Send for your free Pharmacia P-L Biochemicals Catalogue now.



Austria
Pharmacia Ges MbH,
Wien-Voesendorf
0222-67 05 07-0

Belgium
Pharmacia Belga,
Brussels
02/7369990

France
Pharmacia France SA,
Bois D'Arcy
043-9919

German Federal Republic
Deutsche Pharmacia GMBH,
Freiburg (0761) 49 03-239

Netherlands
Pharmacia
Nederland B.V., Aa Woerden
03480-16464

Sweden
Pharmacia Fine
Chemicals AB, Uppsala
018/165450

Switzerland
Pharmacia-Rolf-
Ag, Dübendorf
01/821 18 16

United Kingdom
Pharmacia Ltd.
Milton Keynes
0908-661101

All other countries contact your local Pharmacia representative.

Pharmacia
P-L Biochemicals

Circle No.17 on Reader Service Card.

Mycoplasma Detection and Identification

Can You Really Afford to Wait?

In five days, you can know whether mycoplasmas are infecting your cells. In five hours, you can know which kind.
BRL introduces MYCOTECT™ and MYCOSPEC™.

Detecting mycoplasmas was always a lengthy, tedious and expensive procedure. Therefore, most laboratories tested cell lines only when they looked infected. By that time, the infection frequently had already spread to other cells.

Determining the mycoplasma species is an important step in tracking down its source and preventing future infections. Until now, however, the process was too long, complicated and expensive to be practical.

BRL has changed all that.

With MYCOTECT and MYCOSPEC, available *exclusively* from BRL, you can detect and identify mycoplasmas easily and inexpensively, right in your laboratory.

MYCOTECT

MYCOTECT is cytotoxic to mycoplasma-infected cells. After incubation of cell samples with MYCOTECT and exposure to crystal violet, cell monolayers in uninfected wells stain more intensely, and therefore can be easily distinguished from the sparse cell layers found in mycoplasma-infected wells. The entire process takes only five days -less than one fifth the time needed for detection by conventional methods. The cost of a MYCOTECT assay is only a fraction of the cost of current tests, and the only equipment needed is a 24-well plate.

MYCOSPEC

MYCOSPEC is a rapid, simple and inexpensive assay for the five major mycoplasma species that account for more than 95% of all laboratory mycoplasma infections. Mycoplasma samples are incubated separately with monoclonal antibodies to each of the five species, then



with a biotinylated secondary antibody and finally with a streptavidin-horseradish peroxidase complex. The sample that stains when diaminobenzidine is added contains the monoclonal to the infecting species. With MYCOSPEC, identification is complete within five hours.

MYCOTECT and MYCOSPEC. Only from BRL. Now you don't have to wait.

BRL

Bethesda Research Laboratories, Inc.
Gaithersburg, Maryland 20877
Telex: 64210 BRL GARG UW
To Order: (800) 638-8992; (301) 840-8000
Technical Services: (800) 638-4045

Bethesda Research Laboratories GmbH
6078 Neu Isenburg, West Germany
Telex: 417699 BRLNI D
To Order/Tech Services: 06102-3204

Bethesda Research Laboratories (UK) Ltd.
Cambridge, CB4 4BE United Kingdom
Telex: 818710 BRLCAM G
To Order/Tech Services: (0223) 315504

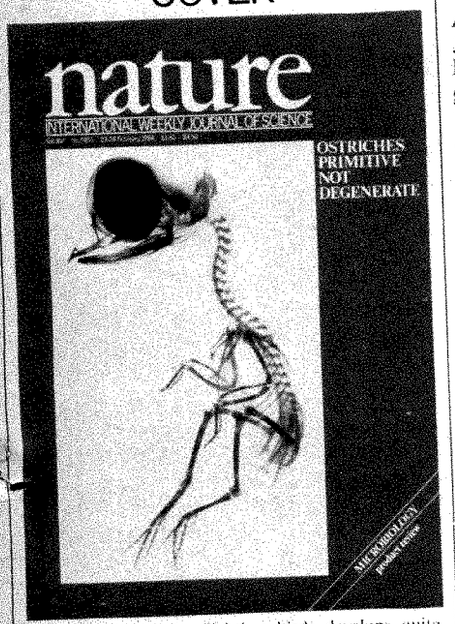
Bethesda Research Laboratories SARL
F-95220 Herblay, France
Telex: 695729 FBIMAGRE
To Order/Tech Services: (3) 978 8200

Circle No.33 on Reader Service Card.

nature

NATURE VOL 307 23 FEBRUARY 1984

COVER



The tarsal region in flightless birds develops quite differently from that in flying birds like the quail shown on the cover, and has a structure similar to that found in theropod dinosaurs. This suggests that flightless birds have arisen from an independent ancestral stock and are not derived from flying birds (see p.733).

OPINION

Secrecy beyond reason 671
Time to back Europe
Supercomputermania 672

NEWS

Illmensee inquiry 673
Orlov appeal
US biotechnology 674
Harvard's Ptashne patent disappoints
Nuclear Pakistan
Nuclear power 675
West German universities 676
China
Italy's biotechnology
Satellite launchers 677
US space station
High-energy physics 678
UK pollution control
Acid rain 679
Conservation

CORRESPONDENCE

Nuclear arms/Psychiatry/ Ice-forming bacteria 680

COMMENTARY

Polygraphic interrogation
D T Lykken 681

NEWS AND VIEWS

Abundant phase transitions
John Maddox 685
DNA supercoiling and gene expression
L Mark Fisher 686
Cryptozoology
Robert M May 687
New data upset ice age theories
Philip Campbell 688
When deviants are favoured: evolution of sex determination
Paul H Harvey and Linda Partridge 689
Ions caught in their tracks
Jon Darius 691
Chemically generated spatial structures
Irving R Epstein 692

BOOK REVIEWS

Rutherford: Simple Genius *by D Wilson*
R V Jones 761
Unity of Forces in the Universe *by A Zee*
Alan H Guth 762
Techniques in Molecular Biology
John M Walker and W Gaastra, eds
Roger Patient 763
Liposome Letters *A D Bangham, ed.* and **Liposomes** *M J Ostro, ed.*
J A Lucy
Mathematical Models in Molecular and Cellular Biology
L A Segel, ed.
Athel Cornish-Bowden 764
Men and Women: How Different Are They? *By John Nicholson*
Stuart Sutherland
History of Staining, 3rd Edn
by G Clark and F H Kasten
Robert Olby 765
Geology of Scotland, 2nd Edn
G Y Craig, ed. and **Scotland's Environment During the Last 30,000 Years** *by R J Price*
W S McKerrow 766

REVIEW ARTICLE

Calcium-activated potassium channels and their role in secretion
O H Petersen & Y Maruyama 693

ARTICLES

Revised Sm–Nd systematics of Kambalda greenstones, Western Australia
J C Claoué-Long, M F Thirlwall & R W Nesbitt 697
Alteration of c-*myc* chromatin structure by avian leukosis virus integration
W Schubach & M Groudine 702
Induction of altered chromatin structures by simian virus 40 enhancer and promoter elements
J Jongstra, T L Reudelhuber, P Oudet, C Benoist, C-B Chae, J-M Jeltsch, D J Mathis & P Chambon 708

LETTERS TO NATURE

A new mine determination of the newtonian gravitational constant
S C Holding & G J Tuck 714
Hyperion: 13-day rotation from Voyager data
P Thomas, J Veverka, D Wenkert, G E Danielson & M E Davies 716
Spatial structures generated by chemical reactions at interfaces
D Avnir & M Kagan 717
A new chemical oscillator containing neither metal nor oxyhalogen ions
M Burger & R J Field 720
Evidence for stabilization of the Pilbara Block, Australia
T S Blake 721
Cambrian and Ordovician conodonts from the Robertson Bay Group, Antarctica and their tectonic significance
C F Burrett & R H Findlay 723
Biomass conversion to liquid fuels: potential of some Arizona chaparral brush and tree species
E A Davis, J L Kuester & M O Bagby 726
Shallow-water sclerosponges on Jamaican reefs and a criterion for recognition of hurricane deposits
T P Scoffin & M D Hendry 728
Real and apparent movement nulled
R L Gregory & J P Harris 729
Induction of the ipsilateral retinothalamic projection in *Xenopus laevis* by thyroxine
S G Hoskins & P Grobstein 730

Contents continued overleaf

Nature (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Annual Index (mailed in February). Annual subscription for USA and Canada US $230 (for subscription prices elsewhere, see next page). USA and Canadian orders only to: *Nature*, Subscription Dept PO Box 1018, Manasquan, New Jersey 08736, USA. All other orders to *Nature*, Brunel Road Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY10010 and additional mailing offices. US Postmaster send form for 3579 to: *Nature*, 15 East 26 Street, New York, NY 10010. Published in Japan by Macmillan Shuppan K.K., 5th Floor Eikow Building, 1-10-9 Hongo, Bunkyo-ku, Tokyo, Japan. 1984 Macmillan Journals Ltd.

New ligands

- Affinity labelling reagent for oestrogen receptors
  [*ring*-^{3}H]Tamoxifen aziridine — TRK.788
- Calcium channel blocker
  (+)-[*methyl*-^{3}H]PN 200–110 — TRK.787
- Selective ligand for opiate receptor
  [*tyrosyl*-3,5(n)-^{3}H]Dynorphin (1-9) — TRK.795
- Muscarinic agonist
  [*N-methyl*-^{3}H]Oxotremorine M acetate — TRK.794
- Dopamine agonist
  [^{3}H]5,6-DPAT — TRK.792
- Selective α_2 antagonist
  [^{3}H]RX 781094* — TRK.799
- Exclusive adenosine agonist
  (−)-[^{125}I]HPIA — IM.153
- Receptor grade [^{125}I]insulin, human
  [^{125}I]-(A14)-monoiodinated human insulin — IM.166
  [^{125}I]-(B26)-monoiodinated human insulin — IM.167

*prepared by agreement with Reckitt and Colman plc.

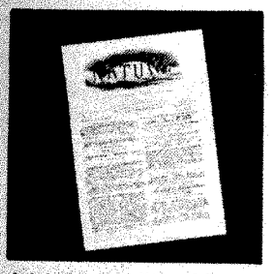
Amersham International plc
Amersham England HP7 9LL
telephone Little Chalfont (024 04) 4444
telex 83141 ACTIVA G

Amersham

Amersham Australia PTY Limited Sydney **Amersham Belgium SA/NV** Brussels **Amersham Buchler GmbH & Co. KG** Braunschweig W Germany
Amersham Corporation Arlington Heights USA **Amersham Denmark ApS** Birkerød **Amersham France SA** Paris **Amersham Japan** Tokyo **Amersham Nederland BV** Utrecht

Circle No.50 on Reader Service Card.

Fascinating reading and an unusual gift



A high-quality, 40-page facsimile of the first issue of Nature is available, including articles on:

* **The recent total eclipse of the sun** by Sir Norman Lockyer
* **Science Teaching in Schools** by W. Tuckwell
* **Triassic Dinosauria** by T. H. Huxley

Prices and address for orders are set out below.

Annual Subscription Prices

UK & Irish Republic		£98
USA & Canada		US$230
Australia & NZ	Airspeed	£150
Continental Europe	Airspeed	£120
India	Airspeed	£115
Japan	Airspeed	Y85000
Rest of World*	Surface	£120
Rest of World*	Airmail	£180

**(not USA, Canada, Europe & Japan)*

Orders (with remittance) to:

USA & Canada
Nature
Subscription Dept
PO Box 1018
Manasquan
NJ 08736
USA

UK & Rest of World
Nature
Circulation Dept
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to:
Japan Publications Trading Company Ltd
2-1 Sarugaku-cho 1-chome
Chiyoda-ku, Tokyo, Japan
Tel: 03-292-3755

Personal subscription rates
These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
15 East 26 Street
New York,
NY 10010, USA
Tel: (212) 689-5900

UK & Europe
Felicity Parker, Nature
Brunel Road
Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

Credit card orders only (in USA):
Call toll-free: (800) 824-7888 (Operator 246)
In California: (800) 852-7777 (Operator 246)

Back issues
UK, £2.50; USA & Canada, US$6.00 (surface), US$9.00 (air); Rest of World, £3.00 (surface), £4.00 (air)

Binders
Single Binders: UK, £4.50; Rest of World, $10.00
Set(s) of 3 Binders; UK, £12.00; Rest of World, $25.00

Annual indexes (1971–1982)
UK, £5.00 each; Rest of World, $10.00

Nature First Issue Facsimile
UK, 75p; Rest of World (surface), $1.50; (air) $2.00

Nature in microform
For Information:
University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

Evolutionary relationships of ratites and carinates: evidence from ontogeny of the tarsus
C McGowan 733

Molecular genetic evidence for early evolutionary origin of budding peptidoglycan-less eubacteria
E Stackebrandt, W Ludwig, W Schubert, F Klink, H Schlesner, T Roggentin & P Hirsch 735

Possible artefactual basis for apparent bacterial growth at 250°C
J D Trent, R A Chastain & A A Yayanos 737
Reply: J A Baross & J W Deming 740

Role of an upstream regulatory element in leucine repression of the *Saccharomyces cerevisiae leu2* gene
A Martinez-Arias, H J Yost & M J Casadaban 740

Expression and regulation of human low-density lipoprotein receptors in Chinese hamster ovary cells
R D Sege, K Kozarsky, D L Nelson & M Krieger 742

Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells
W J King & G L Greene 745

Nuclear localization of unoccupied oestrogen receptors
W V Welshons, M E Lieberman & J Gorski 747

Novel κ light-chain gene rearrangements in mouse λ light chain-producing B lymphocytes
J Durdik, M W Moore & E Selsing 749

A variant translocation places the immunoglobulin genes 3′ to the c-*myc* oncogene in Burkitt's lymphoma
G F Hollis, K F Mitchell, J Battey, H Potter, R Taub, G M Lenoir & P Leder 752

Novel mannosidase inhibitor blocking conversion of high mannose to complex oligosaccharides
U Fuhrmann, E Bause, G Legler & H Ploegh 755

A new myosin fragment: visualization of the regulatory domain
D A Winkelmann, S Almeda, P Vibert & C Cohen 758

MISCELLANY

100 years ago 690
Product review: Microbiology 767

NATURE CLASSIFIED

Professional appointments — Research posts — Studentships — Fellowships — Conferences — Courses — Seminars — Symposia: Back pages

Next week in *Nature*:

- What economists can tell governments
- The effects of nuclear war
- Laboratory model of Jupiter's Red Spot
- Polar glaciation and ice ages
- Insight in pigeons
- Benzodiazepines and postsynaptic GABA sensitivity
- Homology of human T-cell leukaemia virus envelope
- Cryo-electron microscopy of viruses
- Transepithelial transport of immunoglobulins
- H.N.V. Temperley reviews S.J. Brams's *Superior Beings: If They Exist, How Would We Know*

GUIDE TO AUTHORS

Authors should be aware of the diversity of *Nature*'s readership and should strive to be as widely understood as possible.

Review articles should be accessible to the whole readership. Most are commissioned, but unsolicited reviews are welcome (in which case prior consultation with the office is desirable).

Scientific articles are research reports whose conclusions are of general interest or which represent substantial advances of understanding. The text should not exceed 3,000 words and six displayed items (figures plus tables). The article should include an abstract of about 50 words.

Letters to *Nature* are ordinarily 1,000 words long with no more than four displayed items. The first paragraph (not exceeding 150 words) should say what the letter is about, why the study it reports was undertaken and what the conclusions are.

Matters arising are brief comments (up to 500 words) on articles and letters recently published in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted.

Manuscripts may be submitted either to London or Washington (decisions being made only in London). Manuscripts should be typed (double spacing) on one side of the paper only. Three copies are required, each accompanied by copies of lettered artwork. No title should exceed 80 characters in length. Reference lists, figure legends, etc. should be on separate sheets, all of which should be numbered. Abbreviations, symbols, units, etc. should be identified on one copy of the manuscript at their first appearance.

References should appear sequentially indicated by superscripts in the text and should be abbreviated according to the *World List of Scientific Periodicals*, fourth edition (Butterworth 1963–65). The first and last page numbers of each reference should be cited. References to books should clearly indicate the publisher and the date and place of publication. Unpublished articles should not be formally referred to unless accepted or submitted for publication, but may be mentioned in the text.

Each piece of artwork should be clearly marked with the author's name and the figure number. Original artwork should be unlettered. Suggestions for cover illustrations are welcome. Original artwork (and one copy of the manuscript) will be returned when manuscripts cannot be published.

Requests for permission to reproduce material from Nature *should be accompanied by a self-addressed (and, in the case of the United Kingdom and United States, stamped) envelope.*

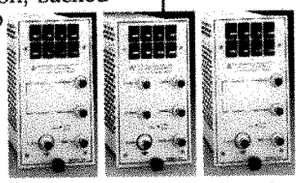
We Make Transients Talk.

Transient analysis is a complex job: capture, digitizing, storage, display, analysis and data transfer. It's harder still if you need remote control capability. Most general purpose instruments can't handle more than one or two of these steps. But the Data 6000 Universal Waveform Analyzer does them all...with speed and power you won't find in any other single instrument. In fact, the Data 6000 offers more powerful capabilities than you could obtain from an entire roomful of equipment at many times the cost. Nothing else even comes close to the Data 6000, which offers you:

Faster Data Acquisition

No matter how short-lived the event, you'll capture it with high resolution, thanks to the Data 6000 100 kHz and 100 MHz digitizing modules. Sample times as short as 10 nanoseconds, with full 8-bit resolution, give you the accuracy you need. Only Data Precision, backed by Analogic's leadership in A/D conversion and data acquisition could have offered the Data 6000's powerful acquisition capabilities.

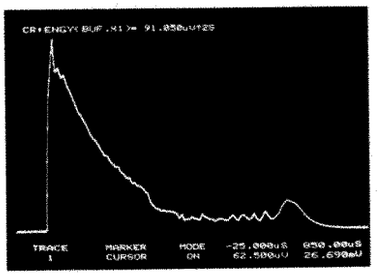
And that's only the front end of the Data 6000's power.



Model 610 2ch 100KHz | Model 611 4ch 100KHz | Model 620 2ch 100MHz

Quicker on the Trigger

Trigger level may be set with minimum 8-bit precision, to eliminate false triggering on noisy signals without sacrificing sensitivity. And event display can begin precisely at the moment you select. View pre- and post-trigger events up to 100% of selected time-base away from the moment of triggering...from milliseconds to months before *and* after. You'll never miss a shot with the Data 6000's smart trigger.



Compute and display a wide variety of functions from the captured signal. Useful functions for transient analysis are Rise, Fall, Settling, and Display Times; Overshoot, Maximum, Minimum, Peak to Peak, Area or Energy under the curve; Real and Complex Magnitude Spectra; Real and Complex Log Magnitude Spectra; and many more...all available with a single keystroke!

More Digital Storage

Up to 56,000 points of data may be stored in on-board storage memory, with unlimited expansion available on accessory floppy disk or your mass storage media. Data acquisition memory holds up to 56,000 points per channel (with the 610 and 611 plug-ins; 16,000 points for the 620).

And data storage is accurate, supporting full 5-decimal place readouts. Double precision accumulators are standard. The high resolution CRT display lets you view waveforms as accurately as they are stored, and a comfortable screen angle permits easy use. The large display has full alphanumerics for on-screen labelling and documentation of functions.

On-Board M68000

How does the Data 6000 do so much more than an ordinary A/D converter, digital waveform recorder, digital storage oscilloscope, and engineering microcomputer? The answer is inside: a fast, powerful M68000 microprocessor, armed with the fastest Fast Fourier Transform Algorithm, and capable of quick, user-friendly communications and remarkable computational power. And when you need to go beyond the Data 6000's stand-alone capabilities, versatile data transfer is available through the RS-232 port. And IEEE-488 bus compatibility allows full integration of the Data 6000 with your system.

IEEE-488

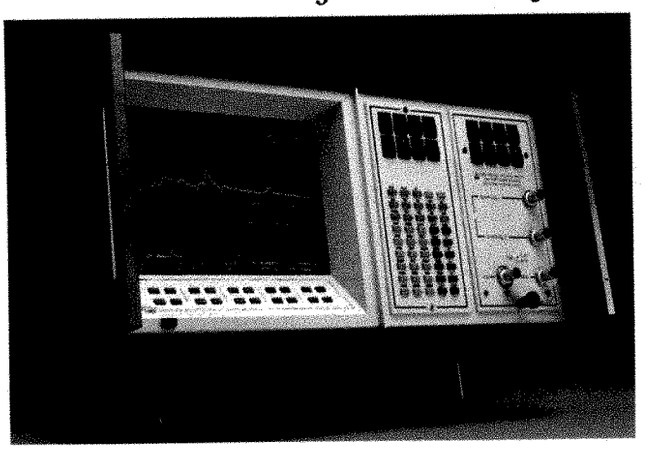
Superior. Demonstrably Superior.

The Data 6000's unique capabilities with transient waveforms are reason enough to have it on your bench. But if you need further reasons, the Data 6000 has them. An instrument this powerful can do much more than transient analysis. Consider it as:

- ☐ A Storage Oscilloscope
- ☐ A Spectrum Analyzer
- ☐ An Auto/Cross Correlator
- ☐ A Transient Recorder
- ☐ An RF or Audio Signal Analyzer
- ☐ An ATE Subsystem
- ☐ A Vibration Analyzer

For transient analysis with unprecedented ease, and much more, try the Data 6000. For further information, call:
800-343-8150
Or in Massachusetts, 800-892-0528.

The Data 6000
Universal Waveform Analyzer



Maintaining The Integrity Of Measurement

ANALOGIC®

DATA PRECISION®

Data Precision Division of Analogic Corporation, Electronics Avenue, Danvers, MA 01923 617-246-1600 TELEX 6817144

For literature Circle No.34
For demonstration Circle No.35

Code-Breakers and Code-Makers BIOSYNTECH

OUR BUSINESS IS YOUR BASE.

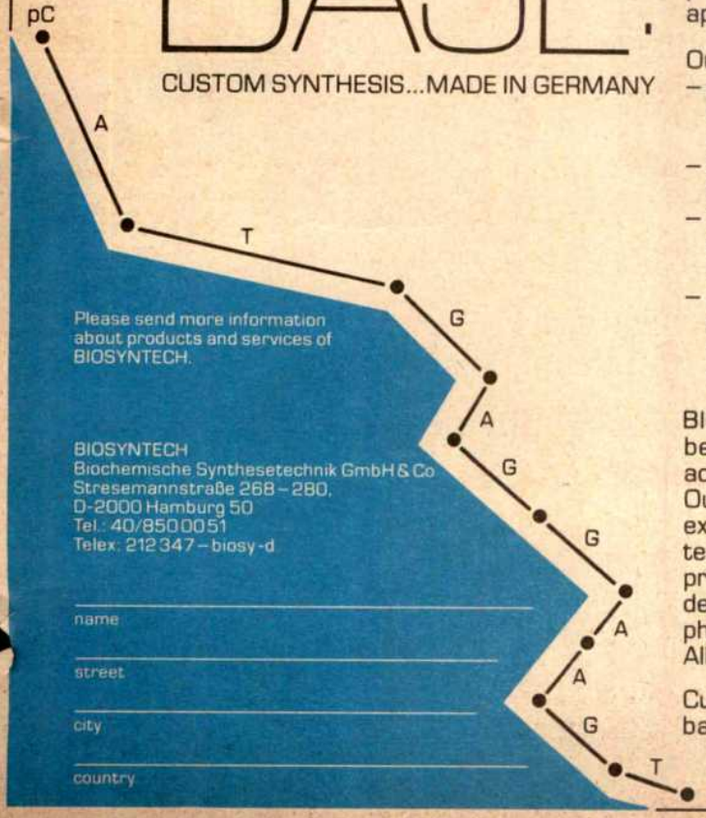
CUSTOM SYNTHESIS...MADE IN GERMANY

BIOSYNTECH is the first company in Western Germany which has an experienced and professional team to support your genetic engineering programmes in either research or industrial application.

Our main product lines
- Custom synthesis of primers and probes (mixed probes) including the incorporation of modified bases.
- Synthesis of linker/adapter molecules also following your individual requirements.
- Total or partial synthesis of structural genes and regulatory DNA sequences (signal structures).
- Design and development of genetic engineered microorganisms for the production of proteins useful for medicine, pharmacy and agriculture.

BIOSYNTECH guarantees all DNA fragments to be of highest purity available. Every shipment is accompanied by a purity certificate.
Our "contract discount" programme offers you exceptionally favourable conditions for long term cooperation. Modern technology and a professional team imply a quick delivery of the desired sequences. You simply order by telephone or telex thus saving time and costs.
All orders will be handled in strictest confidence.

Custom synthesis is our business, and your base.



Please send more information about products and services of BIOSYNTECH.

BIOSYNTECH
Biochemische Synthesetechnik GmbH & Co
Stresemannstraße 268 – 280,
D-2000 Hamburg 50
Tel.: 40/8500051
Telex: 212347 – biosy-d

name

street

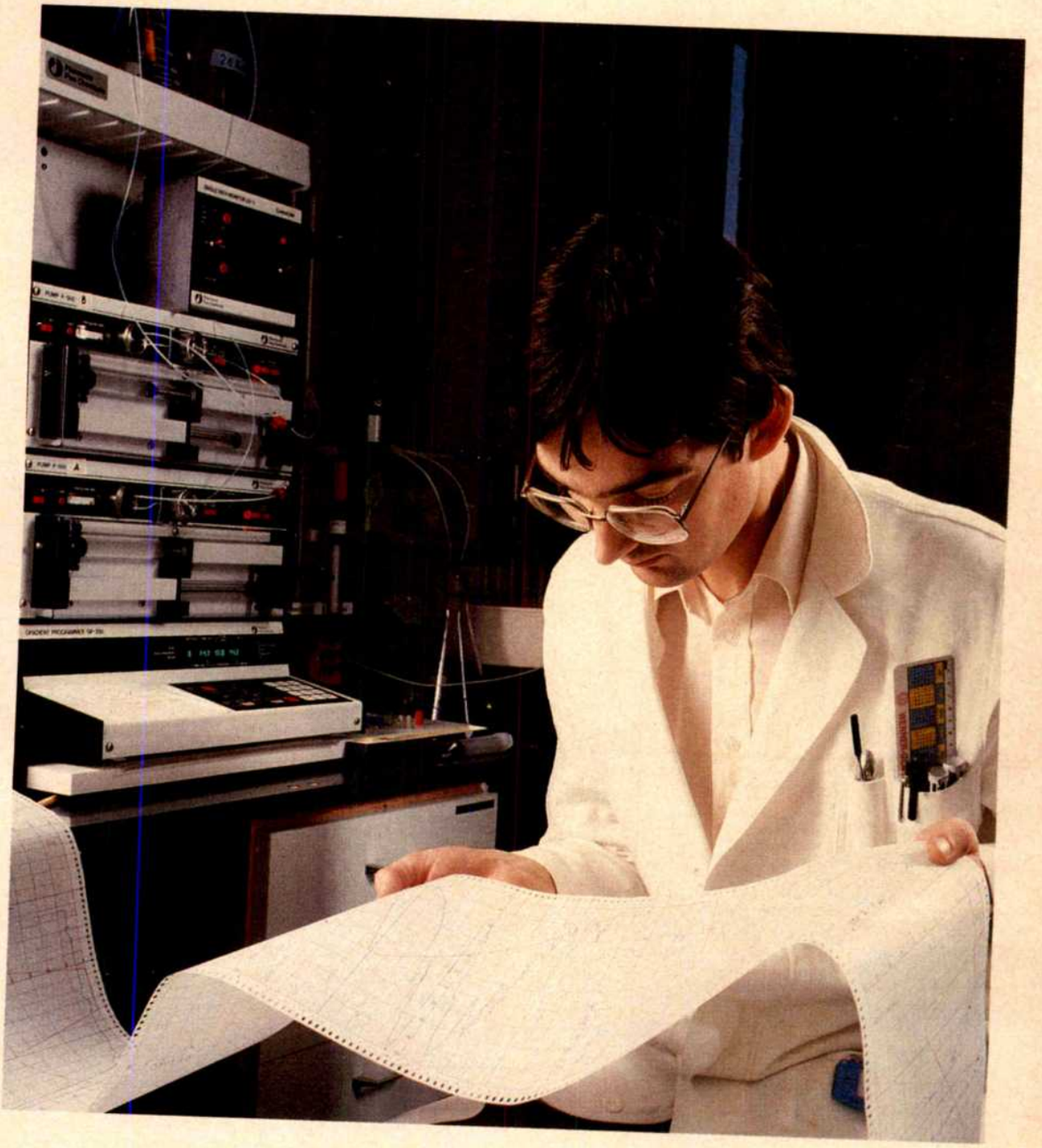
city

country

LOGICON HAMBURG

Circle No.36 on Reader Service Card.

FPLC means ...

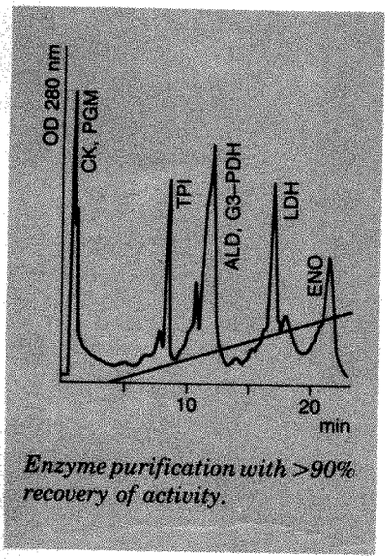


Separation power

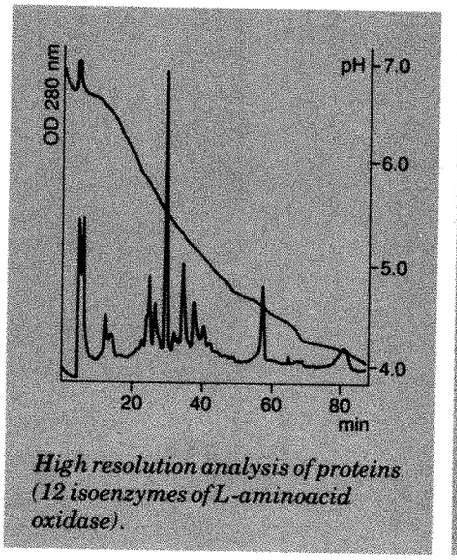
Fast Protein/Peptide/Polynucleotide Liquid Chromatography – but speed is not its only virtue.

FPLC means new unique, hydrophilic, monodisperse media providing high performance resolution and high recovery of biological activity: and new silica based media providing high performance resolution, superior yields of proteins, peptides and polynucleotides – with sample sizes up to 100 mg.

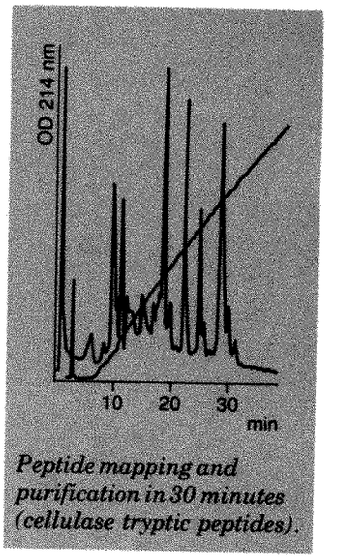
FPLC means new instruments, new media and methods – the combination of which is a high performance liquid chromatography system providing a degree of resolution, capacity and speed previously unattainable for purifying proteins, peptides and polynucleotides.



Enzyme purification with >90% recovery of activity.



High resolution analysis of proteins (12 isoenzymes of L-aminoacid oxidase).



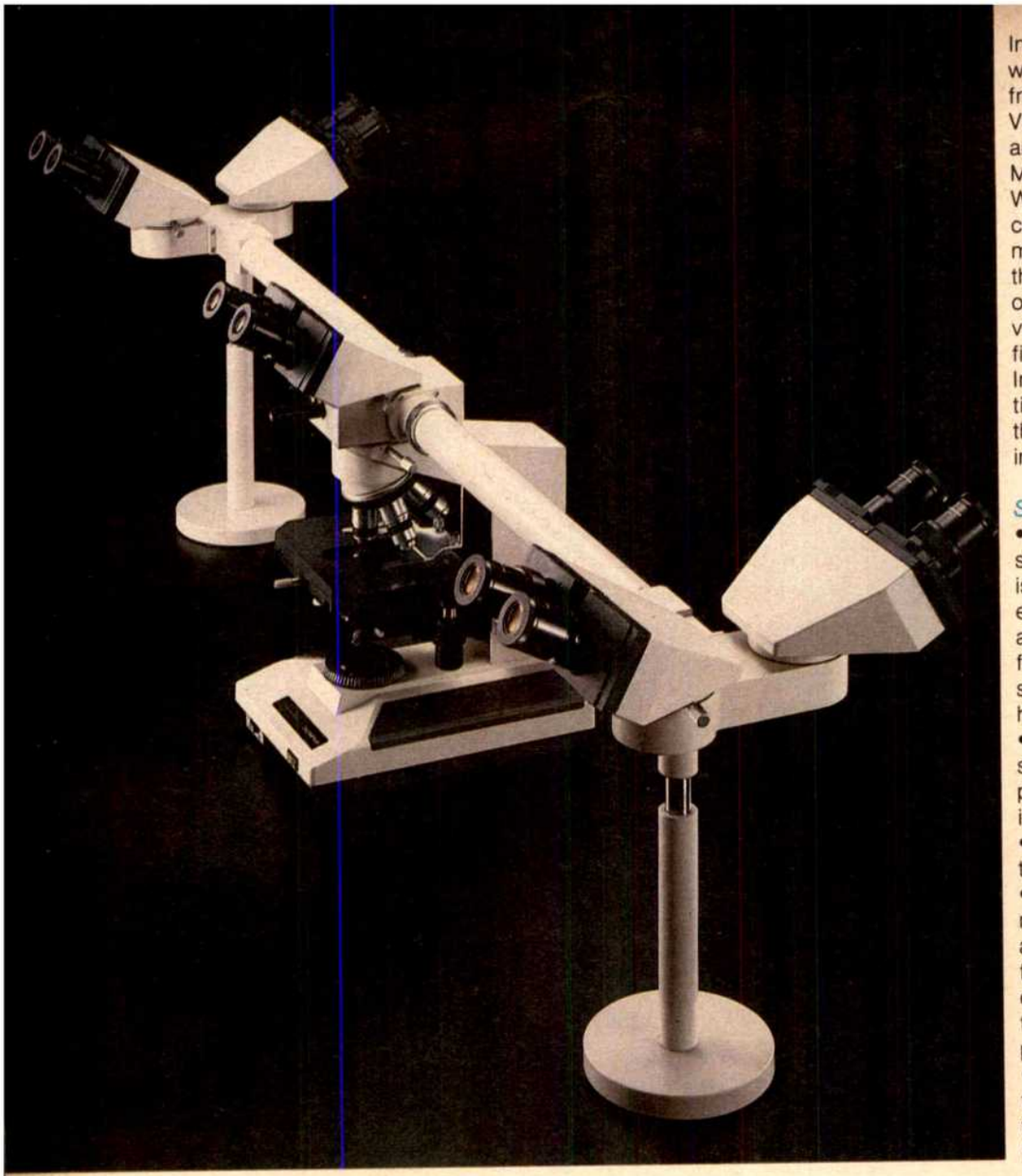
Peptide mapping and purification in 30 minutes (cellulase tryptic peptides).

For further information about the separation power of the FPLC System, please contact your local representative of Pharmacia Fine Chemicals.

Pharmacia Fine Chemicals AB
Box 175
S-751 04 Uppsala 1
Sweden

Pharmacia Fine Chemicals

Circle No.52 on Reader Service Card.



In response to an increasing demand worldwide for a multi-head microscope from Olympus, the BH2-MDO Multi-Viewing Attachment is now available as an accessory of the BH-2 System Microscope.

With the BH2-MDO, up to five observers can view and study the same specimen at the same time. Not only does this provide better efficiency in medical or scientific studies, but it is also highly valuable in educational and industrial fields.

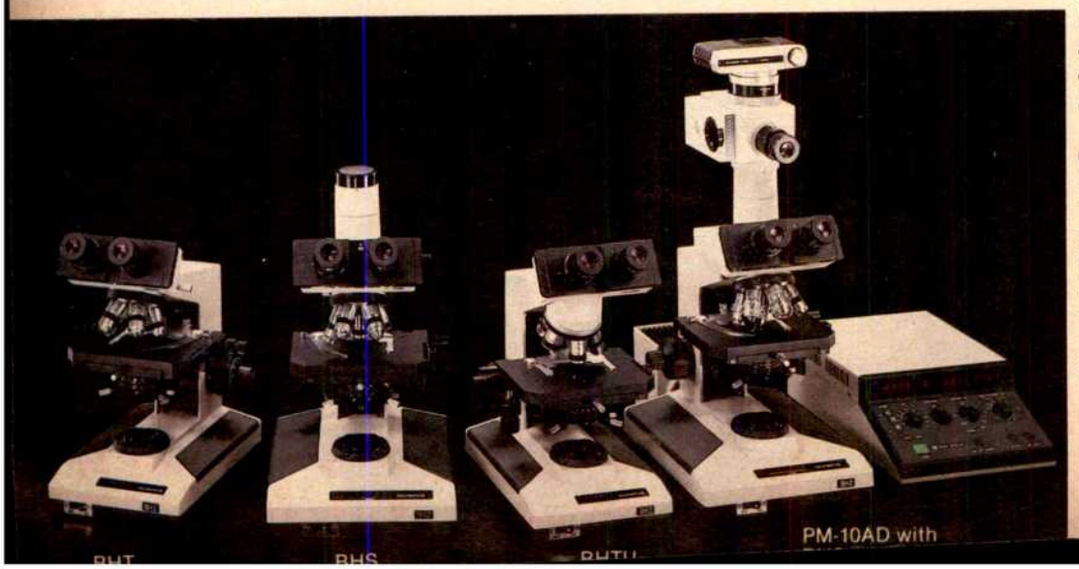
Indeed, microscope users in all scientific and technological areas will find the BH2-MDO an important new tool for instruction, consultation and training.

Special Features:

- All five viewers see the image in the same orientation. Magnification factor is 1x. The field of view is the same for each observer. A unique feature of this attachment, this is particularly useful for instructors and students and represents a major advance over what has hitherto been available.
- Thanks to superior optics and a special coating, every observation tube provides the same uniformly bright image.
- Phase contrast observation is consistently bright up to 40x magnification.
- As with our dual observation attachment, a moveable illuminated pointer appears either green or yellow in the field of view to provide maximum contrast with the specimen stain so the area under discussion can be pointed out easily and clearly.
- The distance between the observation tubes is such that viewers can observe the specimen comfortably, without interfering with each other.

AN ADVANCE IN SCIENTIFIC ACHIEVEMENT

The BH2-MDO–Now, five persons can view simultaneously



The BH2-MDO Multi-viewing Attachment is an advance in scientific achievement—an advance that means greater efficiency.

OLYMPUS

Match your skills with ours.

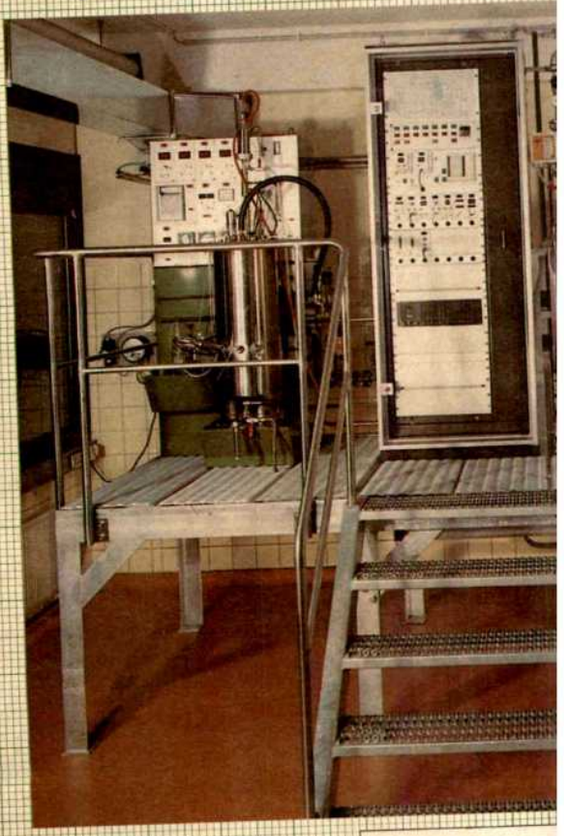
OLYMPUS OPTICAL CO., LTD.
San-Ei Building, 22-2, Nishi Shinjuku 1-chome, Shinjuku-ku, Tokyo
OLYMPUS OPTICAL CO. (EUROPA) GMBH
Postfach 104908, Wendenstrasse 14-16, 2 Hamburg 1, West Ger
OLYMPUS CORPORATION
4 Nevada Drive, Lake Success, N.Y. 11042-1179, U.S.A.

Circle No. 16 on Reader Service Card.

rm ..t tio..
tailore
your speci

You get better results in laboratory and production scale fermentations by using equipment with incorporated know-how. And when this equipment is tailored to your special tasks, you'll be another step ahead.

B.
ar
th
y
e

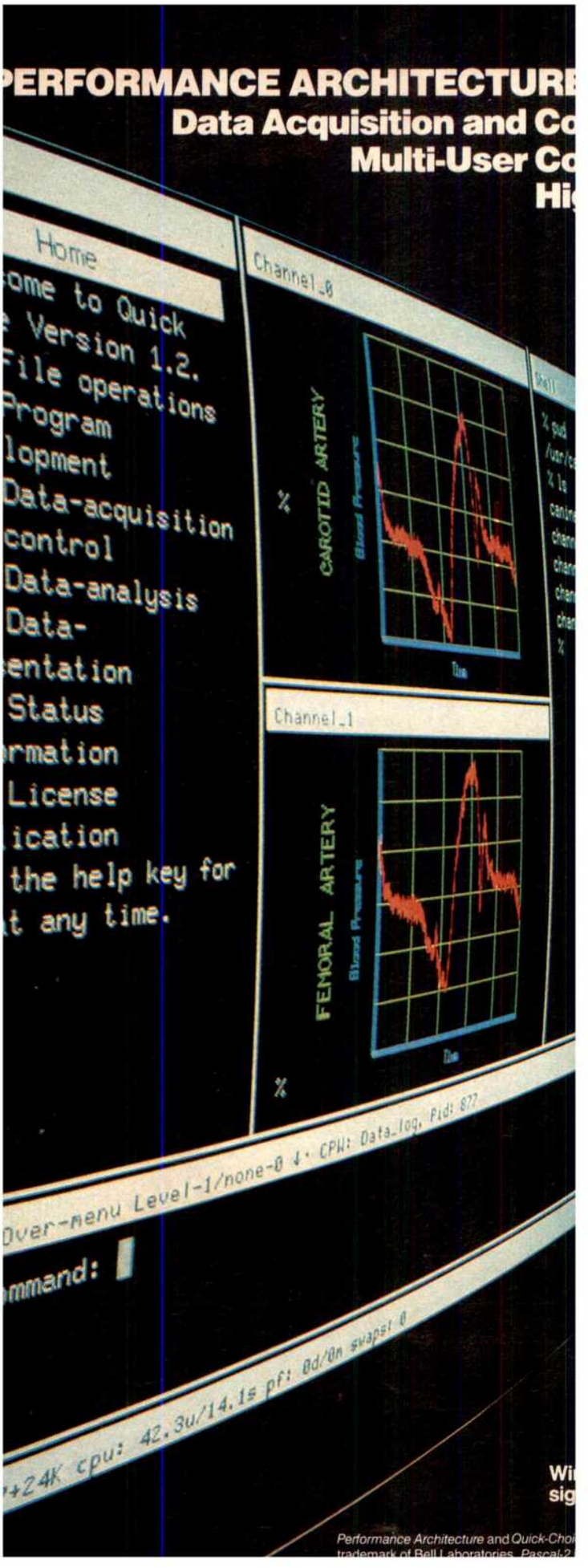


Pictured above:
Custom made
BIOSTAT® 300 D
and BIOSTAT® U 30 D.



B. Braun

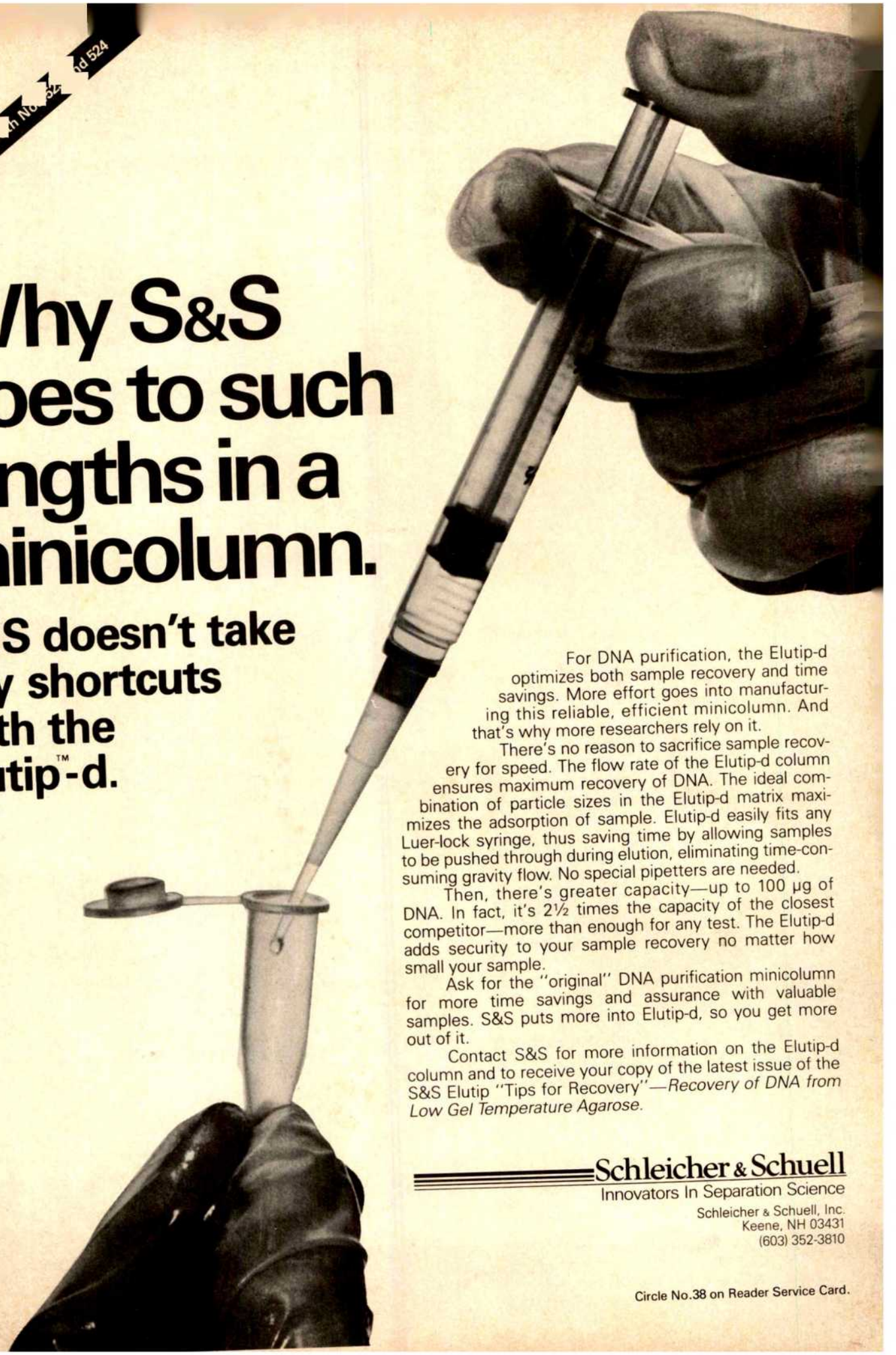
P.O.B. 110 + 12
D-3508 Melsung
Telephone: (0 5
Telex: 9 9 937 b
Cable: INTERB


ERFORMANCE ARCHITECTURE
Data Acquisition and Co
Multi-User Co
Hi
Home
ome to Quick
Version 1.2.
File operations
Program
lopment
Data-acquisition
control
Data-analysis
Data-
entation
Status
rmation
License
ication
the help key for
t any time.
Channel_0
CAROTID ARTERY
Blood Pressure
Time
%
Channel_1
FEMORAL ARTERY
Blood Pressure
Time
%
Over-menu Level-1/none-0 4. CPU: Data_log, Pid: 87?
mmand:
+24K cpu: 42.3u/14.1s pf: 0d/0n swaps: 0
Wi
sig
Performance Architecture and Quick-Choi
trademark of Bell Laboratories. Pascal-2

ee us a... Booth No. ...d 524

Why S&S goes to such lengths in a minicolumn.

S&S doesn't take any shortcuts with the Elutip™-d.



For DNA purification, the Elutip-d optimizes both sample recovery and time savings. More effort goes into manufacturing this reliable, efficient minicolumn. And that's why more researchers rely on it.

There's no reason to sacrifice sample recovery for speed. The flow rate of the Elutip-d column ensures maximum recovery of DNA. The ideal combination of particle sizes in the Elutip-d matrix maximizes the adsorption of sample. Elutip-d easily fits any Luer-lock syringe, thus saving time by allowing samples to be pushed through during elution, eliminating time-consuming gravity flow. No special pipetters are needed.

Then, there's greater capacity—up to 100 µg of DNA. In fact, it's 2½ times the capacity of the closest competitor—more than enough for any test. The Elutip-d adds security to your sample recovery no matter how small your sample.

Ask for the "original" DNA purification minicolumn for more time savings and assurance with valuable samples. S&S puts more into Elutip-d, so you get more out of it.

Contact S&S for more information on the Elutip-d column and to receive your copy of the latest issue of the S&S Elutip "Tips for Recovery"—*Recovery of DNA from Low Gel Temperature Agarose.*

Schleicher & Schuell
Innovators In Separation Science

Schleicher & Schuell, Inc.
Keene, NH 03431
(603) 352-3810

Circle No.38 on Reader Service Card.

INTRODUCING

The first commercially available standardized nucleic acid and immunological reagents for oncogene research...



oncor® Hybridization Probe DNAs

- *Pabl*•1™
- *Pmos*•1™
- *Pmyb*•1™
- *Pmyc*•1™
- *Pras*•1™ (v-Ha)
- *Pras*•2™ (v-Ki)

oncor® Immunological Reagents

- AB*myc*•1™
- AB*src*•1™
- AB*ras*•1™
- AB$_{EGF\text{-}R}$ •1™

To receive complete details on our new product line, and the premier issue of **ONCOS**—the newsletter for participants in contemporary cancer biology—fill out the reader service card or write to **ONCOR, Inc.,** P.O. Box 870, Gaithersburg, MD 20877.

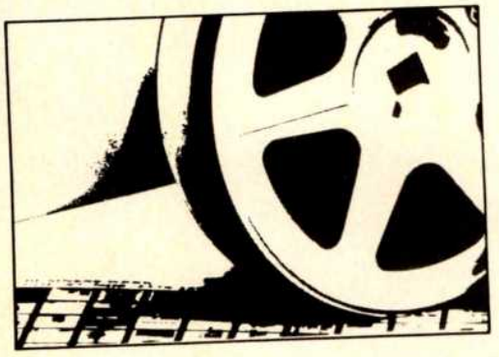
Pmyc•1 and AB*myc*•1 are trademarks of Oncor, Inc.

oncor®

oncor, inc. • P.O. Box 870 • Gaithersburg, MD 20877 • 301/963-3500

Circle No.25 on Reader Service Card.

nature
is available in Microform.



University Microfilms International

Please send additional information

for _____ (name of publication)

Name_____

Institution_____

Street_____

City_____

State_____ Zip_____

300 North Zeeb Road, Dept. P.R., Ann Arbor, Mi. 48106

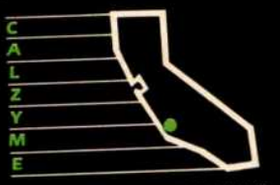
Circle No.66 on Reader Service Card.

CALZYME
LABORATORIES, INC.

Manufacturers of enzymes and related biochemicals, human proteins, and enzymatically prepared substrates of the highest possible purity.

With confidential agreements, offers custom work scaling up your procedures from laboratory scale to industrial scale.

3443 Miguellto Court
San Luis Obispo, CA 93401
(805) 541-5754 Telex: 4994640

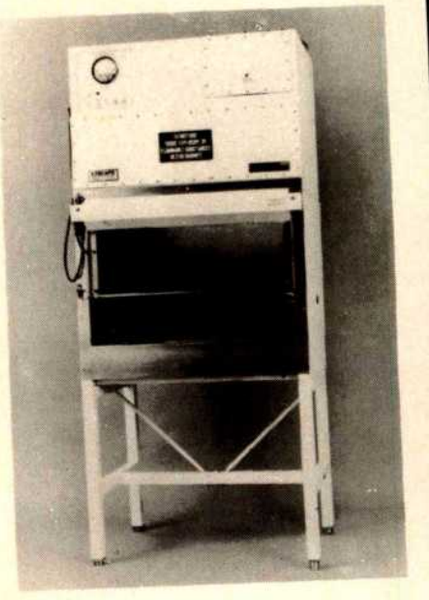


LOCATED ON THE CENTRAL COAST
BETWEEN SAN FRANCISCO AND LOS ANGELES

Write or phone for free catalogue

Circle No.18 on Reader Service Card.

IF YOU NEED A BIOLOGICAL SAFETY CABINET, THEN YOU NEED A NUAIRE!



"WE'RE QUIETLY BECOMING THE LEADER"

nuAire, inc.

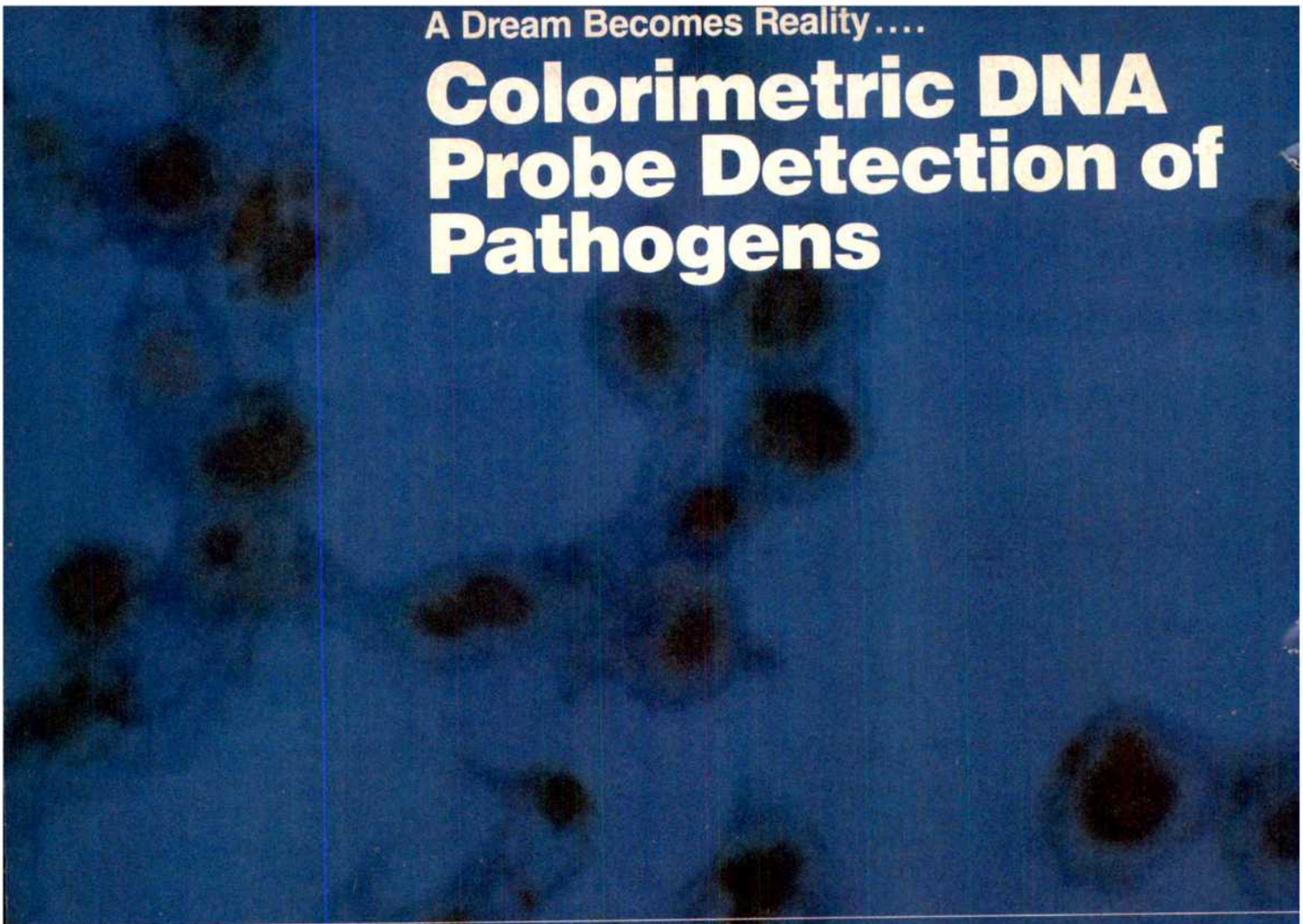
NOW... CALL TOLL FREE **800-328-3352** IN MINNESOTA **(612) 553-1270**

2100 FERNBROOK LANE, PLYMOUTH, MINNESOTA 55441 TELEX 29-0781

Circle No.11 on Reader Service Card

A Dream Becomes Reality....

Colorimetric DNA Probe Detection of Pathogens



Visualization of Herpes virus using an Enzo Patho-Gene™ Kit.

Introducing....

Enzo Patho-Gene™ Kits

For detection of HSV I & II, CMV, EBV, HBV, and Adenovirus.

For years researchers have sought a means for recognition of unique genes as the ultimate method of pathogen identification.

Now, for the first time, this dream is realized. The direct detection of specific viral DNAs can be quickly and safely carried out with Enzo Biochem's Patho-Gene™ Kits, the newest additions to The Enzo Bio-Probe™ System. Currently, kits are available for the identification of Herpes Simplex Virus I and II, Cytomegalovirus, Epstein Barr Virus, Hepatitis B Virus, and Adenovirus 2.

Patho-Gene™ Kit detection is based on DNA hybridization and visualization of hybridized probe DNA by color producing enzymes. The viral DNA probe is tagged with biotin a safe, stable reporter molecule. Upon hybridization to viral target DNA, detection is accomplished using Enzo's streptavidin-horseradish peroxidase detection complex.

The kit provides ready to use biotinylated probe DNA and enzymatic detection complex.

Enzo Patho-Gene™ Kits are for research purposes only.

Herpes Simplex Virus I and II Patho-Gene™ Kit	EBP-HSV-855
Cytomegalovirus Patho-Gene™ Kit	EBP-CMV-852
Hepatitis B Virus Patho-Gene™ Kit	EBP-HBV-853
Epstein Barr Virus Patho-Gene™ Kit	EBP-EBV-854
Adenovirus 2 Patho-Gene™ Kit	EBP-AdV2-851

ENZ

Enzo Biochem, Inc.
325 Hudson Street
New York, N.Y. 10013

Toll-Free: (800) 221-7705
In N.Y.: (212) 741-3838
Telex: ENZOBIO 424683


AnalaR
BDH 1934~1984
50 years
of the gold standard
sting symbol of quality and purity was founded – AnalaR.
ly became accepted as the internationally recognised
nalytical reagents. Today in its Golden Jubilee year of
over 400 AnalaR reagents, of guaranteed purity and
ole through BDH, the largest and most research
acturer of laboratory chemicals
Britain.
112 4NN. Tel: 0202 745520.
ETRADOME POOLE.
BDH
AnalaR
BDH Chemicals Ltd Poole England
Nitric acid sp. gr. 1.42

From Pharmacia
Two Power Supplies
500 Volts and 3000 Volts

for all your gel electrophoresis needs

ECPS 3000/150

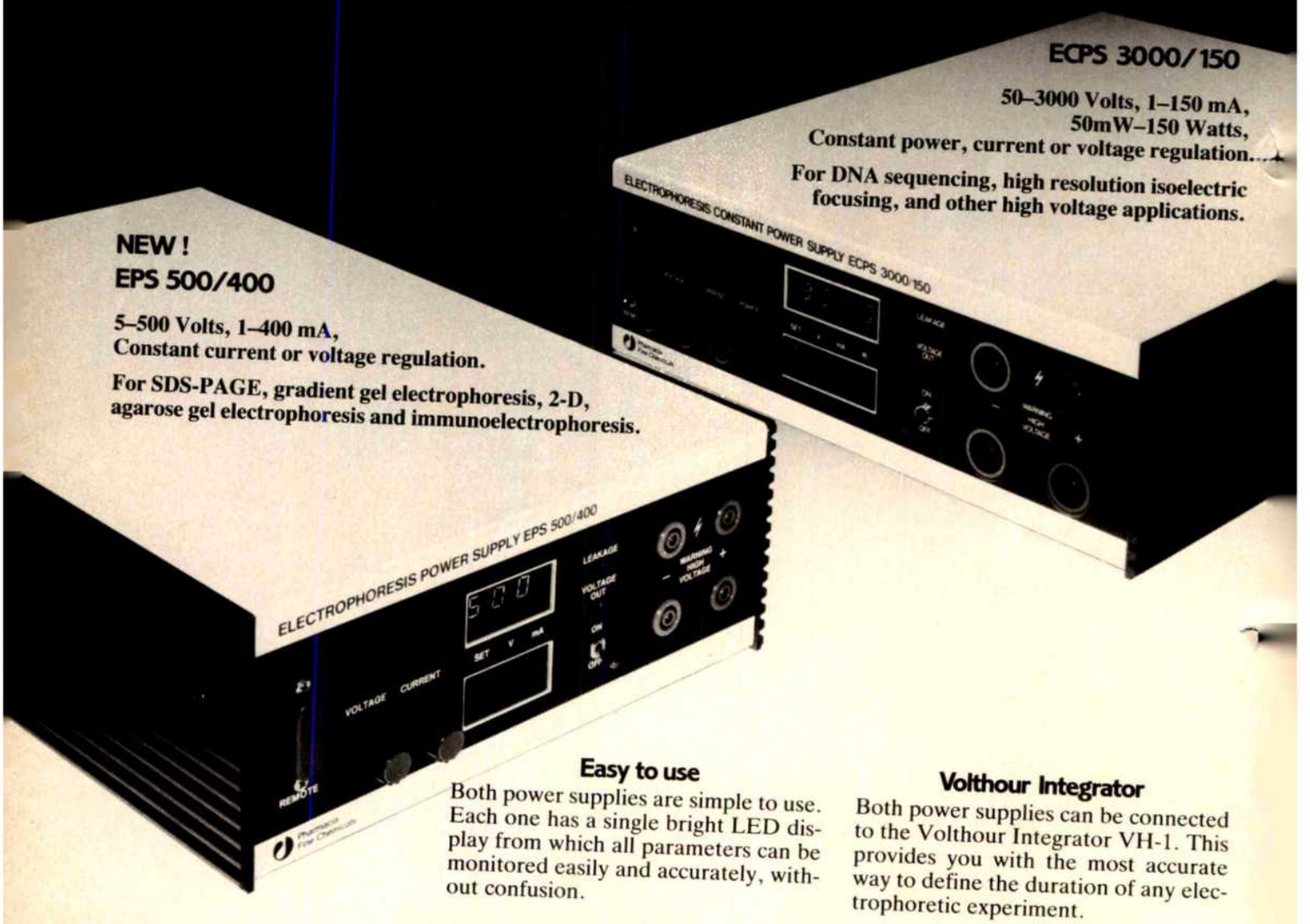
50–3000 Volts, 1–150 mA,
50mW–150 Watts,
Constant power, current or voltage regulation.

For DNA sequencing, high resolution isoelectric focusing, and other high voltage applications.

NEW !
EPS 500/400

5–500 Volts, 1–400 mA,
Constant current or voltage regulation.

For SDS-PAGE, gradient gel electrophoresis, 2-D, agarose gel electrophoresis and immunoelectrophoresis.



Easy to use

Both power supplies are simple to use. Each one has a single bright LED display from which all parameters can be monitored easily and accurately, without confusion.

Safe

When working at high voltages, operator safety is of prime concern. Both power supplies incorporate inbuilt safety features which include a ground leakage circuit, and a unique load fault detector.

Volthour Integrator

Both power supplies can be connected to the Volthour Integrator VH-1. This provides you with the most accurate way to define the duration of any electrophoretic experiment.

For further information on the Pharmacia Power Supplies and the VH-1 contact your local Pharmacia representative.

Pharmacia Fine Chemicals AB
Box 175
S-751 04 Uppsala 1
Sweden

Pharmacia Fine Chemicals

Circle No.44 on Reader Service Card.

Raven Press

The definitive textbook of basic immunology... a landmark contribution....

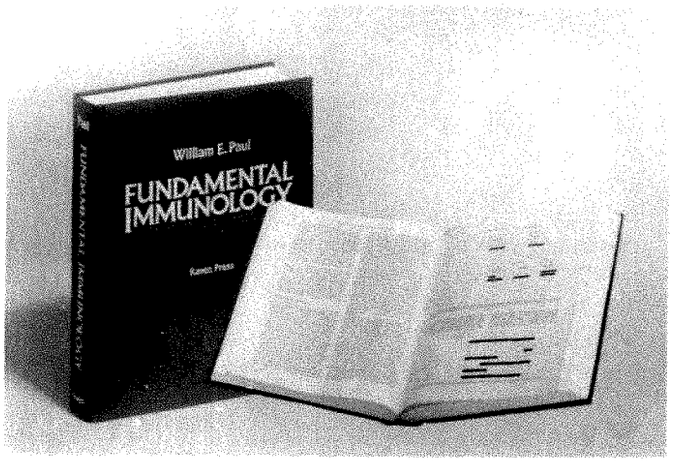
PAUL'S FUNDAMENTAL IMMUNOLOGY

Edited by
William E. Paul, M.D.
Chief, Laboratory
of Immunology
National Institutes
of Health
National Institute of
Allergy and Infectious
Diseases
Bethesda, Maryland /
U.S.A.

The unprecedented growth of the field of immunology has created a need for a definitive fundamental text—a source that can serve as a complete introduction for the student and a detailed, sophisticated reference for the researcher. FUNDAMENTAL IMMUNOLOGY is the first book available to meet this need. Under Dr. Paul's superb editorial direction, a roster of experts sets forth the key concepts of contemporary immunology.

The book begins with a coherent discussion of the organization of the immune system and the history of immunology. Building on this framework, the text offers thorough coverage of:

- cells of the immune system
- antibodies and receptors
- immunogenetics
- regulation of the immune response
- effector mechanisms of immunity



Reflecting the contributions of modern genetics and cell biology, the authors describe such methods as hybridomas and monoclonal antibodies, cellular identification and separation, and long-term culture of immunocompetent cells.

No other source provides such intensive coverage of the basic issues of modern immunology. Whether you're a newcomer to this exciting discipline or a researcher seeking an up-to-date refresher, this landmark text is a book you will find indispensable.

1984 / 880 pp. / 206 illus. / 165 tables / $80.00
Order Code 1307

(Note: Price slightly higher outside the U.S.)

Contents

Foreword, *B. Benacerraf*

Introduction: The Immune System: An Introduction, *W. E. Paul* / The History of Immunology, *A. M. Silverstein*

The Cells of the Immune System: B Lymphocytes, *M. D. Cooper, J. Kearney, and I. Scher* / T Lymphocytes, *H. Cantor* / Macrophages and Related Cell Types, *E. M. Shevach* / Lymphoid Tissues and Organs, *E. C. Butcher and I. L. Weissman*

Antibodies and Receptors: Immunoglobulins: Structure and Function, *D. J. Jeske and J. D. Capra* / Immunoglobulins: Molecular Genetics, *E. E. Max* / Immunoglobulins: Allotypes and Idiotypes, *J. B. Fleischman and J. M. Davie* / B Lymphocyte Receptors, *E. S. Vitetta, K. Brooks, P. Isakson, J. Layton, E. Puré, and D. Yuan* / T Lymphocyte Receptors, *C. A. Janeway, Jr., R. E. Cone, and F. L. Owen* / Lymphocyte Activation, *R. F. Ashman*

Immunogenetics: The Major Histocompatibility Complex, *D. H. Sachs* / Major Histocompatibility Complex Antigens, *T. J. Kindt and M. A. Robinson* / Regulation of Cellular Interactions by Major Histocompatibility Complex Gene Products, *R. H. Schwartz* / Immune Response Genes, *W. E. Paul* / Lymphocyte Antigens, *P. M. Hogarth and I. F. C. McKenzie*

Regulation of the Immune Response: Help, Suppression, and Specific Factors, *T. Tada* / Control of the Production of Different Classes of Antibody, *J. M. Teale and N. R. Klinman* / Immunologic Tolerance, *G. W. Siskind* / Lymphokine Regulation of T-Cell and B-Cell Function, *K. A. Smith* / Idiotypic Networks, *C. A. Bona and B. Pernis*

Effector Mechanisms of Immunity: Antigen-Antibody Interaction, *J. A. Berzofsky and I. J. Berkower* / Complement, *E. J. Brown, K. A. Joiner, and M. M. Frank* / Cell-Mediated Cytotoxicity, *C. S. Henney and S. Gillis* / Delayed Hypersensitivity, *M. I. Greene, S. Schatten, and J. S. Bromberg* / Mediators: Release and Function, *C. W. Parker*

Critical Techniques for the Study of the Immune System: Hybridomas and Monoclonal Antibodies, *J. F. Kearney* / Cellular Identification and Separation, *I. Scher and M. G. Mage* / Long-Term Culture of Immunocompetent Cells, *C. G. Fathman and F. W. Fitch*

Order Today From:
Raven Press

Please send on 30-day approval:

___ copies FUNDAMENTAL IMMUNOLOGY
Paul, editor / $80.00
Order Code 1307

☐ To save postage and handling charges, I enclose payment (or charge my credit card below). N.Y. residents add sales tax.

Check one: ☐ American Express ☐ VISA ☐ Diners Club
☐ MasterCard 4-digit interbank no.: ____ ____ ____ ____

Credit card number: _____ Exp. date: _____

Signature: _____

☐ Bill me—cost plus postage and handling.

Name (please print) _____

Address _____

_____

Country _____ Postal Code _____

L7

Prices subject to change without notice. Prices slightly higher outside the U.S. and Canada.

Raven Press 1140 Avenue of the Americas, New York, N.Y. 10036 U.S.A.

Circle No. 15 on Reader Service Card.

INTRODUCING

Centricon™

THE INGENIOUS NEW DISPOSABLE MICROCONCENTRATOR

1. Fast

Pipette your 1-2ml sample into the top reservoir and spin in a centrifuge. That concentrates the sample down to 25-40µl in less than one hour—with no danger of spinning to dryness.

2. Simple

Then invert the top half and spin again briefly. That brings your retentate out where you want it—perfectly accessible in the cone-shaped cup.

3. Efficient

You get high recovery. Desalting or buffer exchange is performed in the same Centricon simultaneously with concentration. And all with no special equipment, no awkward procedures, no cleanup. Centricon is disposable.

Centricon is ideal for concentrating small biological samples containing proteins, peptides, nucleic acids, viruses, bacteria, and other macromolecular solutions. Send for the complete story on this unique new timesaver. Or call Technical Service: 800-343-1397.



Patent Pending

amicon

Scientific Systems Division Cherry Hill Drive. Danvers, MA 01923
To order call: 800-343-0696 (In MA and international:617-777-4550)

Visit AMICON at ASM Booth 126-128 and FASEB Booth A42-A46.



Sometimes a good idea begins with a product or service you didn't know about. That's where our technical representatives can help.

There's always a possibility that something you're doing could be accomplished faster, or better, or more efficiently if you had the needed information.

Often enough your NEN representative has that information—whether it be a new method, a better product, or one of NEN's extra services.

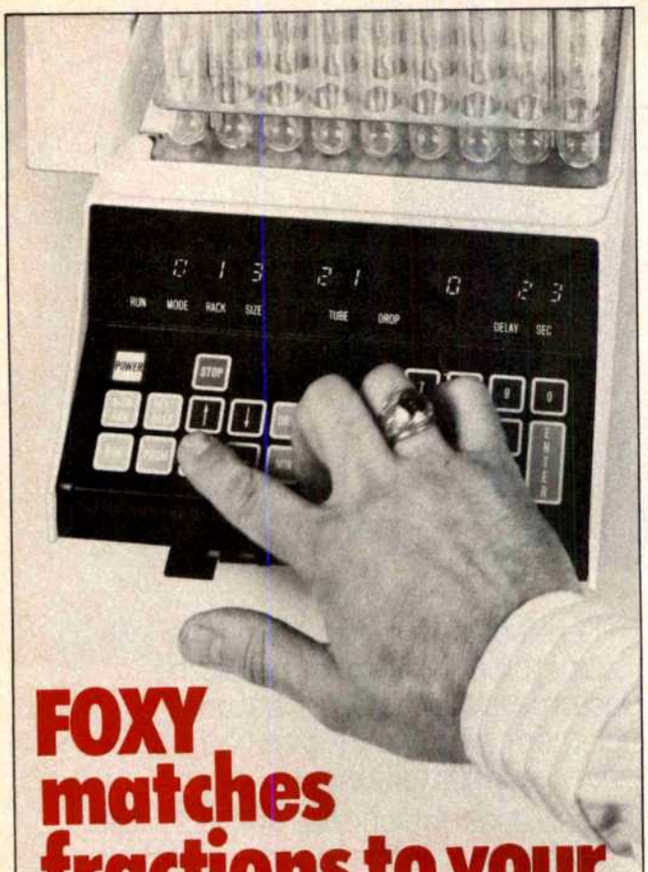
Just ask. The answer will come from solid training and experience. More detailed technical answers are quickly available, too. Our representative will see that the question is handled by the most appropriate of several sources at NEN, such as our Technical Services Group, who specialize in customer assistance.

But first talk with the person who represents it all. A good idea may be only a conversation away.

NEN New England Nuclear®

DUPONT

Circle No.10 on Reader Service Card.



FOXY matches fractions to your chromatogram.

Not to the clock.

Program your collection by absorbance peaks, retention times, timed intervals, counted drops, or pumped volumes. You can even enter fraction size directly in ml if you have an ISCO WIZ peristaltic pump. FOXY rejects void volumes, locates and saves desired peaks, controls valves and pumps to make gradients or automate your system, and changes racks and volumes according to peaks or time. For HPLC, collect successive injections without resetting, and overlay repeated injections of the same sample to collect identical peaks in the same tubes. The sloped keyboard, 12-digit display, and logical programming make it all easy to do.

FOXY takes practically any size test tube, plus 28 mm scintillation vials and even 500 mm bottles. It holds up to four columns and 288 tubes on just a narrow slice of your bench. The most capable fraction collector ever made costs less than you'd think. For a complete catalog, write: ISCO, Box 5347, Lincoln, NE 68505, U.S.A.

See us in booths 9017-10018 at Pittsburgh Conference

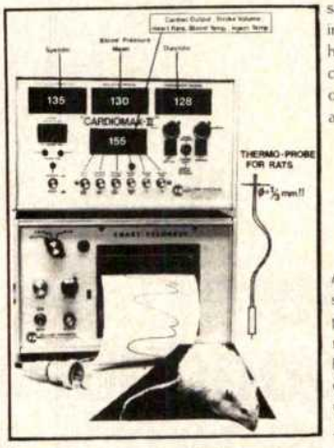
Instruments with a difference

Circle No.61 on Reader Service Card.

RATS!!!
CARDIAC OUTPUT BY THERMODILUTION

"CARDIOMAX-II-R" is the only computer which can measure cardiac output of rats (also guinea pigs, rabbits, dogs, chickens, horses and humans). For rats, it uses unique Columbus Instruments thermodilution microprobes **1/3 mm in size!** It also measures systolic, diastolic and mean blood pressures and heart rate up to 1000 beats per minute. Stroke volume is also automatically computed.

Why Measure Cardiac Output of Rats?



Development of salt sensitive and salt resistive strains of rats provided inexpensive models for studying hypertension. Dynamics of rat circulatory system is similar to dogs or humans. Studies on rats cost only a fraction of studies using dogs.

Automatic Measurements Without Supervision

All parameters (cardiac output, stroke volume, three blood pressures, blood temperature, heart rate, time of day) can automatically be measured periodically and printed when using **"Cardiomax-II-R"** with Columbus Instruments Automatic Injector and Printer.

For more information, call or write to:

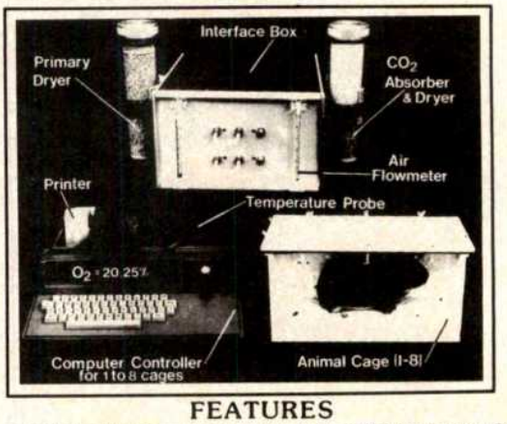
COLUMBUS INSTRUMENTS INTERNATIONAL CORPORATION

950 N. HAGUE AVE., COLUMBUS, OHIO 43204 U.S.A.
PHONE: (614) 488-6176 TELEX: 246514

Circle No.05 on Reader Service Card.

O_2-consumption
CO_2-production
Respiration Rate

Simultaneously measured in 1 to 8 animal cages



FEATURES

• One controller for 1 to 8 animal cages • Continuous measurement of O_2, CO_2 and Respiration Rate over a 24 hour period in ml/kg of body weight/hour in STP units. • Printer prints results every four (4) minutes for each of 1 to 8 animals as well as oxygen percent level in each animal cage • System adaptable to small or large animals • Continuous airflow through system with oxygen level measurements at the output and input • High accuracy, >98% of full measuring range e.g. typical error ±30 ml oxygen/kg/hour in 3000 ml oxygen/kg/hour range • User adjustable alarm warns user of possible hypoxia • Reuseable absorbants • Auto reference assures high accuracy, system periodically measures ambient air and corrects itself • Optional RS-232 interface for communication with other computers • Insensitive to temperature variations in the animal cage.

Columbus Instruments also supplies monitors for: Animal Activity, Cardiac Output, Drinking and Feeding, Respiration Rate and Startle Response.

For more information, call or write to:

COLUMBUS INSTRUMENTS INTERNATIONAL CORPORATION

950 N. HAGUE AVE., COLUMBUS, OHIO 43204 U.S.A.
PHONE: (614) 488-6176 TELEX: 246514

Circle No.06 on Reader Service Card.

GIVE YOUR T-CELLS ONE TO GROW ON.

Our Human T-Cell Growth Factor (Interleukin-2) makes the best sense of all when you want to achieve active, longterm proliferating growth of T-lymphocytes every time.

It's prepared from large pools of human macrophage-depleted lymphocytes stimulated by PHA. And its activity is enhanced by using active macrophages and by the intensity of the mixed lymphocyte reaction.

We offer three TCGF preparations. Crude. 10X Crude. And Lectin-Free. Each can be relied upon to produce quickly growing T-Cell populations every time.

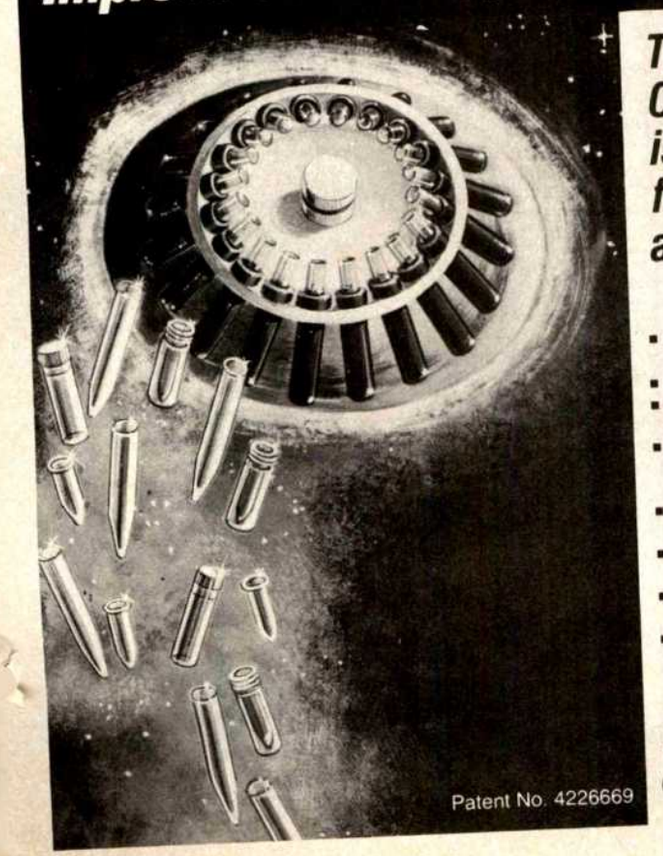
Call us toll free to place a trial order. Or ask for our free brochure.

THE ENZYME CENTER, INC.
36 Franklin St. Malden, MA 02148
1-800-343-2170

©Enzyme Center, Inc., 1982.

Circle No.19 on Reader Service Card.

Improve Your Assays in Any Biochemical Procedure!



The Savant "NO BUMP" Speed Vac Concentrator/Evaporator is now standard procedure for the following and many other applications.

Achieve 100% Sample Recovery!

- **HPLC** sample preparation where a dried, concentrated sample is important.
- **GLC** sample preparation . . . as above.
- **DNA** sequencing — evaporation of piperdine.
- **PHOSPHOLIPID** separation by HPLC — total evaporation of Acetonitrile-Water Solvent.
- **TRIETHANOL AMINE** carbonate evaporation.
- **SOMATOSTATIN** concentration by evaporation of acetic acid.
- **INTERFERON** purification by dialysis and evaporation of solvent phase.
- **TRI-CYCLIC** anti-depressant drug blood levels measured after a series of extractions and evaporations to dryness.
- **NEUROTRANSMITTERS** such as Acetylcholine, Dopa, etc. Now include the Speed Vac Concentrator for the essential evaporation step.
- **AMINO ACID** and Peptide analysis.
- **SUBSTANCE P** concentration and assay.
- **HORMONE** and Enzyme assays.
- **TISSUE EXTRACTS** and Column eluates concentrated by "NO BUMP" solvent removal.
- **ELECTROPHORESIS AND CHROMATOGRAPHY** is greatly improved by concentration prior to application of sample to the support medium.

For additional information and literature contact:

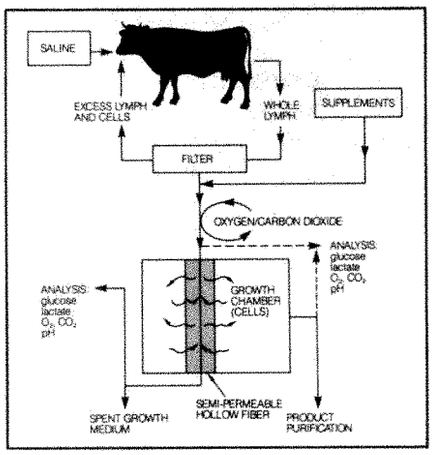
Savant

SAVANT INSTRUMENTS, INC.
221 Park Ave., Hicksville, NY 11801
(516) 935-8774

Circle No.03 on Reader Service Card.

NATURE'S BIOREACTOR

The New Bio-Response MCT* (Mass Culturing Technique) System Delivers Monoclonal Antibodies And Other Mammalian Cell Products When You Need Them—From Grams To Kilograms.



Insufficient quantities of desired peptides are a key cause of expensive research and production delays. The MCT system is a *new* and *unique* mammalian cell culturing process designed to overcome the obstacle of insufficient quantities of peptides by delivering the quantity you need...*when you need it!* Additionally, every step in MCT production has been developed to contain costs now and in the future while maintaining exceptional purity and quality in mammalian cell proteins.

Quantity

Unlike conventional cell-culture methods, the MCT system uses "fresh," free-flowing lymph directly from a cow. Following treatment, the lymph is continuously diffused into and out of a growth chamber. This nearly *in vivo* growth environment stimulates continuous and optimal protein secretion. The result? Large quantities of desired proteins enabling you to move to the clinic or market...*faster.*

Cost

Unlike conventional cell-culture methods that require extensive labor forces and expensive growth mediums, Bio-Response's MCT is a closed, steady-state system. In essence, the MCT system starts with a low-cost lifeline—a feeding cow—and ends with a pure, quality product. MCT ...*an optimal system at low cost.*

Purity

Unlike conventional cell-culture methods, the MCT system is designed to insure purity without tedious processing. By directing the lymph across semi-permeable membranes, MCT provides both separation of media protein from desired cell products, and a continuous nutrient flow. The ability to add or withdraw from the chamber to maintain optimal cell density permits continuous harvest of a highly pure product.

Quality

Unlike conventional methods of production that attempt to create biologically active mammalian cell products, the MCT system provides a pure-mammalian product—an exact copy of the original protein and, in the case of non-antibody polypeptides, uncompromised by inappropriate glycosylation and disulfide bond formation. There is a growing consensus favoring products produced in a mammalian cell-culture system, such as the Bio-Response MCT system.

Circle No.14 on Reader Service Card.

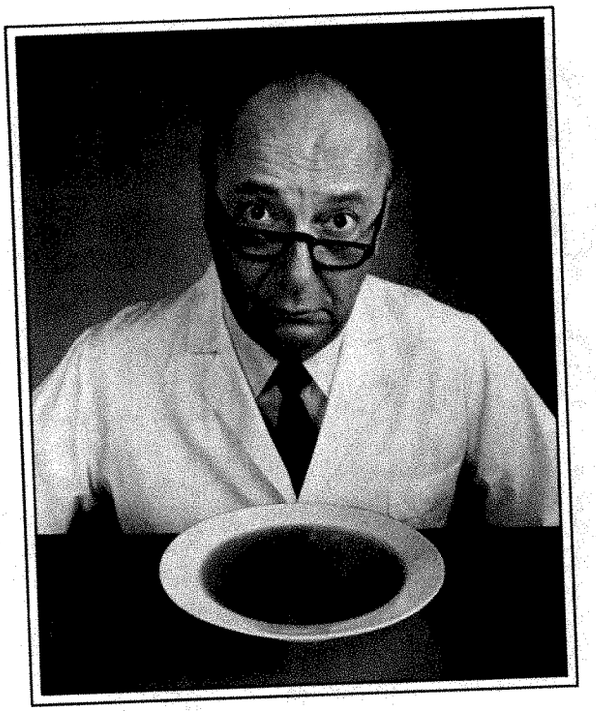
For further information, call Bio-Response, Inc. at (415) 786-9744.

BIO-RESPONSE, INC.

*MCT (Mass Culturing Technique) is a trademark of Bio-Response, Inc.

© 1984 Bio-Response, Inc. All Rights Reserved.

Peptide Soup.



Peptide Soup. That's all you'll get if you use inferior bioactive peptides in your research.

Avoid costly, time-consuming, and embarrassing errors by using quality bioactive peptides from Vega Biotechnologies, Inc.

Vega's peptides are subjected to multiple testing — HPLC, TLC, Amino Acid Composition, and Optical Rotation — to assure their purity. Typical specifications include 97-99% purity by HPLC, Amino Acid Analysis ±4% of theory, and Optical Rotation ±5% of literature value.

A Certificate of Analysis is available with each lot to verify the test results. This certificate also assures you that Vega bioactive peptides meet our strict quality control standards.

Vega synthesizes its own peptides using the highest quality reagents. We routinely check purity at frequent intervals during inventory storage. This ensures that product quality is maintained on each lot produced.

To show you how confident we are of our purity, Vega bioactive peptides are guaranteed. If you're not completely satisfied, we'll replace your order or refund your money in full.

As an added benefit, our efficient bulk manufacturing saves you time and money. The peptides you need are available when you need them... at significant savings. Peptides like CRF, GRF, Enkephalins, Substance P Analogs, Bradykinins, Pituitary peptides, Angiotensins, Neurotensins, and many others of current research interest.

Call us toll-free at 1-800-528-4882 and ask for a complete list of our bioactive peptides and biochemicals, all found in the 1983/84 Vega Catalog.

Vega bioactive peptides. They'll get you out of the soup and into consistent research results.

Vega Biotechnologies, Inc.
P.O. Box 11648, Tucson, AZ 85734
(602) 746-1401 (800) 528-4882 Outside AZ
TELEX: 165572 (VEGA BIO TUC)

The link between science and tomorrow.

VEGA™ BIOTECHNOLOGIES

Circle No.23 on Reader Service Card.

© 1983 by Vega Biotechnologies, Inc. All rights reserved.

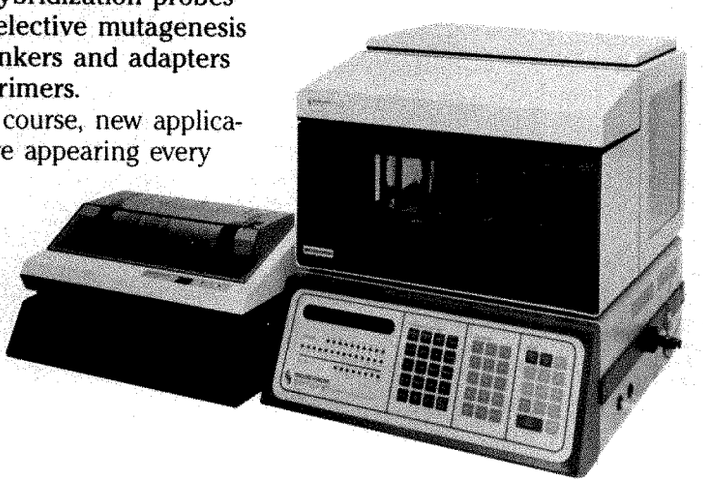
Tomorrow, synthetic DNA will be used as routinely as pipettes are today.

And Biosearch will supply it.

Biosearch is a leader in DNA synthesis, currently supplying synthetic oligonucleotides to molecular biologists who are using them as a routine research tool in such applications as:

- **whole gene synthesis**
- **hybridization probes**
- **selective mutagenesis**
- **linkers and adapters**
- **primers.**

And of course, new applications are appearing every day.



From Our Shelf . . . or Yours!

The question is, will you know where to turn when you need DNA?

To meet our own demand for synthetic DNA, Biosearch *more than a year ago* invented the fully-automatic, chemically-programmable SAM ONE DNA Synthesizer. Today, leading laboratories worldwide are using the SAM ONE to make DNA.

So when the time comes, your choice will be easy: you can order your custom DNA from us . . . or you can make it yourself—automatically.

We'll be ready when you are.

Synthetic chemicals for tomorrow . . . today!

BIOSEARCH

2980 Kerner Boulevard
San Rafael, California 94901
(415) 459-3907
Outside California: 800-227-2624
Telex: 703077 BIOSEARCH

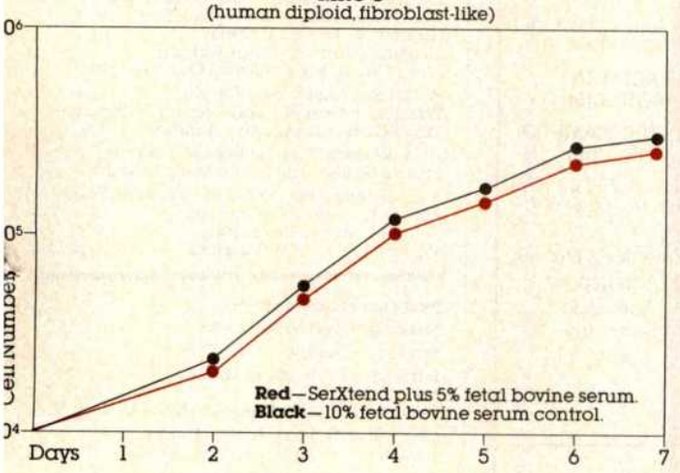
Circle No.26 on Reader Service Card.

Reduce serum requirements by 50%

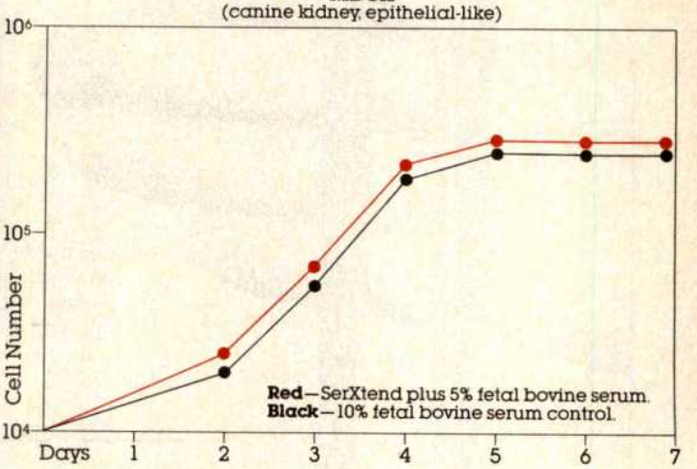
SerXtend™

DEFINED GROWTH FACTORS IN A CERTIFIED, CONSISTENT COMBINATION THAT CUT COSTS AND SAVE SPACE TOO.

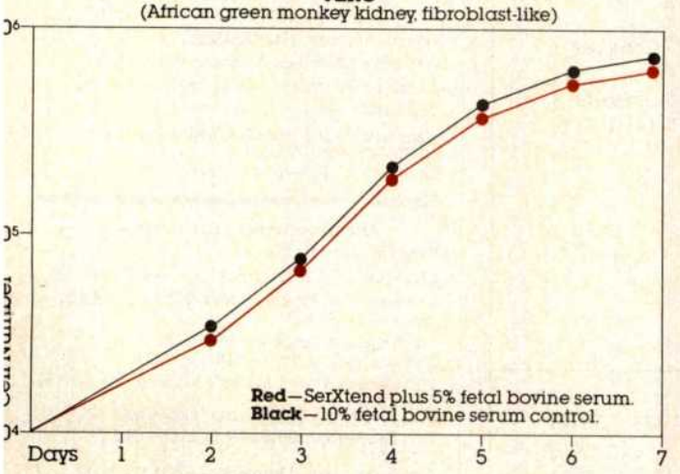
MRC-5
(human diploid, fibroblast-like)



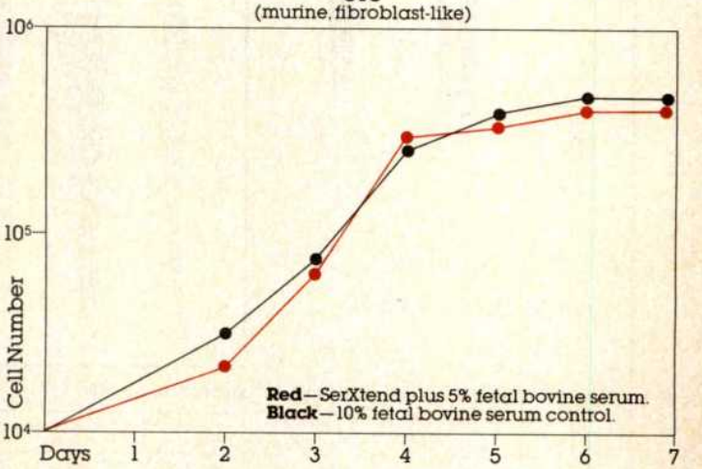
MDCK
(canine kidney, epithelial-like)



VERO
(African green monkey kidney, fibroblast-like)



3T3
(murine, fibroblast-like)



Data was generated using: Hyclone[1] Fetal Bovine Serum, lot 100427; Gibco[2] DMEM, lot 10N4930; and Falcon[3] 24-well cell culture plates. (Experimental data available upon request.)

[1]Hyclone is a trademark of Sterile Systems, Inc.
[2]Gibco is a trademark of Dexter Corporation.
[3]Falcon is a trademark of Becton-Dickinson, Inc.

For more information or to order, use our toll-free number:
800-772-4262

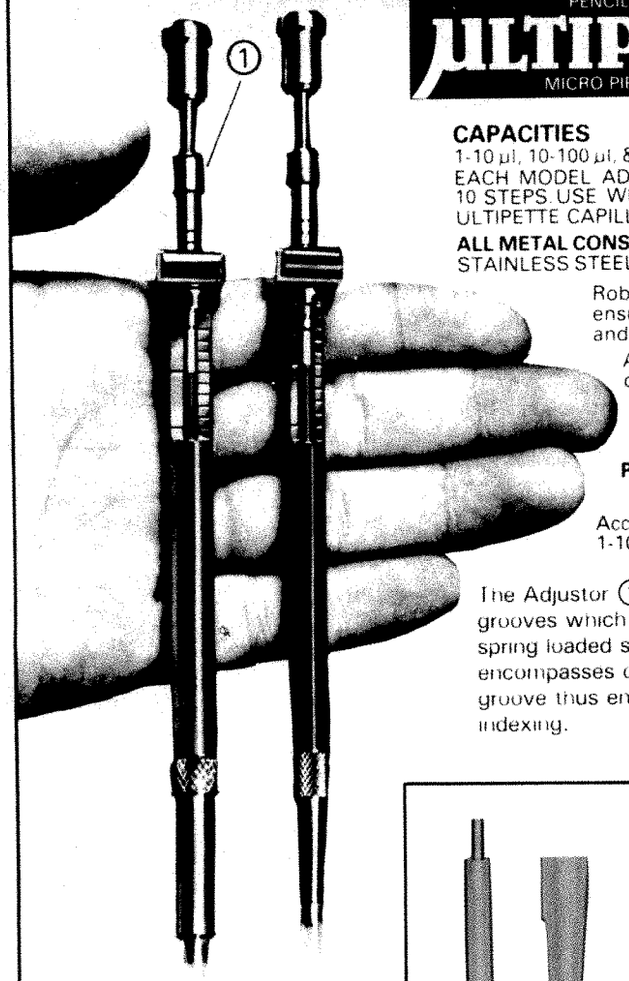
Hana Media, Inc.
A subsidiary of Hana Biologics, Inc.
626 Bancroft Way
Berkeley, California 94710 (415) 549-0874

Circle No.01 on Reader Service Card.

PENCIL SLIM

ULTIPETTE

MICRO PIPETTERS



CAPACITIES
1-10 µl, 10-100 µl, & 25-250 µl.
EACH MODEL ADJUSTABLE
10 STEPS USE WITH UNIQUE
ULTIPETTE CAPILLARY TIPS.

ALL METAL CONSTRUCTION;
STAINLESS STEEL & PLATED BRASS.

Robust Adjustors ensuring long accurate and trouble free life.

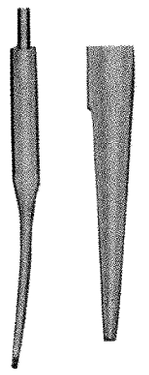
Autoclavable without dismantling.

EXCELLENT REPRODUCIBILITY

PIPETTER DIAMETER ONLY 7mm

Accuracy 25-250 µl ± 1%
1-10 µl + 10-100 µl ± 2%

The Adjustor ① has deep circular grooves which are engaged by a spring loaded slide. This slide encompasses one half of the groove thus ensuring really firm indexing.



Compare the Ultipette 1-10 Tip with the standard tapered tip

PS-250 **PS-100**

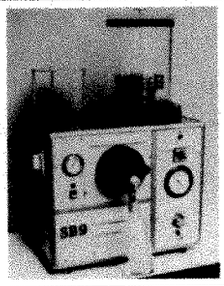
Table of determinations by an independent assessor

種別	PS-10型(1～10µℓ)			PS-100型(10～100µℓ)			PS-250型(25～250µℓ)	
	2 µℓ	5 µℓ	10µℓ	10µℓ	50µℓ	100µℓ	50µℓ	100µℓ
平均測定容量/8回	2.010875	5.02325	10.044375	10.045625	50.221875	100.43862	50.209375	100.42537
誤差 (%)	0.54	0.47	0.44	0.46	0.44	0.44	0.42	0.43
CV (%)	3.25	1.99	1.67	1.13	1.47	0.57	1.27	0.75

SB9 CENTRIFUGAL FREEZE DRYING

Economical Bench Top Unit

The S.B.9 is a 1 litre capacity Freeze Drier with a built in spin freeze facility. Standard carrier plates accommodate either 116 x 0.5 ml or 24 x 2.5 ml ampoules. A demountable shelf is provided for tray drying and a rubber vacuum value, located on the condenser window, provides for flask or bottle drying. The unit is suitable for general laboratory use or small scale production, and the visible condenser is of particular interest to teaching establishments. A vacuum pump is not provided as standard as many laboratories already have spare pumps available.



BARKY
INSTRUMENTS LIMITED
1a Warren Road, Folkestone, Kent CT19 6DE
Telephone: (0303) 50316

variable volumentrics, inc.
18 Cummings Park, Woburn
Massachusetts 01801
Telephone: (617) 933-8170
Telex: 949-329
Attn: Volumentrics

FUNAKOSHI PHARMACEUTICAL CO., LTD.
2-3 SURUGADAI, KANDA,
CHIYODA-KU, TOKYO, JAPAN.
PHONE: TOKYO (03) 293-2341

Circle No.31 on Reader Service Card.

EDITORIAL OFFICES

London Office
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024

Washington Office
1134 National Press Building, DC 20045
Telephone: (202) 737-2355

Tokyo Office
Eikow Building, 10-9, Hongo 1-chome, Bunkyo-ku, Tokyo 113
Telephone: (03) 816 3756/7 Telex: J32121 MSKK

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff: Tim Beardsley, Philip Campbell, Isobel Collins, Peter Gambles, Melanie Kee, Tim Lincoln, Naomi Molson, Sara Nash, Geoffrey North, Peta Pickering, Miranda Robertson, Robert Walgate, Charles Wenz, Nigel Williams, Guil Winchester

Washington Editor: Peter David
Washington Correspondent: Stephen Budiansky
Tokyo Correspondent: Alun Anderson

Art and Design: Joan Godwin, Joel Chernin, Theresa George, Paul Norris, Marie Xeridat

Administration: Mary Sheehan, Jacqueline Cole, Ruth Cutts, Joanna Czechowska, Ruth Goldenberg, Sharon Isaacs and Penny Walker (Washington)

Publisher: Elizabeth Hughes

Marketing Director: Ray Barker

Promotion Manager: Felicity Parker

International Advertising Manager: Andy Sutherland

Features Advertising Manager: Marion Delaney

Group Classified Advertising Manager: John Barnes — Telephone: (01) 240 1101

Nature New York Office
15 East 26 Street, New York, NY 10010
Telephone: (212) 689-5900 Telex: 668497

General Manager: Gary Rekstad

American Advertising Manager: Henry Dale

Classified Advertising Manager: Miss Gene Fein

Circulation Manager: Mirta Soto

Canadian Display and Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1—(416) 690-2423

Display Advertising Representatives

New York: Nature Office

Chicago: Didier & Broderick, Inc.—(312) 498-4520

San Francisco: Jobson/Jordan/Harrison/Schulz, Inc.—(415) 392-6794

Los Angeles & Pasadena: Jobson/Jordan/Harrison/Schulz, Inc.—(818) 796-9200

Philadelphia: Daniel Adams Associates, Inc.—(215) 353-6191

Boston: CEL Associates—(617) 848-9306

Fort Lauderdale: Brinker & Brinker—(305) 771-0064

New Jersey: Mary Grunmeier—(201) 572 9018

West Germany and Austria: Franz Schrecklinger TMW Top Media Werbegesellschaft GmbH Frankfurt—(611) 726046

Holland and Belgium: Adele Struyck G. Arnold Teesing BV Amsterdam—(020) 2636 15

Switzerland: Werner Stähli Agentur Iff AG Schaffhausen—(053) 45821

Scandinavia: Andrew Karnig Andrew Karnig & Associates AB Stockholm—(08) 51 68 70

Japan: Mashy Yoshikawa Orient Echo Inc, Tokyo—541-4923

Australia: Gus Bartel & Associates PO Box 1755, Southport, Queensland 4215—(075) 466 577

Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office
©1984 Macmillan Journals Ltd
Vol.307 No. 5952 23 February 1984

nature

Secrecy beyond reason

The Reagan Administration continues to apply widely its demand for secrecy in scientific matters that have any bearing on national security. It is time for more vocal opposition.

CAN only two years have passed since officials of the Reagan Administration were visiting scientific meetings and warning American researchers that academic science was "the soft underbelly" of national security? In 1982, the administration's apparent obsession with the notion that Soviet spies were tricking American scientists into giving away the nation's technological secrets set the government and the universities at loggerheads. In several celebrated cases, the universities (notably and creditably Stanford University in California) refused State Department requests to act as policemen when Soviet and Chinese scholars visited their campuses. To defuse the situation, the National Academy of Sciences published a report, *Scientific Communication and National Security*, which tried to find a reasonable compromise between the legitimate worries of the defence establishment and the traditional commitment of the United States to free and open communication in science. By 1984, technology transfer — at least insofar as it affects the universities — appears to have become the great forgotten issue of US science policy.

A famous victory for the National Academy of Sciences? Hardly. If little is heard about technology transfer, it is not because the Reagan Administration has promptly accepted the recommendations put forward by the academy's report. In a choice irony, the deafening silence from the White House is a product of a decision that the administration's response to the academy's report on scientific communication should itself be kept secret. Dr Frank Press, the academy's president, originally expected an early response from the White House science office. Later, the job was handed from the science office to a senior inter-agency group meeting under the aegis of the National Security Council. Now, the response is believed to be complete but it has been classified as secret.

It would be tempting to believe that there was little cause for concern. After all, most recent cases of government intervention in technology transfer have consisted of efforts to prevent Eastern bloc nations from illegally importing bits of American hardware, such as sophisticated computers. It was always the contention of the universities that technological transfers of that kind — rather than international communication in academic science — were the only really significant examples of hostile countries gaining militarily useful information. The National Academy panel, chaired by Cornell University's former president, Dale Corson, concluded that academic science was a relatively unimportant source of Soviet poaching, and that the dispute between the universities and the administration could be resolved by building tall fences around a very small number of areas of great sensitivity. The universities have been as good as their word; their voluntary agreement to submit papers on cryptography for prepublication screening has, for example, worked remarkably well.

There is however, little reason to suppose that the administration has decided to accept the general philosophy of the Corson report. The available evidence suggests the opposite. Far from agreeing to confine its attention to a small number of highly sensitive technologies, the administration appears to want to extend its control over scientific communication as far as possible. In a little-noticed recent directive, for example, the Department of Defense has begun to insist that all basic and applied contract research it sponsors in universities must be submitted for prepublication screening. Only if the research cannot be placed within a very loose definition of "sensitivity" can it be submitted for publication without pre-screening.

It is too early to say whether this new policy will result in the suppression of articles that could otherwise be published without compromising national security. One hopeful sign is that the Department of Defense has at last set up an internal appeals board to review borderline decisions. But there are also ominous signs that the department's aggressive attitude towards technology transfer is fostering something akin to a siege mentality in which relatively low-ranking officers are opting for secrecy simply because they want to play safe or, in some cases, because they do not understand the complex rules through which the administration's technology transfer policy is being implemented. Recent meetings on materials science, held at the University of Dayton, Ohio, and the University of California, Los Angeles, have been closed to foreign nationals, including scientists from North Atlantic Treaty Organization (NATO) countries. And last month a meeting of the American Ceramics Society in Cocoa Beach, Florida, was split into two halves with one, technically no longer a meeting of the society, being sponsored separately by the Department of Defense and the National Aeronautical and Space Administration and restricted to US citizens.

The prevalence of secrecy in government departments which sponsor scientific research is bad enough. What is worse is the apparent unwillingness of academic scientists to kick up a fuss when unwarranted restrictions are placed on publications and meetings. In 1982, it was the readiness of scientists to insist on the importance of open scientific communication that prompted the National Academy to produce the Corson report. Now that the report appears to have vanished without trace, the need for vigilance is all the greater. □

Time to back Europe

When research ministers meet in Brussels next week, they should back the Commision.

THE French launched the idea of "a European 'space' for science and technology" in their all-but forgotten memorandum to the Athens summit last December. In Toulouse a couple of weeks ago, socialists from the European Parliament backed the same idea. The meeting was unfocused and hence ineffectual, but at least it indicated concern: Europe is certainly not a "space for science", but a polyglot and divided continent where scientific mobility is almost non-existent.

Something must be done! The cry has echoed around the laboratories of Europe for years — but now there is a new determination to find structures that will work, to avoid institutional atrophy and tired bureaucracy. The goal is real, flexible, European collaboration in science and technology.

Even that temple of bureaucracy and powerhouse of paper in Brussels — the European Commission, the executive arm of the European Economic Community (EEC) — is fired with the new zeal. It burns brightest in the person of research and industry commissioner (and vice-president) Etienne Davignon, a Belgian tipped for the presidency next year, and in his director-general for research, Italian biologist Professor Paolo Fasella. With the aid

0028-0836/84/080671-01$01.00

© 1984 Macmillan Journals Ltd

of their enthusiastic staff, they aim to revitalize research in Europe and to prepare the ground for European industries based on the new technologies. That is why, next week, when European research ministers meet in Brussels to discuss Commission proposals, they should have the courage to back them without regard to the suffocating weight of the "budget problem".

The research ministers, who meet for no more than a few hours a year on European issues, must be prepared to delegate some responsibility — and call for European cooperation. The Brussels hierarchy for science has never been in a better state than now to accept the responsibility. Moreover, the Brussels research budget is tiny, less than two per cent of what is spent on research and development by the ten member states, and only a tiny fraction of the total Brussels budget (two-thirds of which goes to support over-productive European agriculture). A little courage to back excellence, in this situation, would go a long way to remind the world — and Europeans — that Europe can be a constructive as well as a destructive union.

Admittedly, Brussels-backed research has had its faults. Even the new $1,000 million information technology research programme, ESPRIT, a key paper on the ministers' table next Tuesday, is not faultless. (It could pay greater attention to training.) The Commission also has a legacy of *ad hoc* research programmes dating from old post-war treaties, and a sometimes burdensome in-house research centre at Ispra near Milan. But almost everything that can humanly be done with this legacy seems to be being done. Last year council approved the "framework programme", which for the first time relates Brussels research support to a list of political priorities, and moves as far as possible towards a conceptual and practical unity for the work. More imaginatively, the Commision launched CODEST, a small group of illustrous technocrats which aims to spend where the money will get greatest leverage: in part, in creating mobility across almost impenetrable European borders and in part on an eight-laboratory collaboration in optoelectronics. All well and good. But when the Commission asked for more cash, the Council of Ministers only managed to approve the principle.

Another item on next week's agenda is biotechnology, where the Commission has proposed a large-scale programme that faces several Community issues (such as feedstock prices). The Commission does not dare hope for approval, but the faintest nod would be encouraging. And the Council should remember a previous far-sighted biotechnology, first put on the table many years ago, which took literally years to approve. The programme, much trimmed, now goes under the title of "biomolecular engineering" and by all accounts is a great success.

What if the ministers fail to provide backing? Suppose they are so frozen in Europe's recrystallizing nationalism that they can offer Messrs Davignon and Fasella — and Europe — no life-line. Is that the end of European scientific collaboration, and so of any hope of a European high-tech reply to Japan and the United States? No, because frustration is bound to spill over into some solution whatever happens to the EEC.

An example is a body called CERFACS, still a dream at present. This proposed "European centre for advanced research and training in computer sciences" is the result of an astronomer's holiday in the Pyrenees, when Dr Jean-Pierre Poyet of the Observatoire du Pic du Midi near Toulouse met a member of the European Movement (out of which the idea for EEC originally emerged). The European Movement in Toulouse, still rather active, was seeking European projects for the region, and Poyet — frustrated by the death of expertise in vectorial computing in Europe (the kind that needs Crays) — invented CERFACS, which would fill the gap. That was a years ago. The idea has been enthusiastically adopted in France, by researchers and industry alike, but if it shows signs of becoming a reality Poyet would like to see it avoid the EEC maelstrom, by becoming a multilateral project. Even the European Parliament appears to agree: it has given money for a feasibility study. The moral: if research ministers refuse to back imagination in Brussels, there is yet hope that it will emerge elsewhere. □

Supercomputermania

Japanese advances should be welcomed by scientists, not treated as national threats.

THE argument of last resort in US science policy debates is inevitably the invocation of the Soviet peril. If that fails, the Japanese peril will do. It is never a very intelligent way to carry on a debate, but in an administration that feels threatened by the Soviets' use of vacuum tubes in their fighter planes and in a country that has been shaken to the core by Japan's ability to build cars that do not fall apart, it hits home. Thus the news that Fujitsu is soon to produce a supercomputer to rival those made in the United States seems to be doing more to excite interest in supercomputing than all past arguments about the urgent need for more supercomputing facilities for US researchers.

This need was detailed most recently by a working group set up by the National Science Board, the governing body of the National Science Foundation (NSF). The working group recommended that NSF commit hundreds of millions of dollars over the next few years to the establishment of academic supercomputer centres to fill the void left by current facilities, which are either in industry (the oil companies, for example, are very interested in numerical modelling of petroleum reservoirs) or the national weapons laboratories (which are very interested, still, in what happens when a thermonuclear bomb goes off). Meanwhile, astrophysicists and chemical engineers, who could benefit from large-scale numerical modelling, have nowhere to go.

The arguments failed to carry the day in this year's budget proposals. NSF is still apparently reluctant to get back into the business of supporting mainframe computer centres, having abandoned that policy years ago in favour of supporting the purchase of laboratory-sized mini- and microcomputers. Most campus-wide computer centres have fallen into disrepute among scientific researchers — whether as a cause or effect of this policy shift it is not clear. Into the breach have fallen the research projects that demand a massive number-crunching capability.

At least NSF director Edward Knapp has been able to announce a modest programme to support the use of existing supercomputers by researchers — but not without invoking the Japanese peril. And there was the start last week of what will surely become a major stir when reports came trickling in from US scientists who had seen prototypes of Fujitsu's new machine, with *The New York Times* printing what is coming to be the standard "Japan outstrips the West" story.

Yet, in reality, the Japanese development is good news for science everywhere. Whether or not the Fujitsu is faster than US machines is not the point. What the Japanese are commendably doing is to develop the software that will make these machines appealing to business as well as scientific users — the Fujitsu will reportedly be compatible with IBM software. Ninety per cent of supercomputer costs are at present spent in developing software peculiar to non-standard machines. If the Japanese can change that, they will sell a good many more supercomputers, which can only bring the price down and allow more scientists access to them. If supercomputers are broght within the reach of more people, more uses will be developed for them. That will be good news to the computer industry everywhere — even to IBM. □

Nature in Tokyo

***Nature* has opened an office in Tokyo. Dr Alun Anderson, for the past few years in charge of the News and Views section of *Nature*, will be in charge of the office for an initial period of one year. By this means, *Nature* hopes more efficiently to cover news of developments in Japan and also to provide tangible help to Japanese scientists wishing to submit research papers or other communications to *Nature*.**

Dr Anderson's address is *Nature*, Macmillan Shuppan KK, Eikow Building, 10-9, Hongo 1-chome, Bunkyo-ku, Tokyo 113 (Telephone (03) 816 3756/7; Telex JATOK J32121 MSKK). □

0028-0836/84/080672-01$01.00

© 1984 Macmillan Journals Ltd

Illmensee inquiry

Fraud charge unproven, researcher resumes duties

THE commission set up by the University of Geneva to investigate allegations of fraud against a leading embryologist, Professor Karl Illmensee, has concluded that there is no compelling evidence that he fabricated his data. But in a report that is highly critical of Illmensee's experimental records, the six members of the commission are unable to reach agreement on whether "the hypothesis that the protocols were fabricated could be upheld"; some felt it could, others considered "there was no compelling evidence to support or refute the hypothesis". Releasing the full report last week, the university blandly stated that Professor Illmensee is resuming all his duties in the faculty.

The commission had been considering since last August allegations made against Illmensee by three members of his laboratory. The allegations centre on a series of experiments that Illmensee carried out in Geneva between April and August 1982. Growing suspicious of their professor, three members of the laboratory compiled evidence of fraud until, after a talk by Illmensee in January 1983, Dr Kurt Bürki publicly stated that he and his colleagues could not accept the results. An internal investigation of their accusations culminated in what some took to be an admission of fraud by Illmensee (who has since consistently denied it). The rector of the university then publicly announced that the affair would be investigated by a commission which included Dr Anne McLaren of University College London, Professor Pierre Chambon of the University of Strasbourg and professor Richard Gardner of the University of Oxford.

Their report concentrates on three matters — Illmensee's apparent admission of fraud, the numerous errors, discrepancies and corrections discovered in his experimental protocols and errors in a grant application he submitted to the US National Institutes of Health (NIH).

Illmensee's admission amounted to a signed statement that "Protocols of experiments of Dr Karl Illmensee have been manipulated in a way which is contrary to scientific ethics in some period of 1982". It was countersigned by three members of the faculty of sciences of the University of Geneva and dated 17 May 1983. But what exactly did it mean? The faculty members, in a letter that accompanied the statement when it was sent to the dean, said that "Illmensee clearly recognized having falsified ("faked") protocols. . .", a view which they subsequently maintained under questioning by the commission. Professor Illmensee, however, maintained throughout the commission's enquiries and last week that he had admitted only errors in writing up his protocols, not falsification.

Speaking from Geneva, Illmensee said he was so shocked at the accusations made at his meeting with the faculty members that he could not defend himself properly and allowed words to be put in his mouth. Faced with contradictory statements, the commission was finally unable to decide whether Illmensee "did or did not recognize by his signature that experiments were falsified".

It was the day after he signed the admission that Illmensee wrote to NIH withdrawing a sentence, concerned with a particular chimaeric mouse, from his grant application. Faced with contrary evidence, Illmensee acknowledged that the mouse could not be the product of the experiments he had claimed it to be in the application. Subsequently he corrected his "slip" but with a claim that has since become as doubtful as the first. The report concludes: "At the present time, the Commission, as well as Professor Illmensee, do not know which mouse, if any, was the chimaeric male referred to in the NIH grant application." Last week Illmensee disagreed, saying that the words "of two" should replace "if any" since somebody other than himself must have mistakenly mixed up two animals.

As to the accumulation of errors, corrections and discrepancies in his records which, according to the report, "are such as to throw grave doubt on the validity of the conclusions" of the series of experiments, Illmensee admits that his recording system was too prone to error and says that a contributory factor was the stress caused by his impending move to new buildings. He emphasized the report's conclusion that the errors and discrepancies discovered do not bias the results claimed.

Responding to criticism by the commission of his secretive way of working and failure to share techniques with his colleagues, Illmensee said this was exaggerated. He had shared each part of the technique with some colleagues although not every step could be carried out by anyone in his laboratory and certainly not with the level of success he could achieve. The techniques are highly skilled and "it takes Ashkenazy to play Rachmininov properly" said Illmensee.

The report does not reserve its criticism for Illmensee. It is also critical of the university for not making Dr Bürki's written accusations available to Illmensee for several months even though they had been given to other members of the university staff. Moreover, it criticizes those members of the university who felt concerned about the case for not analysing the Bürki report in sufficient detail before drawing their conclusions and not discussing it with Illmensee.

As for Dr Bürki and the two other accusers, the commission acknowledges their sincerity but is mildly critical "of their failure rigorously to assess their evidence" and for their lack of "on-the-spot documentation to support their accusations", because lack of such documentation complicated the commission's task.

Professor Illmensee expressed satisfaction with the way the commission worked (he was allowed to attend all hearings with his lawyer) and with the result. He will now have to reconstruct his laboratory — the accusers and their sympathizers will be leaving — and attempt to repeat the dubious experiments as a collaborative project with full scientific rigour, as suggested by the commission. So far he has no definite plans for that and also has to await the reaction of NIH to his grant application and the Swiss National Fund's decision on a grant that was blocked half way through its 3-year term, pending the outcome of the commission.

Dr Bürki, who did not receive a copy of the report in advance of its publication, said last week that he accepts the report as it is and was glad that the scientific community would be able to read it for themselves. (More than 700 pages of transcripts from the commission's hearings are also open to anyone who wishes to travel to Geneva.) **Peter Newmark**

Orlov appeal

DR Yurii Orlov, the Russian physicist and human rights campaigner, who completed a seven year prison-camp sentence on 10 February (see *Nature* 16 February, p.585), is to serve the second part of his sentence, five years' internal exile, in the Yakut Autonomous Republic, one of the bleakest areas in Siberia.

The decision to send Dr Orlov to Yakutia was taken on 6 February — three days before the death of Mr Andropov. Mr John Macdonald, the London lawyer who has been a noted campaigner for Dr Orlov's release, has therefore made a new appeal to Mr Chernenko "to make a gesture towards the scientific community throughout the world and release Orlov".

To show Dr Orlov that his colleagues abroad have not forgotten him, the members of the "Orlov Committee" at the European Organization for Nuclear Research (CERN) this week applied for a visa for one of their number to visit Orlov in exile, although they realize that the chances of obtaining it are small. More practically, they are organizing their colleagues to send greetings cards and scientific journals to Orlov. The following address should be sufficient: Dr Orlov, Yu. F. Yakutskaya ASSR, Leninskii Raion, Poselok Nurbachan, USSR. Vera Rich

© 1984 Macmillan Journals Ltd

US biotechnology

Information instead of products

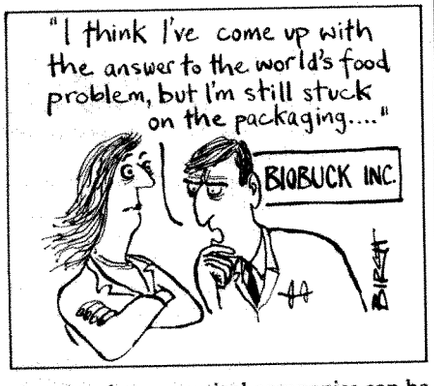
Fort Lauderdale, Florida

THE biotechnology industry has been given warning that in the absence of products or revenue it must keep the investment community up to date with as much information as possible on product development, marketing strategies and other internal management and financial plans.

"Uncertainty is the bane of existence for the stock market", said Nelson Schneider of E.F. Hutton at a meeting here sponsored by the Industrial Biotechnology Association to bring together its member companies and representatives of the investment community. Schneider and other stock analysts at the meeting noted that with so many venture capital companies going public, Wall Street finds it difficult enough as it is to evaluate their worth and that conventional methods could easily conclude that many of the biotechnology stocks are in fact worthless.

Robert Johnston of Johnston Associates pointed out that valuations based on such traditional methods as comparisons with other stocks are of little value in biotechnology, where expectations, supply and demand of the stocks and the "greater fool theory" — the belief that a stock is a bargain because someone else is willing to pay more — operate.

Robert Fildes, president of Cetus, retorted that the new biotechnology companies were being asked by the analysts to "take down their trousers", while the pharmaceutical companies' privacy was respected. And he dismissed the analysts' arguments



that the pharmaceutical companies can be rated by their track record and product stream: "What you're saying is that they have a bigger war-chest than we do", he said, "but they'll go under too, eventually, if they don't produce".

Many of the industry's representatives noted that going public is an essential step in raising the funds needed to become a business selling products rather than being merely a research and development firm. Hugh D'Andrade of Schering-Plough said that his company has a sales force of over 5,000. "It's more than brilliant scientists who turn discoveries into products." D'Andrade said that manufacturing expertise, quality control, packaging and management are crucial elements that many of the new biotechnology companies are only beginning to assemble.

The hazards of going public, however, are sufficient to stop some, at least for the time being. Gabriel Schmergel, president of the Genetics Institute, said his company would remain private for the "foreseeable future". He was conscious of the pressures of going public, one of which is the very uncertainty the market feels for the industry. "The long-term nature of our business is not fully appreciated by the public", he said. Fluctuations in the value of stock on the market can be particularly unnerving, he said, both for management and for the staff who own stock.

A number of biotechnology companies, including Genex, Agrigenetics and Genentech, have resorted recently to commercial bank loans to support their operations, which Schneider said may indicate "a useful new movement".

Stephen Budiansky

Nuclear Pakistan

"Islamic bomb" scare resurfaces

THE vexed question of Pakistan's nuclear capability has surfaced again, first in rumours widely reported last month in the Asian press, but since strongly denied, that China had supplied Pakistan with nuclear bombs and second through a published interview with a leading Pakistani nuclear scientist, Dr Abdul Qadeer Khan, director of the Kahuta uranium enrichment plant.

In this interview, which appeared first in the newspaper *Nawa-e Waqt,* and was repeated next day on Lahore radio, Dr Khan said that he felt "cornered" by questions about a possible Pakistani bomb, and did not know whether to answer yes or no. This remark immediately produced a counterblast from Soviet television which alleged that Dr Khan had boasted that it was within Pakistan's capability to create its own bomb. (The Soviet commentator incidentally identified Dr Abdul Qadeer Khan as head of the Pakistani Atomic Energy Commission — a

Harvard's Ptashne patent disappoints

Washington

HARVARD University is embarrassed in its efforts to sell the rights to a fundamental genetic engineering process developed by Professor Mark Ptashne, a Harvard faculty member. After approaching hundreds of companies, Harvard has succeeded in interesting fewer than a half dozen in purchasing a licence to the pair of patents covering Ptashne's invention.

Harvard was trying to duplicate Stanford University's success with the Cohen–Boyer genetic engineering patent, which currently has 68 licensees, each paying a fee of $10,000 a year against future royalty payments of ½ per cent of net sales. The Ptashne patent covers another fundamental step in genetic engineering: the method for attaching a bacterial promoter to a gene of non-bacterial origin, such as that coding for human growth hormone. Harvard, which also wanted $10,000 a year and ½ per cent royalty on sales, apparently misread both the importance of the Ptashne patents and the mood of the industry. Ironically, Genetics Institute, the Boston-based biotechnology company that Ptashne founded and with which he remains closely associated, did not even take out a licence; its president, Gabriel Schmergel, said that Harvard's terms were "onerous" and that "alternative routes to achieve the same end result" of the Ptashne process are available.

Albert Halluin, patent counsel for Cetus Corporation, attributed the poor response to the reluctance biotechnology companies to head down a path of a whole series of basic process patents, each taking a ½ per cent bite out of sales. And he says that the companies have become much more knowledgeable about patents. "After the history of the Cohen–Boyer patent" — the validity of which has come under considerable question since the companies first signed up — "people are saying maybe we should take a hard look at these. Now people have learned that any patent may have flaws if you look hard enough."

Halluin also noted that many companies took licences to the Cohen–Boyer patent at least partly to show that they were "one of the club" of new biotechnology companies. On that basis, and with a cut-price initial licensing offer, Stanford collected more than $3 million from licensees even before the first products were sold. Harvard also announced that stiffer licensing terms would operate after termination of the initial offering — at the end of 1983.

Schmergel cited another reason behind his company's decision: he said Genetics Institute was "upset" by what he termed Harvard's preferential treatment in the granting of exclusive licences on some of its other biotechnology patents to companies founded by the original inventors of the patents — in particular Dr Walter Gilbert of Biogen and Dr Max Essex of Cambridge Biosystems.

Schmergel said Genetics Institute had discussed with Harvard the possibility of obtaining an exclusive licence on the Ptashne patent but had been unable to reach an agreement.

Stephen Budiansky

0028-0836/84/080674-01$01.00

post actually held by Dr Munir Ahmed Khan, who claims to have little to do with his namesake from Kahuta.)

Dr Abdul Qadeer Khan, in fact, has stressed the entirely peaceful intentions of the Pakistani nuclear programme. He said that unlike India, where the nuclear power programme had been "based from the very beginning on sound foundations" under the patronage of the late Pandit Jawaharlal Nehru, Pakistan had made a relatively late and initially ill-organized start. Moreover, India, at least initially, could get encouragement and help from developed countries, but fear of an "Islamic bomb", which could be disseminated to all Muslim countries, had led the developed countries to impose restrictions on the export to Pakistan of even "the most minor products".

A bomb-making capacity depends, ultimately, on a uranium enrichment facility. On this point Dr Khan did not explicitly commit himself. He claimed only that Pakistan was far in advance of India in uranium enrichment and "not far behind foreign experts" in any field. The Pakistani Government is at present, he said, trying to purchase a 900 MW light-water reactor, and the uranium processing plant at Kahuta was being used to produced the necessary fuel.

Since it would take at least 10 years to obtain, install and operate such a reactor, the Atomic Energy Commission was "positive" that the fuel supply would be ready when needed. Those who thought that the plant was being used for uranium enrichment were deluding themselves, he said. A few minutes later, however, he observed that "as far as the supply of enriched uranium is concerned, we shall supply the Atomic Energy Commission, God willing, with as much as it needs."

Had they come from a politician, Dr Khan's words might well, for reasons of national prestige, be trying to imply a self-sufficiency which has not yet been attained. Dr Khan, however, is a scientist, and moreover, something of a special case. He was sent to study in Europe and them worked at the Almelo uranium enrichment centre in the Netherlands, returning to Pakistan in December 1975. He was subsequently charged, *in absentia,* by the Dutch government with industrial espionage, and he was sentenced, still *in absentia,* to four years imprisonment — a verdict which for prestige reasons, the Pakistan Government is still trying to get reversed.

As Dr Khan admitted in his interview, a special Dutch parliamentary committee appointed to investigate the affair stressed that Pakistan had gained much capability and saved considerable research expenditure on uranium enrichment as a result of his activities — although he maintains that the Dutch judgment was "unjust", "contrary to all the canons of law" and inspired by "anti-Pakistan and anti-Islam" elements.

Vera Rich

Nuclear power

French, plentiful and cheap

THE French national electricity utility EDF is making money at last, despite the enormous cost of the French nuclear power programme, which left EDF with debts of FF 150,000 million (£12,500 million). So claimed EDF director-general M. Jean Guilhamon during a visit to London, in a talk orchestrated by Sir Walter Marshall, chairman of the British Central Electricity Generating Board. Sir Walter, despairing of the future of nuclear power in the United Kingdom, used the opportunity of Guilhamon's visit to sign a fast breeder agreement (*Nature* 9 February, p.495) to ask him to demonstrate that in France, at least, nuclear power works. M. Guilhamon kindly obliged.

The giant EDF debt — in order of magnitude not far from the national debt of Brazil — has been amplified over the past two years by the halving of the value of the French franc against the US dollar — in which currency EDF borrowed much of its capital. But even allowing for making repayments and interest at commercial rates and for depreciation of plant, EDF is now making a profit, says Guilhamon.

France now produces 54 per cent of its electric power by nuclear plant (mostly pressurized water reactors), and with 26 more reactors already under construction or on order, the figure will be 70 per cent by 1990. That is near the practical maximum, given that it is difficult to switch off nuclear power to cope with nightly falls in demand — although EDF is offering customers cheap rates if they will take power nearly continuously.

Opponents of nuclear power have condemned the cost of building the stations, but the tables are now turning, Guilhamon claims. During the construction programme, the government set a low electricity price which made EDF unprofitable, he says. But the price encouraged new electricity consumers: for example, two-thirds of housing starts in France are now all-electric. Now, as more and more nuclear power stations turn on, displacing costly oil- and coal-fired plant, the profits of the programme are beginning to appear.

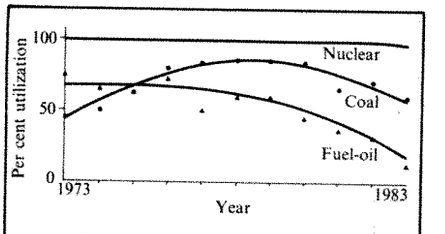
"In 1976 we were burning 14 million tonnes of oil. In 1984 we will burn less than 2 million tonnes", said Guilhamon. Coal burning has also fallen from 18 million tonnes to 11–12 million tonnes. "So we are now getting a very good return on our investment" at electricity prices at around 30 per cent below those of other countries in Europe.

Guilhamon compared the whole French nuclear power programme, which by 1990 will amount to some 50,000 MW and spare France the purchase of some 50–60 million tonnes of oil, with the scale of British North Sea oil production.

Sir Walter Marshall, meanwhile, openly feared the loss of key British industries to French soil, when in the 1990s French commercial electricity could cost only half the price of British power. Imperial Chemical Industries (Britain's biggest chemicals company) and British Steel might decamp to France, Marshall suggested.

Meanwhile, however, EDF does have a few problems caused by over-capacity and strong political pressure to burn French coal, and so keep the French miners happy. The surplus to traditional French markets will reach 50,000 million kWh in 1990, according to EDF, or around 15 per cent of total production. EDF will seek to place 20,000 million kWh in new markets it hopes to create in industry (for example, electricity to power boilers) and to export the other 30,000 million kWh. Certainly, at present French prices, electricity exports are increasing: 13,000 million kWh last year compared with 4,000 million kWh in 1982.

The pressure from coal, however, may be more difficult to withstand. Last week the government was attempting to arbitrate



Utilization of the average net operating power available from France's power stations. Coal and oil powered plants are used well below their capacity. The 100 per cent figure for nuclear power plants hides the loss in capacity due to structural defects and engineering problems.

between the French mining company Charbonnage de France (CdF) and EDF over a dispute in which CdF insists that EDF must buy fixed amounts of coal, or pay a fixed amount of money to CdF, for the next four years. EDF calculates this would cost it FF 2,500 million (£200 million) a year, essentially to support CdF. EDF does not wish to pay the bill (and since both companies are nationalized, the transfer of funds is somewhat notional); but it may have to.

Robert Walgate

● Meanwhile, accountants commissioned in 1982 by the then British energy secretary, Mr Nigel Lawson, to look into CEGB's pricing policy have argued forcefully that British electricity was not only over-priced (Sir Walter Marshall would agree with that — the government has set the price too high, he says) but ineptly priced (a comment less palatable at CEGB). The complex calculations by which the CEGB set its rate to bulk industrial customers are not based on sound economics, the accountants say. A proper pricing formula would result in cuts of 5–10 per cent followed by a few years' freeze. □

© 1984 Macmillan Journals Ltd

West German universities

Minister responds to Knopp

LAST week, West German Minister of Education, Dr Dorothee Wilms, announced her intention to recast the federal university law in line with the recent Knopp report (*see nature* 26 January, p.305). The existing law, the 1976 *Hochschulrahmengesetz* which was designed to provide a unifying framework within which the *Länder* could develop their own legislation, was brought in by a social democratic (SDP) government. Now in opposition, SDP finds the commission asked the wrong questions and came up with the wrong answers. "Partly useless, partly unnecessary, and partly reactionary", commented Eckart Kuhlwein, SDP spokesman.

On the other hand, the West German Rectors' Conference (WDRK), representing the heads of universities, agrees with the report that modest change is needed to create more flexibility within which the *Länder* can act, especially in the areas of personnel, career grades and financing from sources outside the universities.

Controversy still centres on the power of the professors — of feudal proportions before the 1976 law. The report suggests strengthening faculty representation and ensuring professorial majorities in decision-making processes. Both the government and WDRK favour this change. The vice-president of the Free University of Berlin, however, has pointed out that this is tantamount to a return to hierarchy and less equality, towards scientific freedom away from communal control in the coordination of teaching and research. He points out that the universities could not have met the challenge of increasing student numbers without the middle grades of academic staff, which the report suggests should in future assume a still lower position relative to the professors.

A recent conference at the Berlin Aspen Institute pinpointed the failure of West Germany's universities to produce the quantity and quality of trained people the country needs. Large numbers of students, straitened finances, long periods of study, and centralized administrations have created a system in which it is often impossible for the gifted student to distinguish himself. Employment bottlenecks on the academic ladder make it difficult to maintain the pool of academic talent. One highly controversial remedy was presented by the Free Democratic leader and Foreign Secretary Hans-Dietrich Genscher, who restated his party's support for private universities and institutes. A positive comment on the private university at Wittne/Herdecke suggests that the Christian Democratic administration warms to this idea. Others point out that private can mean second-rate.

Policy formation for West German higher education is inscrutable to the point of opacity, emerging from a fog of relationships between the *Länder* and federal institutions and a series of quasi-official advisory bodies, including WDRK, the Wissenschaftsrat, the Conference of Ministers of Culture and the Federal-Land Commission for Educational Planning. These bodies are criticized for being outside parliamentary control, for their obscure nomination procedures, for overworking senior personnel, for consensus policies, and for a general lack of democratic legitimacy and openness.

This system will produce the new *Hochschulrahmengesestz*, which must also be seen as one facet of the administration's overall drive for industrial growth based on science and technology. **Sarah Tooze**

China

Storm over beancurds

AN attempt by the director of a Chinese research institute to add "technological pilferage" to the list of ideological shortcomings against which there is a current drive in the country has backfired. As a result, Ge Fengheng, director of the Liaoning Provincial Commercial Science Research Institute, has found himself the object of a campaign against "local monarchs in science" that may be widely applied.

The incident, known as the "beancurd storm", began last October when an engineer from the Liaoning Institute, Wu Wentao, read to a meeting on food additives a report about a new variety of "liquid beancurd" he had developed. This evoked widespread interest, since beancurd, a staple protein source, is in short supply in many places in China, and the new product could do much to fill the gap. A special meeting of the institute's academic council was accordingly convened, at which director Ge congratulated Wu and immediately accused him of "pilferage". According to Ge, Wu had presented his work without asking the institute or reporting to it afterwards, worked on a project not assigned by the institute, included foreign data in his report, and placed special emphasis on his use of additives to distinguish between his beancurd and other types already in production.

The Liaoning Commercial Department ordered an investigation, and on 7 December reported that the production of such beancurd was unnecessary. By this time the distinguished journal *Shipin Gexue* (Food Science) had published Wu's preliminary research report. This apparently brought the matter to the attention of the party newspaper, the *People's Daily*, which discovered that during the past few years, Ge had repeatedly criticized the work of the institute's researchers as "pilferage", forbidding them to discuss the line their research should take, and used his position and power to penalize those who showed signs of opposing him. As a result, *People's Daily* concluded, there had been "serious losses" to the prestige of the party and to the "party's cause of scientific research".

The "beancurd storm", says *People's Daily*, has implications far beyond Liaoning, raising the issue of the personal qualities needed in the head of a research institute. Beijing radio went even further: "local monarchs" like Ge would not have been able to function were there not also "mistakes and a bureaucratic style of work" in the bodies responsible for administering the country's science. **Vera Rich**

Italy's biotechnology

FAST action

Milan

ALTHOUGH basic biotechnological research is good in Italy, the same cannot be said of its application in industry. Thus concludes a report on Italian biotechnology published by the Federation of Scientific and Technical Associations (FAST), which calls for the establishment of a study committee to decide on some priority applications.

That call was endorsed by Luigi Granelli, Minister of Research, in welcoming the report. At last, he said, Italy has an exhaustive survey of the state of the art of biotechnology in the 150 Italian institutes where relevant research is carried out. The FAST report, added Granelli, is a realistic guide to combining public and industrial efforts in biotechnology, particularly at a time when Trieste is likely to be chosen as one of the two sites of the United Nations Industrial Development Organization's International Centre for Genetic Engineering and Biotechnology (see *Nature* 16 February, p.583).

The report was edited by Professor Gabriele Milanesi of the Biochemical Genetics Institute in Pavia. The message of the survey is that basic research in biology in Italy compares well with other European countries, thanks to recent university law and to the projects supported by the Consiglio Nazionale delle Richerche, Italy's principal research council, in continuance of the policy implemented since the 1950s by the late Professor Adriano Buzzati-Traverso. By contrast, industrial applications are poor despite the involvement of three public companies (Farmitalia-Carlo Erba of the Montedison group, Assoreni and Sclavo of the Eni group) and five private companies (Sorin Biomedica of the Fiat group, Lepetit, Recordati, Soreno Institute and Societa Produzione Antibiotici) and despite the fact that industry cooperates with public centres by investing in basic research. **Paola De Paoli**

0028-0836/84/080676-01$01.00

Satellite launchers

Ariane backers confident

Evry, France

IN France, where they build Ariane, Europe's answer to the US space shuttle, one newspaper last week reckoned the score in own goals to be: Shuttle 3, Ariane 2. That is, the shuttle has lost one more communications satellite than Ariane, although it has regained one too. Certainly this leaves the space insurance business equally nervous of both systems.

But at Evry, south of Paris, from where the French space agency CNES and the commercial launcher company Arianespace run the Ariane programme, there is an increasing sense that Ariane may prove to be a winner in the billion-dollar market for communications satellites.

Without any sense of crowing over shuttle problems — for Ariane has had its own, and may yet have more — it is pointed out at Evry that launch from the shuttle into geostationary orbit is more complicated and more expensive than launch by Ariane. A communications customer on the shuttle must also buy a substantial rocket — the "perigee motor" — to lift the satellite from the low shuttle orbit to the elliptical geostationary transfer orbit (GTO), which reaches up to the parking height of geostationary satellites. It is these motors that failed in the recent shuttle flight. Ariane reaches GTO in one shot, using liquid fuel stages which are theoretically more accurate and reliable.

However, even if there is some delay in the shuttle's geostationary launch programme, Arianespace is not in a position to gain any immediate benefit. Its order book is almost full for two years, with room (perhaps) for only one more "good" customer in 1985.

The limiting factor is the Ariane production line, which can turn out no more than six launchers a year. From 1985, the launch site at Hourou, French Guiana, will be able to cope with 12 launches a year. So Arianespace, which is desperately undercapitalized (it could be bankrupted by a single launch failure), is seeking new capital in Europe to construct a second production line.

In the long run, however, much depends on the Ariane development programme. So far, Ariane launches have used only the first version of Ariane. A week ago, the last tests were completed on the second and third versions (Ariane-2 and Ariane-3, identical except that the latter has two extra strap-on solid boosters in the first stage). The first new version launched will be an Ariane-3, due to take the European Space Agency's ECS-1 and the French Telecom 1-A aloft this July. To maintain confidence in Ariane, of course, the new rocket should not suffer the teething troubles of Ariane-1 — which suffered high frequency vibrations in the first stage (causing failure of the second launch test) and then lubrication failure in the upper stage cryogenic fuel pump (that knocked out the fifth). According to the man in the hot seat, the *Chef de Projet* for Ariane-3 (and a future Ariane-4), André Van Gaver, the problems that hit Ariane-1 are now solved — or as solved as anything can be in the space business.

After Ariane-3 will come Ariane-4, the first parts of the first flight model of which are to be completed this year. Ariane-4, to fly in March 1986, is designed around the next generation of communications satellites led by Intelsat VI: 4,200 kg and approaching 4 metres in diameter, comparable with the shuttle bay's 4.5 metres. The future of Arianespace depends on the company's success in gaining contracts for the Intelsat VI series, but knowing this, Intelsat can afford to wait. Around half the Intelsat business would be ample to give Arianespace a living, says Van Gaver.

The United States "made a mistake" in dropping conventional launchers in order to push business towards the shuttle, Van Gaver believes. Now the United States is trying to commercialize the old launchers (Thor-Delta and Atlas) "but we have yet to see any contracts".

Ariane's other competitor is probably Japan — Van Gaver does not see the Soviet Union or China entering the Western telecommunications market. Japan's space programme is at present uncompetitive, but the Japanese space agency is developing a large cryogenic engine (such as Europe would only see on Ariane-5, now at the planning stage). "We must watch Japan carefully" Van Gaver says.

Robert Walgate

● Intelsat, the international communications satellite organization, has finally decided to go back to an old design for Intelsat V F8, due for launch on Ariane on 5 March. Problems with the L-band maritime communications system (see *Nature* 2 February, p.401) had pushed the launch back two months. Inmarsat, the organization which uses the L-band system, has complained that Intelsat V F7 is unworkable and has refused to accept it. Unable to solve the problem on F8, Intelsat has gone back to the F6 design, which still has problems but not so bad as those of F7. F9, which was to have been launched by Ariane this summer, has now been put back to 1985. □

US space station

Manufacturing plans launched

Washington

WITH a masterly sense of timing, the National Aeronautics and Space Administration (NASA) has announced that a major company, the Minnesota-based 3M Corporation, is planning to embark on a new programme of space research with the aim of eventually producing commercial products in orbit. The corporation's decision comes fast on the heels of President Reagan's announcement that the United States will build a manned space station within a decade, and is being portrayed by NASA as vindication of its claims that a space station could become the nucleus of a sizeable space-based manufacturing industry.

At a formal ceremony announcing 3M's plans, NASA administrator James Beggs did his utmost to link the company's decision with the President's approval of the space station. 3M, he pointed out, was the first non-aerospace corporation to sign a memorandum of understanding with NASA since the space station was approved. He said the deal would hasten the day when US industries were as comfortable with factories in space as they were with those on Earth.

NASA's enthusiasm is understandable. To persuade Congress to fall in with its plans for a space station, the agency must show that private industry (and foreign governments) are prepared to pick up a share of the tab. Propaganda value apart, the agreement with 3M looks decidedly one-sided. In return for expressing an interest in space, 3M scientists will receive free access to NASA laboratories where materials processing research is under way. 3M also gets two free flights on the shuttle, the first as early as August.

3M's research programme will focus on processing organic materials in the low-gravity, high-vacuum environment available in space. Its vice president for technology services, Dr R. M. Adams, said the company's first experiment on board the shuttle would look at the growth of organic crystals and the development of thin films with novel physical and chemical properties.

Directed by both President Reagan and Congress to encourage commercial investment in space, NASA faces an uphill struggle in extending commercialization beyond its one great success story — the satellite communications industry which does more than $2000 million of business a year. The most visible commercial interest in space-based manufacturing has come from Johnson & Johnson and McDonnell Douglas, which are developing a prototype instrument for the large-scale production of drugs by electrophoresis in space.

To garner more investment, NASA plans to offer an expensive array of incentives including free or subsidized flights on the shuttle and access to NASA expertise. That could queer NASA's pitch with academic scientists, many of whom have been grumbling for years about the length of the waiting list for space on the shuttle.

Peter David

© 1984 Macmillan Journals Ltd

High-energy physics

Future accelerator plan

Chicago, Illinois

AN enormously powerful colliding particle accelerator, the Superconducting Super Collider (SSC or the "desertron"), may still be only a gleam in the eye of US physicists but last week a workshop on the options for the proposed machine brought its construction a little nearer. SSC, at 20 TeV per beam, would be 20 times more powerful than even the Fermilab collider, which is still under construction.

The workshop, held at the University of Chicago and co-sponsored by Argonne National Laboratory, brought together about a hundred high-energy experimenters and theorists to sharpen the arguments on the relative merits of proton–proton colliders and antiproton-proton colliders. Either could still be the basis of SSC, which was recommended last year by the US high-energy physics community as the next big step in US accelerators.

Antiproton–proton colliders — such as the one at the European Organization for Nuclear Research (CERN) at Geneva and that being built at Fermilab — can circulate both types of particles within the same beam pipe and thus need only one ring of magnets. At 90 km in circumference, a one-ring SSC could cost considerably less than a two-ring proton–proton machine. However, antiprotons cannot be produced as copiously as protons. Antiproton colliders thus have lower luminosities and produce fewer particle interactions.

But the disadvantages of an antiproton collider may not be so great as had been thought. For example, the antiproton source group, headed by James Simpson of Argonne and Thomas Collins of Fermilab, concluded that antiproton sources can be built that would provide luminosities greater than 10^{32} per cm^2 per second. This approaches the 10^{33} that has generally been assumed possible in a proton–proton machine and necessary for much of the expected physics. In addition, most of the groups reported that luminosities much above 10^{32} would usually be unnecessary.

A proton collider can always be made more luminous than an antiproton machine, Collins told his audience. "But let's not choose a machine based on capabilities that we don't really need." However, the groups studying tests of electroweak interactions and potential new particles and interactions decided they could sometimes use all the luminosity they could get. Others, according to Robert Cahn of the Lawrence Berkeley Laboratory, really would like both types of collisions. But when the next major workshop on SSC takes place in June at a three-week meeting in Snowmass, Colorado, the participants will have two strong candidate accelerators to consider. **Larry Arbeiter**

UK pollution control

Commitment urged

THE Royal Commission on Environmental Pollution, in its 10th report, published this week, urges the British Government to "play a more positive role" in promoting environmental issues in the European Community. In a survey of its 14 years of offering advice on matters of pollution, the commission concludes that British commitment to international action "has not always been as strong as it might be". And on the vexed question of acid precipitation, concern over which is currently spurring several EEC draft directives on air pollution, the commission says that the Central Electricity Generating Board (CEGB) should within the next five years introduce pilot plants to investigate the various options for reducing emissions of sulphur dioxide from its power stations.

The EEC draft directives on air pollution controls, which are being pushed hard by West Germany, are currently the subject of an inquiry by a House of Lords Select Committee. This week the committee will hear a CEGB claim that one of the major proposed directives (on large combustion plants) misquotes figures from a cost-benefit analysis of sulphur emission reductions. The Confederation of British Industry and the Chemical Industries Association will join CEGB in condemning the draft directive as "excessive" and "very damaging". It calls for, among other things, a 60 per cent reduction in total national emissions of sulphur dioxide and fixed emission limits for sulphur dioxide from large plant. A separate draft directive on air quality limits for nitrogen oxides is also attacked for being too stringent.

The Royal Commission on Environmental Pollution has very widely drawn terms of reference and has never been afraid to use them. The commission, whose present chairman is Professor Sir Richard Southwood, Linacre Professor of Zoology at the University of Oxford, delivers a well-aimed swipe at Whitehall officials who have allowed ministers "unfettered discretion" over timetables to frustrate the wishes of Parliament. Implementation of the 1974 Control of Pollution Act has been piecemeal and incomplete, according to the commission, and "reflects little credit on successive administrations". Such action as has been taken is ascribed to pressure from the EEC. The lack so far of any government response to several of the commission's earlier reports is described as disappointing.

Official secrecy over pollution control has long been a controversial issue in Britain, and the latest report from the commission reaffirms its position firmly on the side of the "right to know" camp. No less than ten detailed recommendations urge greater public access to information compiled by pollution enforcement authorities. The commission says that " a guiding principle for all legislative and environmental controls . . . should be a presumption in favour of unrestricted access" with provision for secrecy only in exceptional cases. Observing that secrecy fuels fear, the commission draws attention to public anxiety over nuclear power and calls for greater public representation on bodies concerned with managing nuclear waste.

Recognizing that there is a growing awareness of the urgency of international pollution problems, the Royal Commission advocates more forward planning of measures designed to avoid pollution before it becomes a problem. Thus it recommends government support for research into new potential pollutants from high-technology electronic and biotechnology processes, as well as more "traditional" pollutants in air and water. The government should also study how pollution control equipment is subsidized in other countries with a view to introducing subsidies in Britain. The commission says the politician faced with decisions on pollution control "cannot wait for rigorous proof": prudent abatement practices must be instituted when perceived risk of damage justifies the cost of alleviation. It therefore proposes that decision makers should be guided by principles of the best practicable environmental option and the best practicable environmental timetable. The commission ends by lamenting the decreasing emphasis on environmental protection in Britain and the concomitant decrease in resources. **Tim Beardsley**

The Times 6 February

QUEEN MARY COLLEGE
UNIVERSITY OF LONDON

APPLIED MATHEMATICS DEPARTMENT

Theoretical Astrology Unit

Applications are invited for 1 or 2 year RESEARCH FELLOWSHIPS (with possibility of extension for 3rd year). The core staff of the Unit comprises 6 permanent members of the Academic Staff (Prof. Roxburgh, Drs MacCallum, Papaloizou, Rowan-Robinson, Schwartz, I. D. Will[illegible] staff

The Times 15 February

Astrology Unit. Was this just a typographical error or some mysterious intervention that defies scientific explanation?

I suspect it is now too late to stem the flood of applications from would-be experts in the occult, but may I use your columns to point out to them that, had they been in possession of such skills, they would have known that their applications would be rejected: they should therefore not be surprised if they hear nothing further from us.

Yours etc,
IAN W. ROXBURGH,
Theoretical Astronomy Unit,
Dept of Applied Mathematics,
Queen M[illegible] College

0028-0836/84/080678-01$01.00

Acid rain

Canada must act alone

Washington

THE Canadian Government, exasperated by the Reagan Administration's decision to do no more than continue research on the acid rain problem, is considering substantial unilateral curbs on its sulphur dioxide emissions.

In 1982, Canada unilaterally decided to institute a 25 per cent reduction by 1990 in sulphur emissions. It has since been hoping to negotiate further cut-backs as part of an overall agreement with the United States in which both parties would work to reduce transboundary sulphur pollution. Although sulphur emissions from both countries cross into the other, Canada suffers most from transboundary pollution.

The Reagan Administration has consistently refused to order reductions in US sulphur emissions, arguing that scientific uncertainties must first be resolved. That position was reiterated most recently by science adviser George Keyworth in his briefing on Reagan's 1985 budget proposals, which include funds for more research on acid rain, but no regulatory action. The administration, in maintaining this position, has ignored the recommendations of both the National Academy of Sciences and a panel of scientific experts assembled by the White House Office of Science and Technology Policy. The White House group last summer found that although scientific uncertainties do exist, new regulatory action is justifiable because of the possibility of "irreversible damage" to the environment by acid deposition.

Canada's current programme involves reducing sulphur emissions 25 per cent below the levels already required to meet ambient air quality standards instituted in the mid-1970s. Canada's ambient standards are at least as strict as US standards and, like them, are designed to protect human health from acute effects of sulphur dioxide rather than to control acid rain.

Bruce Jutzi of the Canadian Embassy in Washington says that while Canada had not yet decided to institute new curbs, such action is under serious consideration. "Now that it's apparent there's nothing for the foreseeable future to be done together, that puts the onus back on the Canadian Government", he said. Jutzi said that ultimately Canada expects to have to reduce its sulphur emissions by 50 per cent from the ambient standards as its contribution to solving the transboundary pollution problem. "I'd personally be a bit surprised to see us go all the way to that limit without any movement from the United States", he said.

Canada's main source of sulphur emissions is smelters; in the United States it is coal-fired power stations.

Stephen Budiansky

Conservation

Farm policy hits Broads

CONSERVATIONISTS in Britain are anxiously awaiting the outcome of a local dispute that is seen as a major test of the 1981 Wildlife and Countryside Act. The question is who should pay the compensation that the act offers to landowners who agree not to turn valuable habitats into farmland. The growing incentives to produce more farmland were stressed last week at a meeting called to discuss the ecological crisis facing the locality.

The dispute concerns Halvergate Marshes, close to Great Yarmouth in eastern England, and has arisen because the Broads Authority, set up in 1978 to safeguard the whole of the Norfolk Broads of which Halvergate is a part, claims that it cannot afford the level of compensation involved. Two thousand acres of grazing marsh have been turned over to arable farming in the past 3 years and according to one estimate another five thousand acres could be ploughed within the next 5 years, unless compensatory payments of up to £200 per acre per year are found.

The problem of much of broadland is that it is very expensive to protect, having a high amenity value to tourists (whose boats are eroding the river banks) and to farmers (who drain and turn to the plough the prized grazing marshes in order to take advantage of the generous guaranteed prices laid down in Brussels). And so the area tends to throw into sharp relief conflicts of interest, and tests to the limit the legislation designed to resolve them.

The essence of the Wildlife and Countryside Act is that designated authorities wishing to protect a site from some planned action must, if persuasion is ineffective, be prepared to pay the owner for loss of projected profits. For arable farming these can be high, and the Broads Authority says it cannot afford to pay for these "management agreements". Half of the authority's income comes from the local councils of the region, while the other half is from central funds through the Countryside Commission. The authority, which comprises local authority representatives, has refused to continue making payments on these terms.

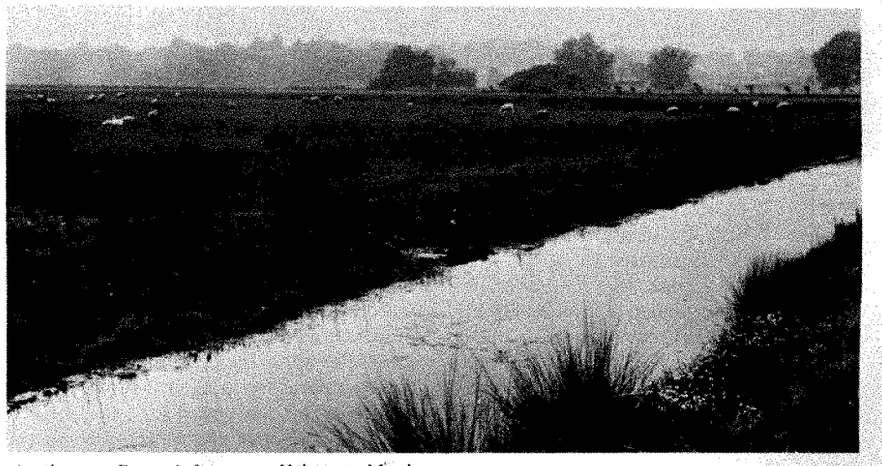
In areas designated as National Parks (which the Broads are not), 75 per cent of local compensation agreements are met from Government. But the Countryside Commission, itself the government's adviser, has argued strongly that 90 per cent reimbursement would be more appropriate for the Broads Authority. Representatives of the authority are soon to meet Mr William Waldegrave, Under-Secretary of State in the Department of the Environment, to thrash out a solution.

The powers of the Broads Authority have recently been the subject of a searching review by the Countryside Commission, which next month will formally recommend to government that it should be given special statutory powers to coordinate a rescue plan for the area.

The main problems facing broadland are that raised nitrate and phosphate levels cause algal blooms which shade out bottom-living plants. Consequently, banks erode more quickly, much wildlife disappears and sedimentation rates increase. Some ecologists fear that the expansion of arable farming may have wider consequences in the region.

Last week, two learned societies spent a day discussing the ecological crisis facing the Broads and heard speakers express concern that the European Community's Common Agricultural Policy and grants from the Ministry of Agriculture, Fisheries and Food will encourage the loss of much of the remaining grazing marsh in the area. At the end of the day, representatives of the British Ecological Society and the Linnean Society of London were told by their chairman that while it would be "entirely inappropriate" for them to pass a resolution expressing concern on the issue, it would be in order for a report on recent research "showing the scientific consensus" to be prepared and despatched to relevant government officials.

Tim Beardsley



Anathema to Europe's farmers — Halvergate Marshes

0028-0836/84/080679-01$01.00

© 1984 Macmillan Journals Ltd

McNamara's plan

SIR — We suggest a strategy for global disarmament of nuclear weapons: that all land-based missiles carrying nuclear warheads be destroyed. We refer to a statement by Robert S. McNamara in *Newsweek* of 5 December 1983 and repeated at the meeting of the Bellerive Group in Geneva last December that nuclear weapons have no military use whatsoever other than to deter one's opponent from their use. With the words of Professor Robert Neild, Cambridge, at that same meeting: "we could draw back the nuclear weapons to where they belong, deep in the oceans, where they could serve the sole purpose of deterring nuclear attack or threat". We note that there seems to be general agreement that a limited nuclear exchange cannot be confined, but will lead to a general exchange of strategic weapons. If so, land-based missiles do not add to deterrence over and above what submarine-based missiles can achieve.

The nuclear arms deployed on land thus only reduce the security of the nations possessing them. The risk of nuclear war triggered by accident or misinterpretation (such as a computer error or an astrophysical phenomenon such as the 1908 Tunguska event misinterpreted as a nuclear attack) is greatly increased by the mere presence of stationary nuclear weapons on the continents which, being vulnerable, call for a fast response. Submarine-based nuclear missiles cannot be easily found or destroyed by enemy forces and thus do not invite overreaction in response to or in anticipation of a presumed first strike.

The specific disarmament measure we suggest can be undertaken unilaterally without any loss of security. Any nuclear power implementing this strategy will gain a colossal political victory.

ANDERS BOSERUP
Institute of Sociology,
DK-1361 Copenhagen K,
Denmark

OVE NATHAN
University of Copenhagen,
DK-1168 Copenhagen K,
Denmark

TOR NOERRETRANDERS
Science Department,
The Weekly Weekendavisen
DK-1147 Copenhagen K, Denkark

Improper psychiatry

SIR — I was interested to read (*Nature* **304**, 293; 1983) that ". . .remaining members (of the World Psychiatric Association) should put their houses in order. . .". You meant, I suppose, that neither unconditional nor automatic legitimacy should be granted to WPA when, as now, the bad boys are out. I cannot but agree with such a view. However, in the same article, you use the term "mental illness" when referring to several types of anomalous human behaviour. The nature and/or the very existence of such "illness" remains a matter of debate, as you make clear, so I find it misleading when such "illness" is referred to later in the article. This shows how hard it is to avoid the use of an ambiguous term, even in a scientific journal.

This confusion could lead the reader to believe that scientifically unequivocal correlations have been found between truly existent mental illnesses (of a total physical origin), social danger (as an effect of such an illness) and treatments (scientifically based). Of course, if such correlations exist, they are far from being unequivocal and, beside mental "illness", social danger seems to be of a very doubtful consistency too. Even more doubtful is the therapeutic value of treatments based on physical modifications of the human brain (electroconvulsive therapy, lobotomy, heavy drugs) which, it is claimed, work simply as repressive tools. This is as close to torture as to therapy.

Put simply, the problem of ill use of psychiatry is psychiatry itself. The alleged use of psychiatry in repressing political dissent — an extreme degeneration of an already strongly criticized activity — provides an opportunity for pushing WPA and the United Nations to face their responsibilities. As for the Soviet Union's resignation from WPA, it should not be welcomed unconditionally, as it can be justified as a way of maintaining social prestige, credibility and income for the remaining members of WPA. Moreover, unpleasant though it is, we must face the possibility that such a resignation can be represented as a protest or a Soviet psychiatrist's refusal to play the role of scapegoat.

Finally, can we be sure that the type of abuse of physichiatry as documented by various international human rights organizations happens only in the Soviet Union? Should we draw the sad conclusion that what in the Soviet Union is done for political reasons is done elsewhere for ill conscience and/or for material benefit?

FERRUCCIO APRILE
via Franco Bartoloni 40,
cap 00179 Roma, Italy

Frost tolerance

SIR — Current ideas on the protection of plants from frost by alteration of the characteristics of the bacteria normally resident on the leaves raises an environmental question because of the differences between resistant and non-resistant plants, and the role of supercooling in each of these when they are exposed to frost.

The cultivated potato, some beans and the dahlia are examples of non-resistant plants which are damaged by ice forming within their tissues but not by sub-zero temperatures if no ice forms. This is possible if the water they contain supercools as the temperature passes below freezing point. In the great outdoors, the surfaces of leaves are normally quite dirty with all manner of material, some of which may be expected to be of the sort which encourages water to freeze when the temperature falls below zero. The connections between events on the surface of leaves and the freezing of water within are unclear, but it is certainly true that the freezing of dew on the surface can lead quickly to ice formation within. It has been shown that some bacteria normally resident on leaves are able to encourage ice formation by virtue of some property of their coat. Genetic engineering has succeeded, it is claimed, in creating forms of these bacteria which lack the ability to trigger ice formation in water below its freezing point. It is also being suggested that if the natural population of bacteria on leaf surfaces of frost-susceptible crops could be replaced by these altered forms, then the plants would be less likely to freeze during frosts and so avoid damage.

Some cases of frost resistance by avoidance, apparently involving a thick water repellent cuticle, are known in natural vegetation. However, the majority of frost-resistant plants are quite different, they are tolerant of freezing dew and the formation of ice within the leaves and stems. Although like non-resistant plants their cells are killed if ice forms inside them, there are mechanisms which ensure that ice forms only between the cells and in less important tissues; the cells become desiccated due to water loss to the intercellular spaces, but few freeze. These mechanisms take time to work, and it is essential that in these plants the process of ice formation begins as soon as possible after the temperature falls below zero. Frost-tolerant vegetation is usually inoculated by freezing dew which sets off internal ice formation, and if this triggering fails, as it does when there is no dew because the air is too dry, then the subsequent "black" frost is more than usually damaging. The reason is that the plants have supercooled to a temperature well below zero, and when ice formation eventually begins it is a very rapid process. This is damaging because the movement of water out of the cells to the growing ice crystals cannot keep up with the rate of ice formation, and the ice grows into the cells and kills them.

Because of the different role of supercooling in the survival of these two main categories of plants, the expected results of any treatment which encourages supercooling will be different too. The question is, what may happen if such a treatment, carried out on a crop, also affects the surrounding natural vegetation? If the treatment works at all, the crop may be less damaged but the natural vegetation may be more damaged than would have been expected from the severity of that particular frost, unless the frost were so slight that both types of plant survive by supercooling.

D. B. IDLE
Department of Plant Physiology,
University of Birmingham,
Birmingham B15 ZTT, UK

Polygraphic interrogation

David T. Lykken*

The polygraph ("lie detector") test is wrong one-third of the time overall, biased against innocent and conscientious persons, and can be "beaten" by sophisticated liars. Increasing use of this technique, in the United States and soon in Britain, is a cause for alarm.

THE origins of lie detection are lost in the mists of prehistory. When *Homo sapiens* developed language, one suspects that he soon began to use that language to mislead and deceive, mendacity following hard upon capacity. This in turn required all of us to become human lie detectors.

Although most of us can probably detect lying with better than chance success using just our eyes and ears and commonsense, some are less skilful than others. It is interesting that English, at least, is replete with terms descriptive of the low end of this dimension of talent ("gullible", "credulous", "sucker" and so on) yet apparently empty of descriptors for the skilled end ("suspicious" is biased, "perceptive" is too broad). The ancients developed rituals and ordeals for identifying liars, some of which, like modern lie detectors, relied on the autonomic arousal that accompanies fear. One often-cited trial required suspects to chew a mouthful of rice and then attempt to spit out a moist bolus. When the rice grains clung to the dry mucosa or dribbled individually from the parched and trembling lips, the priests believed that they had found their culprit[1].

In London in 1730, Daniel Defoe published a tract entitled *An Effectual Scheme for the Immediate Prevention of Street Robberies and Suppressing All Other Disorders of the Night.* Defoe argued that "there is a Tremor in the Blood of a Thief, that, if attended to, will effectually discover him". "Some are so hardened in Crime", he continued, "that they will . . . outface even a Pursuer; but take hold of his Wrist and feel his Pulse; there you will find his Guilt."

The first modern to take up Defoe's idea was also a writer of popular fiction, the inventor of the comic-strip character *Wonder Woman*. William Moulton Marston, a Harvard-educated psychologist and lawyer, realized, however, that fear symptoms alone are ambiguous for there may be a tremor in the blood of an innocent accused as well. Marston, who either coined the term "lie detector" or took up the phrase from one of the journalists whom he cultivated, believed that there is an involuntary physiological response, like the elongation of Pinocchio's nose, that we show when, and only when, we lie. This specific lie response, Marston claimed, is a transitory elevation in systolic blood pressure brought about by the interaction of fear of detection and the attempt to maintain outward composure[2].

The polygraph

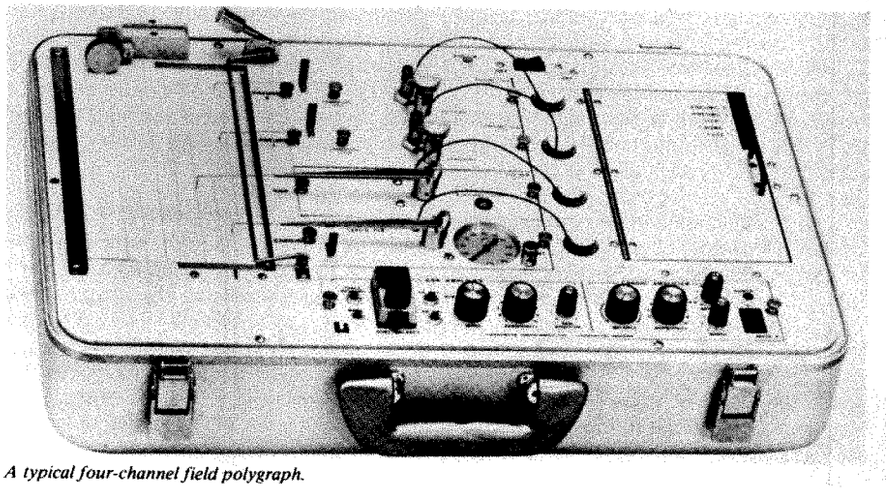
A Californian police officer, John Larson, soon developed the first recording polygraph which made continuous tracings of breathing movements and "average" blood pressure. On a moving paper chart, the "cardio" pen, connected pneumatically to a standard blood pressure cuff on the subject's arm, deflects briefly with each heart beat and the entire "cardio" tracing moves up or down with transitory changes in blood pressure. While it soon became obvious to Larson that Marston was mistaken, that innocent persons too may show blood pressure responses to accusatory questions, he nonetheless found his polygraphic lie detector to be a useful investigative tool. When questioning a series of possible suspects in some crime, it often happened that one of them would display a really strong reaction to the relevant or accusatory question and, moreover, when confronted with this evidence of internal disquiet, such suspects would frequently confess.

Like the rest of us, police have always looked for blushing, trembling, averted eyes and other signs of guilty nervousness when questioning someone whose testimony is in doubt. Here was a scientific instrument that could record internal blushes and other "tremors in the blood" and which seemed to be effective. The standard modern polygraph is not much different from Larson's. Two pens are driven by pneumatic tubes placed about the subject's chest and stomach, recording thoracic and abdominal breathing movements. The "cardio" pen is still attached to a blood pressure cuff which, during the test, is inflated midway between systolic and diastolic pressure, partially occluding blood flow to the arm. The fourth pen is connected electrically to a pair of metal electrodes on the subject's fingers: it records changes in the electrical resistance of the skin, electrodermal responses which reflect sweating of the palms.

A single polygraph chart is a continuous record of these variables over the 4 or 5 minutes required to ask a series of about 10 questions; at the end of each chart it is necessary to release the cuff pressure for a time to restore circulation in the arm. Most polygraph tests involve three charts, three repetitions of the same list of questions.

The relevant/irrelevant test

Leonarde Keeler, Larson's assistant, developed a portable polygraph instrument and, in 1930, moved to Chicago where he established the Keeler Polygraph Institute, the first school devoted to the training of polygraph examiners. There are now dozens of such schools in the United States,



A typical four-channel field polygraph.

*Department of Psychiatry, University of Minnesota, Minneapolis, Minnesota 55455, USA.

0028-0836/84/080681-04$01.00

© 1984 Macmillan Journals Ltd

more than 20 in Florida alone, most of which offer a curriculum lasting from six to eight weeks or about one-sixth of the study time required by the average barber college.

The standard Keeler polygraph test involved a series of relevant or "Did you do it?" questions ("On 15 May, did you take the $1,000 from the safe?") intermixed with a number of innocuous irrelevant questions ("Is today Tuesday?"). Since the relevant questions can span a lot of territory ("Did you tell the truth on your application form?"; "Have you been in contact with a foreign intelligence agency?"), this is the test format commonly used in screening job applicants or vetting people for security clearances. It is estimated that a million people in the United States were subjected to polygraph testing in 1983. President Ronald Reagan's National Security Directive, due to take effect in April 1984, will require millions of federal employees and employees of civilian defence contractors to submit to such tests on demand or face "adverse consequences".

All anyone can determine from a polygraph chart is that the subject was more aroused or disturbed by one question than by another; one cannot tell why he was disturbed, or whether the question elicited guilt or fear or anger. Nor is it meaningful to compare Jones's reaction with Smith's response to the same question, since individual differences both in emotional and physiological lability are so great that a small response for one person may be a large response for another. Moreover, it is generally agreed that it is the question, rather than the subject's answer to the question, that determines the recorded response. In the relevant/irrelevant test, therefore, it must be assumed (1) that an innocent person will be no more disturbed by the accusatory relevant questions than by the innocuous irrelevant questions and (2), the corollary, that any relatively stronger response to a relevant question indicates deception rather than, say, embarrassment, fear or indignation.

A young engineering professor, engaged in secret research for the Central Intelligence Agency (CIA), was recently tested by a CIA polygrapher in connection with his security clearance. The question "Have you ever engaged in homosexual activity?" produced in him more physiological arousal than the interpolated irrelevant questions. It was concluded that this young scientist was a "closet" homosexual subject to blackmail and his clearance was withheld. It is possible that this examiner actually assumed that deception was the only reasonable explanation for the physiological disturbance evoked by this question. On the other hand, many polygraphers regard themselves as the real "lie detectors" and their understanding of the facts plus their interpersonal impressions are combined, according to unspecified rules, with whatever they think they can see in the charts in arriving at their diagnosis. Moreover, many polygraphers continue to believe, against all evidence, that certain patterns of reaction are specific evidence of lying; "stair-step" respiration, for example, or an increase in blood pressure in anticipation of a question, followed by a "relief response" after the question has been answered. A polygraph chart is noisy and complex; like the tea leaves at the bottom of a cup, it invites flights of interpretive fancy.

	Polygrapher's diagnosis			
Status of suspect	**Deceptive**	**Truthful**	**Total**	**Accuracy**
Guilty	98.1	19.9	118	83%
Innocent	38.2	50.8	89	57%
Accuracy*	72%	72%	20%	72%

Aggregated findings of the three credible field studies of polygraph accuracy[6,8,9] in which polygraph charts of 207 criminal suspects were scored independently by 14 polygraphers. Average accuracy on guilty suspects was 83%; however, 47% of innocent suspects were erroneously diagnosed as "deceptive".

*The accuracy of tests that are "failed" or "passed" depends on the proportion of liars in the group tested. Thus, if 800 of 1,000 persons tested are truthful, a test that is 72 per cent accurate overall will "fail" 144 liars and 224 truthful persons.

Polygraphic interrogation

Reacting to President Reagan's plans vastly to expand federal use of the polygraph test, the US Congress asked its Office of Technology Assessment (OTA) to examine the scientific status of this technique. OTA's report[3], published in November 1983, concluded that there is no scientific evidence at all concerning the validity of the type of test used in preemployment or periodic screening. To understand how, in the absence of such evidence, polygraphic screening has become a multi-million dollar industry in the United States, it is necessary to appreciate that the lie detector has been a part of American mythology for 50 years. Every citizen has heard of this device and assumes that it must work; why else would the Federal Bureau of Investigation (FBI) and the Pentagon use it?

Why would politicians and other celebrities, their veracity challenged, volunteer to take lie detector tests? Once established, such a myth feeds on itself. Refusal to submit is interpreted to mean that one has something to hide. As Richard Nixon remarked on the Oval Office tape of 14 July 1971, "Listen, I don't know anything about polygraphs and I don't know how accurate they are but I know they'll scare the hell out of people".

The control question test

Polygraph examiners, too, believe in the myth of the lie detector because they are generally insulated from discorroborative evidence. An examiner may give 1,000 tests a year and yet almost never discover, after having recorded his diagnosis, whether he was right or wrong. In the absence of corrective feedback, wishful thinking flourishes.

Yet, by the late 1940s, some perceptive polygraphers had come to realize that, "Did you take the $1,000?" may be a stronger stimulus than "Is your name John?" even for an innocent suspect. Therefore, a new test format was devised by the late John Reid, an attorney, who operated the Reid College for the Detection of Deception in Chicago. Reid added a third type of question, misleadingly known as "control" questions, to the standard list. Although it does not lend itself to the screening application, the control question test (CQT), in one form or another, has become the standard technique for use in specific issue situations, as in criminal investigation.

For a person suspected of Crime X, a genuine control question might be, "Did you commit Crime Y?", where Y and X are equally serious and where the examinee can be persuaded to believe that he is truly suspected of Crime Y although, in fact, the examiner knows that Crime Y is fictitious. Then the polygraphic reaction to the question about Crime Y will provide an estimate of how this subject should react to the question about Crime X, if he is also innocent of that crime. For a variety of obvious reasons, this type of control is seldom possible. Instead, the examiner tries to develop two or three "probable-lie control questions", questions referring to the examinee's past which he will answer deceptively out of embarrassment or a desire to make a good impression.

This curious concept can most easily be understood in terms of an example. A young mother in Hillsville, Virginia, was awakened at 3 a.m. one night last year by an intruder who, on threat of death to her and her child, proceeded with an elaborate sexual assault which, among other perversities, involved shaving her pubic hair. The local sheriff had read somewhere that this feature was characteristic of lesbian rape and the victim therefore was asked if her assailant had been a woman. In many US jurisdictions it is commonplace to require rape victims to submit to a polygraph test and, when this woman insisted that she had been raped by a man, she was referred for testing. Two of the "control" questions asked were: "Before the age of 15, do you remember playing with your sex organ?"; and "Between the ages of 15 and 25, did you ever have sex with a woman?" The examiner, employed by the state crime

bureau, had been trained at the highly regarded Backster school of polygraphy and had more than 10 years of experience. On the basis of that training and experience, he asumed that this woman's "No" answers to these questions were "probable lies". Therefore, he compared the strength of her physiological reaction to these "control" questions with her reaction to the relevant question, "Was the person who attacked you a woman?" Backster, whose methods are used by FBI and taught at the US Army Polygraph School, teaches that a subject will focus on the source of greatest threat. If he can be truthful in answering the relevant questions, then the examinee is expected to feel that his greatest jeopardy lies in the "fact" that his answers to the control questions are deceptive.

Since the Backster form of CQT is widely regarded as the state of the art of polygraph interrogation, it is appropriate to consider its assumptions with some care:

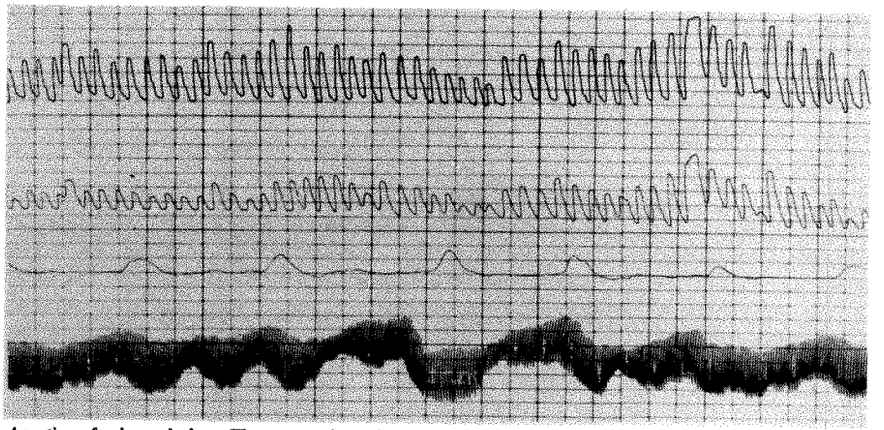
Assumption 1

The examiner will be able to invent several questions referring to the subject's past which the subject will answer deceptively. Is it reasonable to assume, in spite of her denials, that this rape victim practised masturbation before the age of 15 and that she subsequently had at least one homosexual experience? In 1982, in connection with a leak of information to the *Washington Post*, the chairman of the Joint Chiefs of Staff and the Secretary of the Army were given polygraph tests by Pentagon examiners. The questions used in those tests are not in the public domain, yet we can still ask ourselves whether it is reasonable to assume that those examiners were able to invent questions to which these high officials "probably lied".

Assumption 2

A subject who can answer the relevant question truthfully will be more disturbed by the "controls" than by the relevant questions. In the case of this rape victim, perhaps all one can say is that it is unreasonable to predict with any confidence which questions she might find more disturbing.

Floyd Fay, on the other hand, was confronted with a clearer choice[4]. Fay was arrested in Toledo in 1978 and charged with robbing and murdering an acquaintance who, before dying, had stated that the masked robber "looked like Buzz (Fay)". Fay was held without bond for two months while the police searched, in vain, for evidence tying him to the murder. Finally, the prosecutor offered to drop the charges if Fay passed a polygraph test, but required Fay to stipulate that the results should be admissible in court if the test indicated deception. Fay agreed, failed the test, failed a second test by a different examiner, was tried and convicted of aggravated murder and sentenced to life imprisonment. After more than two years, the real killers were caught; they confessed, exonerating Fay who was promptly released.



A section of polygraph chart. The top two channels record thoracic and abdominal breathing movements. The third pen records changes in the electrical conductance of the skin; the wave-like electrodermal responses are related to insensible sweating of the palms. The bottom trace is the "cardio" channel; the pen deflects with each heart beat and the entire tracing moves up and down with changes in blood pressure.

A relevant question in Fay's first test was "Did you shoot Fred?" A "control" question was, "Before the age of 25, did you ever think of hurting someone for revenge?" Because Fay's physiological response to the first question was, in the examiner's opinion, somewhat stronger than his reaction to the second, Fay was diagnosed as "deceptive". What the jury heard was that this examiner had many years of experience, that he had administered a standard control question polygraph test and that in his professional opinion Fay was deceptive in denying his guilt. Such is the strength of the myth of the lie detector that the jury had little difficulty in concluding "beyond reasonable doubt" that this man, who had no previous criminal record and against whom there was no credible evidence, was guilty of murder.

The two tests that Fay "failed" were typical examples of the CQT format which spokesmen for the polygraph industry claim to be accurate at least 95 per cent of the time. F. Lee Bailey, for example, a polygrapher before he became a defence lawyer, asserted recently on national television that, "Out of 100 tests, we expect 96 to be correct, three to be inconclusive and only one to be in error". To accept such a claim, we must be able to agree that an innocent person, accused of killing Fred, will be less disturbed by the question, "Did you shoot Fred?" than by the question, "Before the age of 25, did you ever think of hurting someone for revenge?" — in 96 cases out of 100.

Assumption 3

The third assumption of CQT is that guilty persons, who must answer the relevant questions deceptively, will show stronger reactions on the polygraph to the relevant than to the "control" questions. While this seems plausible, at least in comparison with the first two assumptions, it should not be accepted too readily. John Reid himself showed in 1947 that a sophisticated subject can artificially augment his natural response to a question by self-stimulation — by biting his tongue or pressing on a nail hidden in his shoe — and that the examiner cannot distinguish these augmented responses from normal ones. Thus, a person who can recognize the "control" questions as they are asked might "beat" the lie detector by augmenting his responses to these questions so that they are stronger than his natural responses to the relevant questions. Floyd Fay confirmed this idea while serving time in the London (Ohio) Correctional Facility. That prison routinely used polygraph tests to adjudicate charges against inmates of violating prison regulations. Having studied the literature on the polygraph, Fay decided to attempt his own experiment. By the time he was exonerated and released, Fay had managed to coach 27 inmates, all scheduled to take polygraph tests and all willing to confess to him that they were guilty; 23 of the 27 beat the lie detector.

The evidence

The recent OTA report, which found no credible scientific evidence of the validity of R/I or screening tests, did find 10 field studies of the accuracy of CQT that it considered to be worthy of consideration. The earliest of these[5] compared results of tests administered to criminal suspects with the judgement of a panel of legal experts who later reviewed the dossier on each suspect. Because the polygraphers in this study based their conclusions on what they knew of the evidence and on their impressions of the suspects' demeanour during the interview, as well as on whatever they inferred from the charts, we cannot tell what contribution, if any, the actual polygraph results made to their performance. To assess the accuracy of the lie detector in real life, we must use tests given in real life situations (since we cannot simulate in the laboratory the emotional concerns of an actual criminal suspect) and we must then have the polygraph charts evaluated by a different examiner from the one who administered the test.

Another requirement is to find a criterion by which to identify those

suspects who were actually lying and those who were being truthful. Some individuals will confess their guilt at a later time so that we know they were deceptive on the polygraph. Such confessions often will clear another suspect whose tests are then verified as truthful. Finally, it is plainly improper for a polygrapher to select the tests to be re-scored, choosing only those examples that he did score or would have scored correctly. Several of the other studies considered by OTA appear to have violated this rule.

In one good study[6], for example, 109 successive suspects were tested by one examiner and then the charts were scored by a second polygrapher. A panel of five experts reviewed the final evidentiary files and excluded cases where they felt that the evidence did not permit a decision as to guilt. On the 51 cases where there was both a panel decision and a definite conclusion by the examiner, the average accuracy achieved was 72 per cent (against a 50 per cent chance expectancy). The polygrapher correctly labelled 98 per cent of the guilty suspects as deceptive, but he did this at the expense of the innocent suspects, 55 per cent of whom were also called deceptive. This study is one of the three extant that clearly meet the criteria specified above.

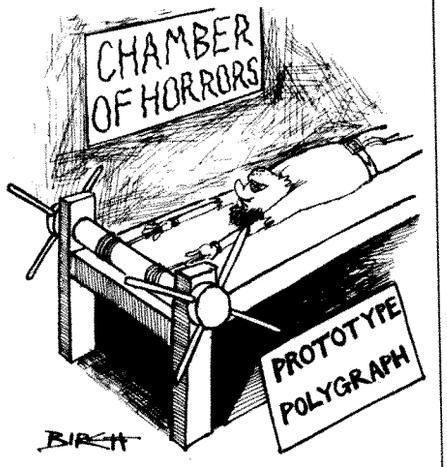
Subsequently, however, the guilt or innocence of 16 of the original 109 suspects was verified by confession and the polygrapher was shown to have been correct in 14 of these cases; he classified the other two as inconclusive. He then submitted these 16 charts to 25 other polygraphers for re-scoring, finding that those who used the same scoring system as he did scored these particular charts as he had done. This small study[7] provides evidence that polygraphers who use numerical scoring will tend to agree with one another, but it cannot be taken as evidence of the accuracy of CQT. The original study, from which these few charts were drawn, provides that evidence and it shows that CQT was wrong 28 per cent of the time overall and that it was biased against the truthful persons, more than half of whom were erroneously scored as deceptive.

Guilty knowledge test

A fundamentally different procedure for the polygraphic interrogation of criminal suspects attempts to detect not lying but the presence of guilty knowledge. In the rape case described earlier, the victim's twisted assailant did a variety of unusual things during the two hours or so that he spent with her. When she chanced to identify him on the street four months later, the memory of most of those acts would still have been with him. The police examiner might have conducted a polygraph test using questions like:

"The rapist in this case poured some sort of fluid between his victim's legs. If you are the man, you will remember what liquid it was. Just sit quietly now and repeat the names of these liquids. What liquid did the attacker pour between the victim's legs? Was it — (1) water? (2) beer? (3) nail polish? (4) urine? (5) perfume? (6) blood?".

Since people tend to respond most strongly to the first item in a series, the first alternative is never correct and never scored. An innocent person might react physiologically to each of the five scored alternatives but would have only about one chance in five of reacting most strongly to the correct alternative (nail polish, in this case). With 10 multiple-choice items of this type, an innocent person would have less than one chance in 10 million of reacting most strongly to all 10 correct alternatives in the absence of guilty knowledge. The guilty knowledge test (GKT), in laboratory experiments with simulated crimes, has repeatedly been found to detect most of the "guilty" suspects while clearing all of the innocent ones. In the absence of research on GKT under real life conditions, however, it would be rash to assume that it



can work equally well in actual criminal investigation. This research has not been attempted, partly because GKT is more difficult to implement than merely asking the suspect, "Did you do it?" and partly because polygraphers continue to believe in the myth of the lie detector. In the actual rape case, the same examiner who had concluded from testing the victim that her assailant was a woman also tested the man she later identified as the rapist. Not too surprisingly, this man was diagnosed as truthful.

Conclusions

The type of polygraph test used for screening job applicants (or federal employees requiring top secret security clearance) assumes that physiological reaction to accusatory questions is evidence of deception. There is no credible scientific evidence concerning the validity of such screening tests. Since highly socialized persons (religious, conscientious persons) tend to fail polygraph tests even when truthful, whereas poorly socialized persons — or sophisticated ones such as the KGB agents trained at their anti-polygraph school in Poland — tend to pass such tests even though lying[10], it is possible that the use of polygraph screening may produce results that actually are opposite to those intended.

The type of test used in criminal investigation or other specific issue situations also is predicated on implausible, even fantastic, assumptions. The three credible studies we have of the accuracy of these tests indicate that they are wrong nearly one-third of the time overall. The three studies show that an innocent person has almost a 50:50 chance of "failing" the lie detector. For this reason, when several persons are tested in respect to the same issue, polygraphers typically withhold judgement until all results are in. In the Pentagon case mentioned above, all 36 members of the Defense Resources Board were tested before one of them was identified as the culprit who leaked the information. Had the tests been scored independently, it is likely that a dozen or more "culprits" would have been identified. (The man who failed the polygraph, it turned out, was innocent; the real culprit apparently beat the lie detector. Even so, polygraphers here can claim 34/36 or 94 per cent accuracy!)

In the wake of the Geoffrey Prime spy case, and on the recommendation of the US security agencies, the British Government has decided to begin using the polygraph in vetting personnel of GCHQ and the Secret Service. Apart from damage done to the careers and reputations of innocent persons, this decision is likely to result in the loss to the government of some of its most conscientious civil servants, replacing them with the undersocialized types who easily pass polygraph tests. Because of the tendency to slightly more expensive but more effective security procedures, once polygraph testing has been introduced, this decision may well open the door to easy penetration of the security services by foreign agents trained to beat the polygraph.

The ever-expanding dependency on this pseudoscientific technique, in the face of both reason and evidence, implies an alarming degree of irrationality among decision makers in private industry, in the criminal justice system and even at the highest levels of government. □

1. Lykken, D.T. *A Tremor in the Blood: Uses and Abuses of the Lie Detector.* (McGraw-Hill, New York, 1981).
2. Marston, W.M. *The Lie Detector Test.* (Smith, New York, 1938).
3. *Scientific Validity of Polygraph Testing: A Research Review and Evaluation* (Office of Technology Assessment, Washington, 1983).
4. Cimerman, A. *Criminal Defense* **8**, 7–10 (1981).
5. Bersh, P. *J. appl. Psychol.* **53**, 399–403 (1969).
6. Barland, G.H. & Raskin, D. *Report No. 76-1, Contract No. 75-NI-99-0001* US National Institute of Justice, Washington, 1976.
7. Raskin, D. *Proc. Stockholm Symp. Witness Psychol.*, 319–371 (1982).
8. Horvath, F. *J. appl. Psychol.* **62**, 127–136 (1977).
9. Kleinmuntz, B. & Szucko, B. *Law Soc. Rev.*, **17**, 84–104 (1982).
10. Waid, W., Orne, M. & Wilson, S. *Psychophysiology*, **16**, 15–21 (1979).



IUPHAR 9TH INTERNATIONAL CONGRESS OF PHARMACOLOGY

29 JULY – 3 AUGUST 1984

LOCATION

Barbican Centre for Arts and Conferences, City of London

SCIENTIFIC PROGRAMME

The scientific programme will extend over five days and consist of symposia, invited lectures, pharmacological debates, an educational meeting and free communications, the latter as both oral and poster sessions. The symposia will consist of three simultaneous sessions morning and afternoon and each day there will be four invited lectures. A new feature of the International Conference is the pharmacological debates. In these the intention is to select topics on which there are clear differences in evidence or its interpretation and have the issue debated between protagonists of each view with sufficient opportunity for the audience to participate. In the educational meeting it is hoped to concentrate on the place of various forms of programmed learning in Pharmacology and the instrumentation available. Finally, there will be free communications and to the fullest extent possible authors will be allowed to select whether to give these as oral communications or as a poster.

PROCEEDINGS

The Congress Proceedings are being published by Macmillan Press and may be ordered in advance on the registration form or at the Congress.

SOCIAL EVENTS

A full social programme is being arranged for delegates and accompanying guests. Details are given in the advance programme.

ACCOMMODATION

Accommodation has been reserved at advantageous rates in a number of hotels in various price categories and in student halls of residence. All accommodation is within easy travelling distance of the Barbican Centre.

DATES TO REMEMBER

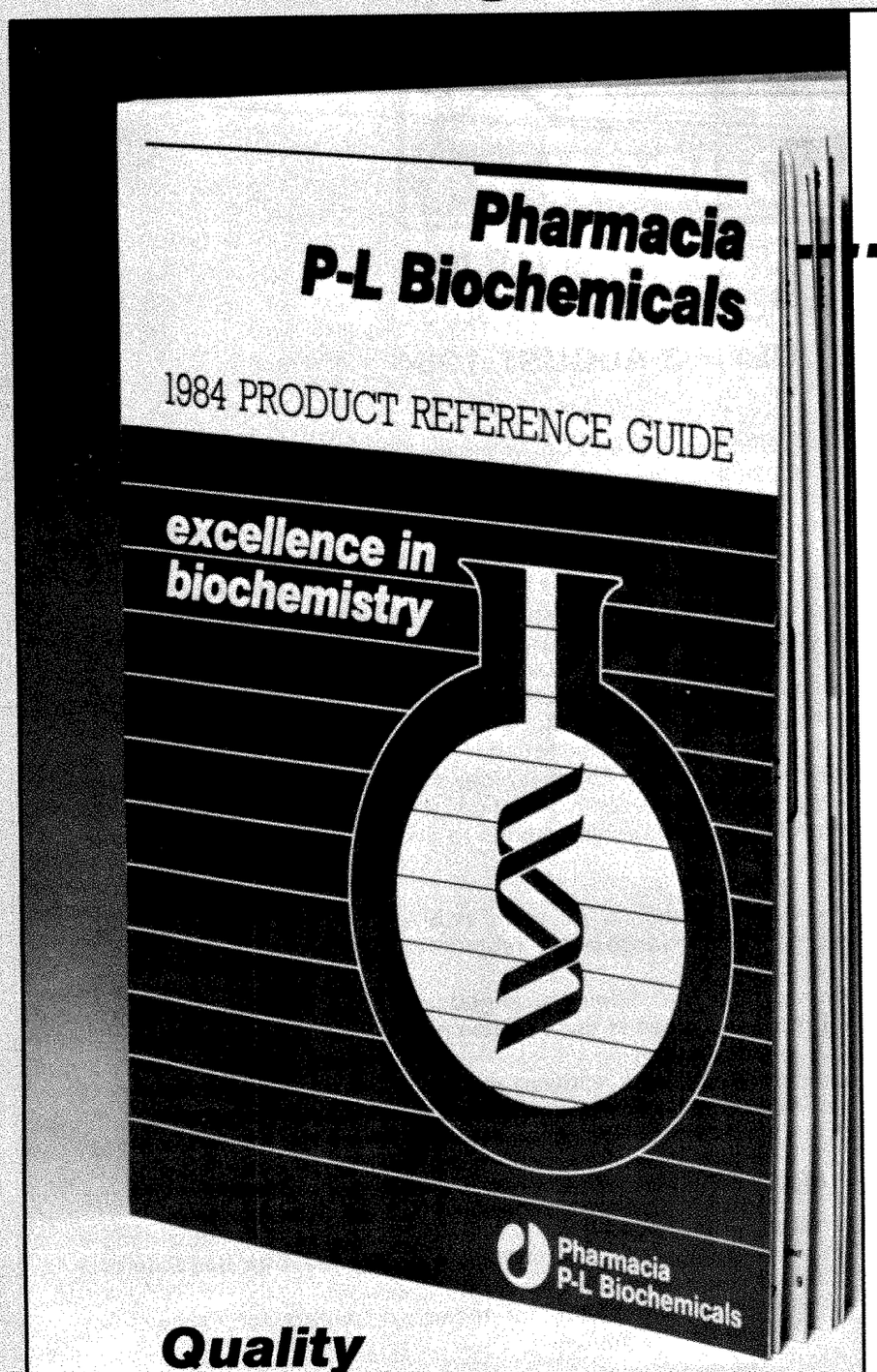
1 May 1984	Deadline for college accommodation reservations.
18 May 1984	Deadline for receipt of registration at the standard fee (£150). There is a late fee after this date of a further £40. Deadline for receipt of papers for Proceedings volumes.

CONGRESS SECRETARIAT

Advance programmes, registration forms and abstract forms can be obtained from the Congress Secretariat:–

Congress Associates ICP
34 Stanford Road
London W8 5PZ
Telephone: 01 937 3163

There is only one product in this catalog...



...Quality

Actually, the Pharmacia P-L Biochemicals Product Reference Guide lists over 2000 products for biochemistry and molecular biology. The products range from restriction nucleases to coenzymes, from lipids to polynucleotides – but our most important product is the quality that goes into each biochemical that we make.

At Pharmacia P-L Biochemicals, we take our commitment to "excellence in biochemistry" seriously. We make our biochemicals with quality – we know no other way.

The 1984 Product Reference Guide from Pharmacia P-L Biochemicals

For your copy, contact your local Pharmacia Fine Chemicals/Pharmacia P-L Biochemicals representative or write to one of the addresses below.

Quality biochemicals for quality research.

Pharmacia P-L Biochemicals Inc.
Subsidiary of Pharmacia Inc.
800 Centennial Avenue
Piscataway, NJ 08854

Pharmacia P-L Biochemicals Inc.
Subsidiary of Pharmacia Inc.
Box 175
S-751 04 Uppsala 1, Sweden

Pharmacia P-L Biochemicals

Circle No.09 on Reader Service Card.

Abundant phase transitions

The bewildering variety in which phase transitions come is neatly illustrated in a collection of papers to be found in a single issue of a journal.

LAST year's final issue (on 26 December 1983) of *Physical Review Letters*, a journal which is no longer the exclusive preserve of high-energy physicists, contains half a dozen new twists in the understanding of phase transitions. What follows is merely a selection.

Simplest, perhaps because it is experimental, is a study of the properties of plutonium antimonide (chemical formula PuSb) which, among other things, has entailed the preparation of what are said to be the first "sizeable crystals" of any plutonium compounds — they come out at 8 mm^3. That in itself has been a substantial undertaking. Crystals of PuSb have been grown and embedded in a matrix of metallic copper (B.R. Cooper *et al. Phys. Rev. Lett.* **51**, 2418) by a four-cornered collaboration involving the Los Alamos National Laboratory, the University of West Virginia, ETH at Zurich and the Transuranium Institute at Karlsruhe (on account of its "unique handling and glove-box facilities"). The trick of surrounding the crystals with solid copper has the advantages of eliminating the toxicity hazard of the plutonium but also of preventing self-heating due to spontaneous fission.

The interest of PuSb is its magnetism. Plutonium is an element whose 5*f* shell is only partly filled with electrons. Indeed, the triply charged plutonium ions in the antimonide can have only five 5*f* electrons, whence the argument that the ion must have much in common with the electronic structure of the trivalent cerium ion Ce^{3+}, which has only one electron in the 4*f* shell.

This expectation seems to be borne out by experiment. PuSb, a cubic crystal, has markedly anisotropic properties which imply that the *f* electrons are not strictly localized on the atoms which contribute them to the lattice but, rather, are stretched out towards their nearest neighbours. Cooper *et al.* argue that this is an interesting case of hybridization between atomic and band electrons. Their discussion of the strength of the magnetic coupling between neighbouring plutonium atoms in the lattice is necessarily incomplete, but does not obscure the striking circumstance that PuSb is a ferromagnetic material below 75K and an antiferromagnetic material above, with a transition to disorder at 85K.

The obvious interest of the data now published is that they offer a way of testing different methods of approximation to interionic forces — and that there are more where these have come from. The antiferromagnetic phase of PuSb appears from neutron diffraction to have long-range order in which magnetic spins cancel out over a distance of about two lattice units.

This is comparable with the nature of the long-range order with respect to translation that occurs in the nematic phase of liquid crystals, where planar (and usually polar) molecules are spatially ordered. In the same issue of *Phys. Rev. Lett.* (p.2386), G. Grinstein and John Toner from the IBM Research Center at Yorktown Heights, New York, claim a remarkable prediction of a previously unrecognized property of those liquid crystals which can exist in the nematic phase and the two smectic phases *a* and *c*, the second of which involves both orientational and translation ordering of the planar molecules.

Which of the three phases will be assumed by any material will be a function of the only two adjustable parameters available — the temperature and the concentration of the material in its solvent. On such a plot, the pairwise phase boundaries should come together at a triple point, but so far this occurrence seems to have been unexplored. Grinstein and Toner argue that there cannot be a simple triple point but, rather, that there must be a fourth phase, a kind of hybrid between the nematic and smectic *c*.

Physically, what this implies is that the geometrical rearrangement of the planar molecules needed to accomplish the phase transition requires more than two steps to be taken in succession, but the point is established by means of A. C. Wilson's correspondence between lattice statistics and the renormalization theories that usually turn up in gauge field theories. None of this, of course, implies that the fourth phase will exist over a wide range of concentration or of temperature — indeed, the predicted effect may turn out to be too fleeting to be recognized.

The solidification of xenon adsorbed in gaseous form on a platinum surface has, however, been neatly observed by the scattering of helium atoms. Bene Prelsema, Laurens K. Verheij and George Comsa of the Low-Temperature and Vacuum Physics Division of the West German nuclear research centre at Julich point out (p.2410) that the cross-section for scattering helium atoms from molecules (for example CO) adsorbed on a metal surface can be very much larger than simple geometry suggests, perhaps as much as 125 $Å^2$. The result is that it is possible to use helium scattering as a measure of aggregation of adsorbed molecules for each possible density of coverage. Hitherto, attempts to measure condensation in adsorbed xenon have been unsuccessful, chiefly because of the need to work with surfaces which have been densely covered with adsorbed molecules. The helium atom technique works, but at the opposite end of the range, with as few as one in a thousand adsorption sites occupied. The result is what must have been hoped for. The transition from two-dimensional gas to two-dimensional solid occurs at lower coverage by adsorbed gas with decreasing temperature (at least down to that of liquid nitrogen). It has even been possible to infer the heat of vaporization of solid two-dimensional xenon (1.1 kcal per mole) and, because the densities of adsorbed gas at which crystallization occurs are indeed low, to explain why the phenomenon has not been previously observed. While xenon itself is of moderate interest, the helium scattering technique is likely to be widely used.

No fewer than three other types of phase transitions crop up in the same issue of *Phys. Rev. Lett.*, of which the transition known as commensurate-incommensurate is perhaps the simplest. A. H. Moudden, D. E. Moncton and J. D. Axe from the Brookhaven National Laboratory describe (p.2390) measurements of the behaviour of the dipolar molecular thiourea in strong electric fields using X rays to look for periodicity in a crystal lattice. At high field strength, greater than 1,000 volts per mm, thiourea crystals behave as if successful slab-like domains in which dipoles are oriented in one direction or the opposite in an alternating fashion along the field. The numbers suggest that under these conditions the slabs may be either 7 or 8 molecular planes thick. Decreasing the electric field allows 9-fold slabs to occur and also suggests how the relaxation to low field conditions may show hysteresis, as is observed.

What else? The process of wetting a solid surface is also a candidate for phase transition while the problem of percolation, in which free moving molecules thread their way through a tortuous lattice, clogging the system if there are too many of them, is dealt with for the first time on a lattice of fractal dimensions, no doubt simulating more accurately what may happen in the percolation of petroleum through, say, a reservoir rock.

John Maddox

© 1984 Macmillan Journals Ltd

Genetics

DNA supercoiling and gene expression

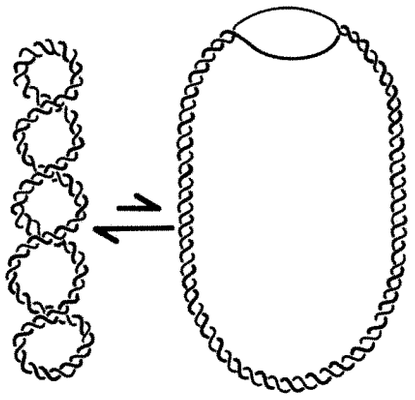
from L. Mark Fisher

SUPERCOILING of DNA — coiling of the DNA helix axis — is now known to have a dramatic effect upon DNA replication, recombination and transcription in bacteria[1-3]. But how are bacteria able to regulate the supercoiling of their DNA? Recent work reveals that the degree of DNA supercoiling depends upon a dynamic balance between two mutually antagonistic enzyme activities: DNA gyrase which supercoils DNA, and DNA topoisomerase I and perhaps other enzymes which remove supercoils. Intriguingly, expression of the genes for one of these enzymes — DNA gyrase — appears to be itself regulated by DNA supercoiling[4,5].

Circular chromosomal and extrachromosomal DNA is actively maintained in a negatively supertwisted state by the ATP-dependent bacterial enzyme DNA gyrase. When gyrase activity is blocked by chemical or genetic means, relaxation of cellular DNA rapidly ensues with pleiotropic biological effects, including the halting of replication[2,3]. The coexistence in *Escherichia coli* of both gyrase and DNA-relaxing activity, predominantly DNA topoisomerase I (omega protein), suggests that both these competing activities may play a part in determining the cellular level of DNA supercoiling. Evidence to support this proposal has come from a series of elegant experiments made possible by the localization of the structural gene for topoisomerase I (*topA*) and the isolation of *E.coli* strains carrying mutations in this gene[6,7].

TopA maps to a region of the chromosome near *cysB* and is not closely linked to either *gyrA* or *gyrB*, which encode the gyrase A and B subunits. Point mutations in the *topA* gene of *E.coli* and their effects on DNA supercoiling have recently been studied in some detail[6,8,9]. When a strain bearing the nonsense mutation *top10* was examined, a level of DNA supercoiling higher than that of the isogenic wild-type strain was found. As the *top10* strain produces only low levels of topoisomerase I activity the simplest conclusion is that topoisomerase I acts in removing DNA supercoils *in vivo*. Deleting the entire *topA* gene leads, however, to an apparently contradictory result — the degree of DNA supercoiling is lower than that of the isogenic Top A$^+$ counterpart[9].

This paradox was resolved with the discovery that deletion of the *E.coli topA* gene is accompanied by the acquisition of a secondary mutation, closely linked to *gyrA* in one strain and to *gyrB* in another strain[8,9]. The respective gyrase A and B subunits purified from these strains are partly defective and are less efficient in promoting DNA supercoiling than wild-type subunits[4]. Apparently, too much supercoiling caused by a total absence of topoisomerase I is as deleterious as too little. Compensation for a lack of topoisomerase I activity is achieved by spontaneous mutations that reduce the supercoiling activity of gyrase. Thus, selective pressure exists to maintain an intermediate biologically acceptable degree of supercoiling.



Underwound circular duplex DNA (right) forms negative supercoils (left). DNA in the form of a closed circle with about one helical turn for every ten base pairs — the same pitch as linear duplex DNA in the B configuration — is said to be 'relaxed'. If the two strands of the duplex circle are intertwined fewer (or more) times than in the relaxed circle, the DNA minimizes its departure from the energetically favoured configuration by coiling about its axis, as in the figure. By convention, the supercoils formed by underwound DNA are referred to as 'negative' and those by overwound DNA as 'positive' (see *News and Views* **294**, 607; 1981).

How is the degree of supertwisting regulated in wild-type cells? Experiments by Gellert, Menzel and co-workers demonstrate that synthesis of gyrase is itself controlled by DNA supercoiling[4,5]. First, they carried out two experiments which showed that gyrase synthesis is activated by conditions that lower gyrase activity. Using antibodies raised against purified gyrase A and gyrase B proteins they measured the relative rates of synthesis of these proteins in various conditions. Treatment of *E.coli* with either of the gyrase inhibitors novobiocin or coumermycin, at concentrations known to induce relaxation of chromosomal DNA, led to a 10-fold increase in the rate of gyrase A and gyrase B synthesis. The time course of gyrase induction closely follows that observed for chromosomal DNA relaxation promoted by the inhibitor. Next, a strain carrying two mutations in *gyrB*, one conferring temperature sensitivity to growth, the other coumermycin resistance, were shown to have normal levels of gyrase A and B proteins when challenged with coumermycin. A shift to the non-permissive temperature, however, resulted in increased synthesis of both gyrase A and B subunits when compared with the isogenic *gyr B*$^+$ strain.

To confirm that DNA relaxation is the cause of gyrase induction, cloned *gyrA* and *gyrB* genes contained in plasmids were used to programme a cell-free protein synthesis system. The *gyrB* plasmid maintained in a relaxed state by the inclusion of novobiocin (to inhibit endogenous gyrase in the extract) produced up to 100 times more gyrase B protein than the plasmid in its supercoiled conformation. Stimulation of gyrase B synthesis was also observed in the absence of gyrase inhibitors when purified topoisomerase I from *E.coli* or HeLa cells was added to the system. Similar effects were seen in experiments using the *gyrA*-containing plasmid. These results show that gyrase synthesis is promoted by a relaxed DNA template and render unlikely the formal possibility that gyrase might regulate its own synthesis by binding to putative repressor sequences on DNA.

It is interesting that both the *E.coli* and the HeLa topoisomerase I enzymes act as positive effectors of gyrase synthesis. The fact that a eukaryotic relaxing enzyme can stimulate gyrase synthesis argues that these enzymes exert their effects by relaxing the DNA template rather than by binding to regulatory sequences on DNA. What controls the expression of topoisomerase I is not yet known although the recent cloning and analysis of the *topA* gene by Wang and colleagues should allow progress to be quickly made[10]. As yet, the possibility remains open that other DNA-relaxing enzymes isolated from *E.coli*, for instance topoisomerase II' (refs 11, 12), or topoisomerase III[13], may operate in concert with gyrase and topoisomerase I.

GyrA and *gyrB* belong to an emerging class of genes whose expression is dependent on DNA conformation[14].

1. Fisher, L.M. *Nature* **294**, 607 (1981).
2. Cozzarelli, N.R. *Science* **207**, 953 (1980).
3. Gellert, M. *A.Rev.Biochem.* **50**, 879 (1981).
4. Gellert, M., Menzel, R., Mizuuchi, K., O'Dea, M.H. & Friedman, D.I. *Cold Spring Harb. Symp.quant.Biol.* **47**, 763 (1982).
5. Menzel, R. & Gellert, M. *Cell* **34**, 105 (1983)
6. Sternglanz, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 2747 (1981).
7. Trucksis, M. & Depew, R.E. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2165 (1981).
8. DiNardo, S., Voelkel, K.A., Sternglanz, R., Reynolds, A.E. & Wright, A. *Cell* **31**, 43 (1982).
9. Pruss, G., Manes, S.H. & Drlica, K. *Cell* **31**, 35 (1982).
10. Wang, J.C. & Becherer, K. *Nucleic Acids Res.* **11**, 1773 (1983).
11. Brown, P., Peebles, C. & Cozzarelli, N.R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6110 (1979).
12. Gellert, M., Fisher, L.M. & O'Dea, M.H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6289 (1979).
13. Dean, F. *et al. Cold Spring Harb. Symp. quant. Biol.* **47**, 769 (1982).
14. Smith, G.R. *Cell* **24**, 599 (1981).
15. Sanzey, B. *J. Bact.* **138**, 40 (1979).
16. Saucier, J-M. & Wang, J.C. *Nature new Biol.* **239**, 167 (1972).

0028-0836/84/080686-01$01.00

Promoters that are activated by DNA supercoiling have already been identified and include those for catabolite-sensitive genes — the maltose operons, the lactose and galactose operons and the tryptophanase gene[15]. The mechanism by which DNA supercoiling enhances transcription from these promoters is not well understood. What is clear, however, is that RNA polymerase unwinds DNA on binding to the promoter — a process which should occur more easily on negatively supercoiled DNA as it is already partially unwound[16]. Why then is gyrase synthesis activated by a relaxed rather than a negatively supercoiled DNA template? At present this problem is unresolved although preliminary evidence again suggests that control is exercised at the level of RNA transcription[4]. Structural and functional analysis of gyrase genes and their promoters should help clarify how DNA supertwisting differentially affects gene expression. □

L. Mark Fisher is in the Department of Biochemistry, St George's Hospital Medical School, University of London, Cranmer Terrace, London SW17 0RE.

Science journals

Cryptozoology

from Robert M. May

OF the 79 *News and Views* pieces I have written over the past ten years, the one attracting far and away the most attention outside the ranks of *Nature* readers was a brief *jeu d'esprit* on 'The ecology of dragons' (*Nature* **264**, 16; 1976). This article was picked up by United Press International, and resulted in telephone calls from radio stations and newspapers in most English-speaking countries, offers to appear in three television shows and an invitation to write a coffee-table book about dragons. Needless to say, none of my other *News and Views* articles has produced anything approaching this reaction. The dragon article differs from the others only in that it deals with the unreal and fanciful, rather than with the real wonders that actually exist in the natural world. To generalize recklessly and gloomily, I think this isolated experience reflects a tendency on the part of the media — and maybe the public — to prefer meretricious marvels to real ones.

Be all this as it may, it is consistent with the fact that, of the plethora of new journals established in 1982–1983 (see the 6–13 October 1983 issue of *Nature*), *Cryptozoology* was the only one reviewed in *Newsweek*. *Cryptozoology* is the official journal of the International Society of Cryptozoology, whose president, Bernard Heuvelmans, defines the subject as "the science of 'hidden' animals". The first and to date only volume of the journal runs to 100 pages embracing articles, research reports, field reports and book reviews. By a rough count, 29 pages deal with 'wildmen' of one kind or another (such as 'Sasquatch' or 'bigfoot' in the Pacific Northwest and 'yeti' in China), 7 pages with mermaids in Papua New Guinea, 33 pages with the mysterious entities in Loch Ness and Lake Champlain, 16 pages with the putative sauropods (Mokele-Mbembe) of the Central African swamp-forest, and 15 pages with general definition and defense of cryptozoology as a legitimate subject for research. Clearly, if you want to be an interesting cryptobeast, you are well advised to be a large vertebrate, and preferably human-like or dinosaur-like.

More than half the pages devoted to human-like creatures are reviews of five books. These books appear to deal mainly with second- and third-hand anecdotes; it seems generally agreed that the hard evidence — photographs, tapes, casts of footprints — is fraudulent. An article by Guoxing on 'The status of wildman research in China' gives an earnest account of available reports, and speculates on whether the Chinese wildmen are surviving descendants of *Ramapithecus, Australopithecus* or possibly *Gigantopithecus*. With commendable honesty, the article concludes "it should be emphasized that many of the participants in Wildman research in China are professional scientific workers. At the same time, however, it has to be admitted that most of them are not well trained in faunal ecology, primatology, vertebrate paleontology, paleoanthropology, physical anthropology and other disciplines concerned with this topic". Wagner recounts tales told him by New Guinea natives about the mermaid-like *ri*, and comes to three conclusions: "they are certainly not dugongs" (it is sometimes suggested that manatees or dugongs gave rise to sailors' stories about mermaids); the reports are more substantial than the "inventories of rather fanciful 'bush' creatures" familiar to anthropologists working in New Guinea; and the reports "strongly suggest that some such creature exists, and that it remains unknown to science".

The papers on dinosaur-like creatures are, on the whole, less silly. A research report by LeBlond employs a photograph of the 'Lake Champlain monster' taken by Mansi in 1977, and uses standard results relating the appearance of the sea surface to wind speed, and thence to the length of wind waves, to estimate that the length of the 'monster' visible in the photograph is in the range 5–17 m. The evidence reviewed in other articles in *Cryptozoology* and in the pages of *Nature* over the past few years suggests that large objects of some kind (logs look like the best guess to me) are present in deep lakes such as Loch Ness or Lake Champlain, and LeBlond's elegant estimate of the physical dimension of one such object is useful.

Mackal, Greenwell and Wilkinson recount persistent reports of Mokele-Mbembe, a large creature with a long flexible neck and a tail like an alligator's, which inhabits the 60,000 square mile Likouala swamps in the remoter parts of the People's Republic of the Congo. These investigators recorded stories from the native inhabitants, including one of a hunter who had tracked a creature that left a path of grass flattened as if by a great tail, and positive reactions to photographs of sauropods. The reports also suggested that the apple-size fruit of the molombo (a tropical vine found in Africa) were the main diet of the Mokele-Mbembe. A separate paper by Weber, Berry and Greenwell therefore analyses the nutrient content of these fruit, and compares it with the nutrient requirements of typical large mammals, to conclude that molombo fruit alone could not sustain the Mokele-Mbembe. Mackal and his collaborators sensibly conclude that "our expedition found no compelling evidence that Mokele-Mbembe exists", although they append the usual appeal for further investigation.

Many people feel that the correct response from a scientist to the kind of enterprise this journal represents should be one of friendly, even encouraging, tolerance. At very least, I am persuaded such may be an expedient image to project. We would be ill-served if everyone studied safe subjects. But, in truth, my reaction to *Cryptozoology* is regret for the money libraries will waste on acquiring the journal (if only as a curiosity) and regret for the dissipated efforts that could be directed more productively to studying some of the species of tropical plants, insects and other organisms that may be going extinct at a faster rate than they are being classified. Even on its own Alice-Through-The-Looking-Glass terms, the journal *Cryptozoology* is marked by a rather arbitrary focus on only two groups — human-like and dinosaur-like — from the full cryptozoological spectrum of animals that may or may not exist; this spectrum is catalogued by Cohen in *The Encyclopedia of Monsters* (Dodd, New York, 1983). Some of the omissions are understandable: for instance, the lack of work on the unicorn is undoubtedly because these beasts are fairly generally agreed to have been hunted to extinction, a process made easier by virtue of their maladaptive attraction to virgins. Less understandable — especially to an antipodean — is the absence of any work on the bunyip, a particularly engaging but elusive member of the Australian cryptofauna. □

Robert M. May is Class of 1877 Professor of Zoology at Princeton University, Princeton, New Jersey 08544.

© 1984 Macmillan Journals Ltd

Carbon cycle

New data upset ice age theories

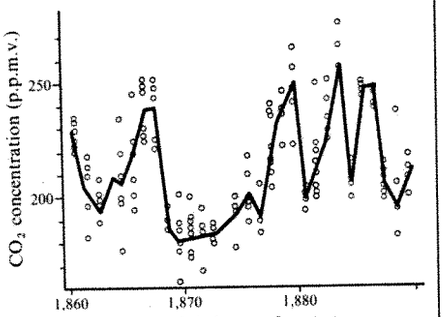
from Philip Campbell

"It was the best of times, it was the worst of times... it was the epoch of belief, it was the epoch of incredulity..."

AT the risk of over-dramatization, the opening lines of Dickens' *A Tale of Two Cities* reflect something of a recent meeting* devoted to the carbon cycle. Little of the meeting dealt with the effects of fossil fuels, the participants encountering enough problems and surprises from the natural cycle before it was perturbed by man. In particular, they were confronted with natural rates of change in atmospheric CO_2 concentrations much faster than previously measured, new data from deep-sea cores indicating that CO_2 variations precede changes in the extent of ice sheets (contrary to prevailing ideas), and theoretical results suggesting that atmospheric CO_2 was to a significant extent responsible for the warmth of Cretaceous climate.

A year or two ago, ice cores yielded the surprising result that at the end of the ice age, between 15,000 and 10,000 yr ago, the atmospheric CO_2 abundance climbed from about 210 to about 280 parts per million by volume(p.p.m.v.) in only a few thousand years[1,2]. W.S. Broecker[3] pointed out that over time scales shorter than the 180,000-yr residence time of CO_2 in the atmosphere and oceans, atmospheric CO_2 is controlled by the physical, biological and chemical state of the oceans. Thus such a 'rapid' increase of 70 p.p.m.v. would seem to have required an unusual perturbation to this system. Broecker suggested that detritus from surface organisms, containing organic carbon and phosphate, was deposited on the continental shelves as they were submerged following the melting of ice sheets. This reduced the phosphate/carbon ratio in the oceans and, phosphate being a limiting nutrient, the fixation rate of carbon by marine photosynthesis would have decreased. In this way the CO_2 concentration in ocean surface water, and hence the atmospheric CO_2 content, would have increased by the amount and in the time scale revealed by the ice cores.

A number of results presented at the conference were consistent with this model. For instance, one of its predictions is that, as a result of the removal of organic carbon from the ocean into shelf sediments, the deeper ocean would have become less prone to dissolve carbonate sediments, leading to enhanced preservation of $CaCO_3$. L.C. Peterson and W.L. Prell (Brown University, Rhode Island) sampled a series of deep-sea piston cores raised from the eastern flank of the Ninety-East Ridge in the east Indian Ocean. The depth range over the flank from 2.9 to 4.4 km encompasses today's lysocline, the oceanic boundary below which foraminiferal tests, or shells, will dissolve. Over the 700,000 yr record, both $^{18}O/^{16}O$ isotope ratios — interpreted as a record of global ice volume — and the proportion of carbonate revealed clear periodicities of 100,000, 40,000 and 19–23,000 yr. According to the now-respected Milankovich theory, ice ages are driven at these periodicities by changes, respectively, in the eccentricity of the Earth's orbit, the tilt and the precession of the Earth's rotation axis. More to the point, the amount of $CaCO_3$ present in the cores was a maximum when ice volume was decreasing most rapidly and was a minimum when the ice sheets were expanding at their quickest. This specific relationship concurs with a recent numerical model[4] based on the phosphate-extraction hypothesis. A correlation of sea level falls and increased carbonate dissolution in the Canary Basin, North Atlantic, was also reported by J.T. Crowley (NSF Climate Program, Washington) using a similar experimental approach.



Atmospheric CO_2 variations from the Dye 3 ice core.

The theoretical impetus provided by Broecker's model was illustrated by several computer models of the ocean–atmosphere carbon cycle. F. Knox and M. McElroy (Harvard University) and J.R. Toggweiler and J.L. Sarmiento (Princeton University) described similar models in which the atmosphere, the intermediate/deep ocean, low-latitude and high-latitude shallow ocean reservoirs are represented by distinct boxes, linked by appropriate carbon fluxes. The purposes of these models is to investigate the roles of high-latitude nutrients and of ocean circulation rates in controlling atmospheric CO_2 levels, bearing in mind that restricted areas of the ocean surface, such as the Antarctic upwelling regions where productivity in surface waters is high, can have a considerable effect on the carbon chemistry of the entire deep oceans and the atmosphere. Both models confirm that an increase in high-latitude surface nutrients is associated with enhanced atmospheric CO_2 levels, demonstrating that changes in large-scale (thermohaline) circulation and in high-latitude surface water productivity (caused, for instance, by changes in light levels) could have a crucial role. A 1,000 yr response-time of the predicted changes is imposed by the circulation time of the deep ocean.

All the results described so far have been consistent with and/or stimulated by the phosphate-extraction hypothesis. It is intriguing, to say the least, that two papers were presented that between them — as Broecker (Lamont-Doherty Geological Observatory, New York) himself pointed out — seem to demolish shelf deposition models.

The first challenge has come from ice cores. B. Stauffer, U. Siegenthaler and H. Oeschger (University of Bern) reported measurements of past CO_2 concentrations from the Dye 3 site in Greenland — a record spanning the period 25,000–40,000 yr ago. Four sharp peaks in CO_2, reaching levels of 250 p.p.m.v. from a 'background' of 200 p.p.m.v., occurred during the last ice age (see the figure). The magnitude of the changes are of the order which the phosphate-extraction model seeks to explain, but the excursions appear to have occurred within a hundred years or so — ten times faster than can possibly be explained by shelf deposition. The excursions are well correlated with oxygen isotope changes, as are accompanying variations in trace chemicals Cl^-, NO_3^- and SO_4^{2-} (R.C. Finkel and C.C. Langway, SUNY, Buffalo). Stauffer *et al.* proposed that climate behaved as a bistable oscillator during the last glaciation. If the rapid CO_2 variations are also found in Antarctic ice cores, then the idea that CO_2 acts as an interhemispheric climatic amplifier will be greatly strengthened.

T. Wenk, Siegenthaler and Oeschger (University of Bern) described a box model constructed on identical principles to the two already discussed to explain these rapid variations. They reported that a 50% reduction from present-day conditions in the exchange flux between high-latitude (Antarctic) surface and deep waters results in a decrease in atmospheric CO_2 levels of 50 p.p.m.v., an effect which is enhanced if the large-scale circulation is increased. These authors maintain that changes in surface circulation could account for rapid CO_2 variation, although this view was challenged by other box-modellers. The role of deep-ocean circulation as an inhibitor of such rapid variations was left unresolved; all three box models were steady-state calculations, and models of the transient responses are now required.

A second nail for the coffin of shelf-deposition models was provided by N. Shackleton (University of Cambridge) and N.G. Pisias (Oregon State University), who presented high-resolution oxygen and carbon isotope records from an equatorial Pacific deep-sea core. Because marine

*The Chapman Conference on 'Natural variations in carbon dioxide and the carbon cycle' was held in Tarpon Springs, Florida on 9–13 January.

0028-0836/84/080688-01$01.00

photosynthesis preferentially extracts ^{12}C from surface waters, the $^{13}C/^{12}C$ ratio in foraminifera can indicate changes in the oceanic carbon reservoirs. (It is essential to distinguish between this effect and that of deep-ocean circulation, which causes a progressive isotopic lightening of dissolved CO_2 in deep waters as they circulate through the rain of organic detritus from surface regions. Several talks at the meeting highlighted different ways of tackling this problem.

Broecker had already showed[3] that the difference in carbon isotope ratios in surface and deep waters varies with atmospheric CO_2 levels. Interpreting planktonic and benthic foraminiferal isotope ratios in this manner, Shackleton and Pisias provided a record of ice volume and atmospheric CO_2 over a 340,000 yr period. The Milankovich frequencies were clearly present with the CO_2 record having a stronger high-frequency content — consistent, perhaps, with the ice-core data. Both ice volume and CO_2 changes lag the orbital (Milankovich) changes but, most significantly, the changes in CO_2 lead those in ice-volume by, on average, 2.5×10^3 yr. This result is completely at odds with shelf deposition models (which predict that sea level changes lead CO_2 changes) and the authors concluded that variations in atmospheric CO_2 are part of the direct forcing of ice ages. They argued that changes in the ocean nutrient cycling and in ocean circulation must lie at the root of the CO_2 changes. However, the field is now open for more specific explanations.

This report has so far been restricted to variations in the carbon cycle over time scales of tens of thousands of years or less. But the Florida meeting covered the entire gamut of time scales up to 10^9 yr; those of 10^7–10^8yr, associated with the Cretaceous and Cenozoic eras, were the subject of particularly lively discussion.

A provocative interpretation of South Atlantic deep-sea carbon isotope data from the last 75 Myr was presented by N. Shackleton. He used the ^{13}C record in bulk sediments to deduce changes in the partitioning between limestone and organic carbon and hence in the global organic carbon reservoir. According to his data, the amount of organic carbon stored in sediments 70 Myr ago exceeded that in today's oceans by about 10^{19} moles. Shackleton concluded that oxygen in the atmosphere must have been correspondingly enhanced over the present-day content by 20%; the dissolved oxygen concentration would have been similar to today's because of the reduced solubility in the warmer ocean waters at that time. H.D. Holland (Harvard University) and R.M. Garrels (University of South Florida) suggested, however, that the excess oxygen would have been accommodated by SO_4 in the oceans, and a debate ensued, hingeing on the differences between the residence time of SO_4 in the ocean and the characteristic time scales within Shackleton's data. J.R. Herring (US Geological Survey, Lakewood) maintained that the putative enhancement in oxygen is rebutted by the absence of a significant increase in charcoal fragments or other sedimentary signatures of natural plant burning.

Over the 100 Myr time scale, geological processes provide significant contributions to the carbon cycle. In particular, the balance between weathering, by which CO_2 is removed from the atmosphere and stored as carbonate, and metamorphism (in which the process is reversed) has a significant effect on atmospheric CO_2. Recently a geochemical box model was published[5] in which the oceans, the atmosphere, calcite, dolomite and Ca–Mg silicate reservoirs were linked by the chemical reactions of weathering and metamorphism. The novel aspect of the model lay in its treatment of the chemical rate constants. Those connected with weathering, for instance, were parameterized in terms of atmospheric CO_2 levels.

A number of problems and uncertainties associated with the published model were raised at the conference. However, these were trumped by its instigators, A. Lasaga, R. Berner and R. Garrels (Pennsylvania State, Yale and South Florida University respectively), who presented an extended model in which organic carbon, evaporates and pyrite reservoirs were incorporated. One of the model's central conclusions eventually remained unchallenged: that in the Cretaceous, 100 Myr ago, the amount of CO_2 in the atmosphere was at least four, and maybe over ten times as great as today's, with average temperatures correspondingly elevated.

This conclusion was supported by S. Schneider, S. Thompson and E.J. Barron (National Center for Atmospheric Research). Using a three-dimensional atmospheric circulation model in which the continental geography resembles that of Cretaceous times, they found that no matter how oceanic circulation is varied, winter temperatures in high-latitude continental interiors remain near or below freezing, a situation belied by palaeobotanical evidence. They suggest that elevated CO_2 concentrations might have been the missing factor that produced the higher temperatures of that era.

These models and much of the rest of the work described in this report reflect one of the major themes to come out of the conference. As one of its organizers, E. Sundquist (US Geological Survey, Reston) emphasized, climate and the carbon cycle appear to have been more tightly linked than previously thought. □

Philip Campbell is Physical Sciences Editor of Nature.

1. Delmas, R.J. *et al. Nature* **284**, 155 (1980).
2. Neftel, A. *et al. Nature* **295**, 220 (1982).
3. Broecker, W.S. *Progr. Oceanogr.* **11**, 151 (1982).
4. Keir, R.S. & Berger, W.H. *J. geophys. Res.* **88**, 6027 (1983).
5. Berner, R.A. *et al. Am. J. Sci.* **283**, 641 (1983).

Evolutionary biology

When deviants are favoured: evolution of sex determination

from Paul H. Harvey and Linda Partridge

A NEW book from J.J. Bull[1] makes a fresh attempt at explaining the evolution of mechanisms that determine sex. What makes the problem a difficult one is the bewildering variety of sex-determining mechanisms found in the animal and plant kingdoms, and the seemingly chaotic way in which they are distributed among taxa. Even in species with separate sexes, male heterogamety (XY male, XX female) can be found in such diverse taxa as mammals, fruit flies and some vines, while female heterogamety (ZW female, ZZ male) occurs in birds, schistosomes and strawberries. Haplo-diploidy (males develop from unfertilized eggs and are therefore haploid, females from fertilized eggs and are diploid) is thought to have evolved at least eight[2] and probably more than twelve[1] times among insects, mites and rotifers. Sex may even be environmentally determined, as in many reptiles, a marine fish, an echiuroid worm, a crustacean parasite of fish gills, some nematodes and at least one species of amphipod.

Bull documents much of the diversity of sex-determining mechanisms but his book differs from previous accounts because it specifically addresses the question of how one type of sex determination can evolve into another. His approach is to consider what combination of selective forces and historical constraints have resulted in the evolutionary changes observed, these being only a limited subset of the theoretically possible transitions. The application of this approach across a broad taxonomic range allows him to draw some very general conclusions about the parts played by selection and by evolutionary constraints.

Haplo-diploidy

Haplo-diploidy appears from its taxonomic distribution to have often evolved from an ancestral diploid condition, as does a functionally equivalent system — paternal genome loss — where males are produced from fertilized eggs which transmit only the maternally derived part of their genome to their offspring. What selective forces are responsible for the evolution of these systems? Hartl and Brown[3] and Bull[1,4] suggest that an allele

© 1984 Macmillan Journals Ltd

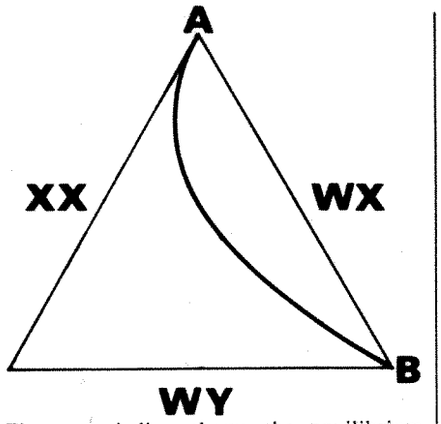
causing a female to produce haploid eggs that will develop into sons will increase in frequency in her grandchildren because haploid sons transmit maternal alleles to all their offspring whereas diploid sons transmit maternal alleles to only half their offspring. As long as the haploid sons leave more than one-half as many offspring as the diploid sons, haplo-diploidy will spread.

But this argument applies equally well to male and female parents — any genetic device in a parent that causes offspring to transmit only alleles from that parent should be favoured. Why, then, is it always the maternal genome that is transmitted? And why is the maternal genome always transmitted by sons rather than daughters? The first question is difficult to answer: selective transmission of the paternal genome could, in theory, be effected via haploid embryos that develop from sperm (which is improbable) or from a fertilized egg that lost its maternal genome. It is not yet clear why the latter mechanism has never evolved (as far as we know), partly because the mechanism of paternal genome loss is not well understood. Perhaps females are in a position to resist male-derived mutants that cause maternal genome loss. The reason that only sons transmit the maternal genome is probably related to the disadvantages of asexual inheritance[5-7]. Transmission by daughters would mean that male-derived alleles would never be transmitted to females and would cause asexual inheritance in the female line. Finally, haplo-diploidy could only arise from a diploid species with a sex-determining mechanism such that aberrant haploid individuals develop into males[1].

Genetic and environmentally determined sex

There have been several evolutionary transitions between genetic and environmentally determined sex. Environmental sex determination is likely to be favoured in a patchy environment where different patches affect the fitnesses of each sex differently, but where parent and offspring have little or no control over the type of patch in which the offspring develop[8].

Take the case of mermithid nematode worms, for example. Free-living adult worms lay eggs in larval insect hosts: the worms hatch and grow to a size determined by the richness of the food source, which is itself determined by the density of the worms and the nutritional status of the host. But female fitness increases with size more than does male fitness and, as a result, selection has operated so that worms developing at high density or in a poorly fed host tend to become males, while those developing at low density or in a well fed host become females[9,10]. In the case of these worms, offspring cannot choose the type of host in which they develop and mothers laying eggs may not be able to detect whether the potential host has already been parasitized by other mermithid nematodes. The two conditions



The curved line shows the equilibrium frequencies of the three different genotypes of female platyfish (see text). When the perpendicular distances from the three axes to any point on the line are scaled so that they sum to unity, their lengths give the frequencies of the three genotypes. The points labelled A and B represent extremes with female heterogamety (WY female, YY male) and male heterogamety (XY male, XX female). Populations at all other points on the line of equilibria contain three female genotypes (WY, XX, WX) and two male genotypes (YY, XY). (Refs 1, 17.)

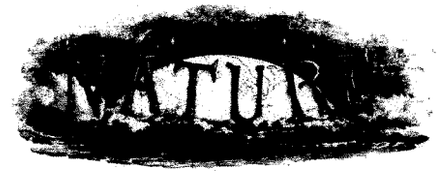
of absence of patch choice and differential fitness of the two sexes thus apply. The model also seems to hold in other cases[11-13], and some preliminary data suggest it may well be relevant to environmental sex determination in reptiles[14,15].

Under what conditions can genes for sex determination invade species with environmentally determined sex? The theory given above suggests that changes in relative fitness of the two sexes must be fairly consistently associated with particular patch types for environmentally determined sex to be favoured. In the wild, this condition will not always hold. If mermithid nematode population density were to vary between seasons or if host nutritional condition also changed dramatically between seasons, then, under pure environmental sex determination, some seasons might produce very high proportions of males and others would produce excess females. This would result in fluctuating fitnesses for individuals of each sex and, in such conditions, genotypic sex determination can invade[1]. (Could some dinosaurs have gone extinct because their sexes were environmentally determined? A sudden change in climate without an appropriate genetic response could provide the conditions for producing populations consisting totally of frustrated males, or females if you will.)

Male and female heterogamety

Although there are conditions in which one sex-determining mechanism can invade a population with an alternative method of sex determination, this does not necessarily mean that the new mechanism will completely replace the old. Indeed, it is possible for two or more sex-determining mechanisms (or sex factors) to be maintained at equilibrium in the same population. Sometimes, instead of a single equilibrium, there is a continuous set of stable equilibria which can be described graphically by lines of 'neutral equilibria' along which any point describes a combination of equilibrium sex factor frequencies. When populations lie on such a line, there is a prospect for indeterminate evolution — genetic drift can carry small populations along the line of neutral equilibria. The commonest mechanisms of sex determination in animals — male and female heterogamety — could evolve from one another in this way.

An example is provided by a tropical poeciliid fish, the platyfish (*Xiphophorus maculatus*), a favourite among aquarium fanciers, of which the earliest captive individuals proved to have female heterogamety while later collections had male heterogamety. It turns out that natural populations are polymorphic for three sex factors (W, X and Y) which could occur in six different combinations: XY, YY, XX, WX, WY and WW. The XY and YY genotypes usually develop as males and XX, WX and WY become females[16]; WW genotypes do not in fact occur because male gametes can never bear W factors. Assuming that fitnesses of the different genotypes are the same within each sex, their equilibrium frequencies can be calculated[17] (see the figure). At one



100 years ago

PROF. HULL, who has returned with his party, brings with him, it is stated, materials for the construction of a geological map of the Holy Land very much in advance of anything which could hitherto be attempted. He is reported to have traced the ancient margin of the Gulfs of Suez and Akaba to a height of 200 feet above their present levels, so that, according to Prof. Hull, the whole country has been submerged to that extent, and has been gradually rising. As one result of this rise, the Professor is of opinion that at the time of the Exodus there was a continuous connection of the Mediterranean and the Red Sea. As regards the Dead Sea, Prof. Hull believes he has discovered that it formerly stood at an elevation of 1400 feet above its present level — that is to say, 150 feet above the level of the Mediterranean. He believes he has also found evidences of a chain of ancient lakes in the Sinaitic district, and of another chain in the centre of the Wady Arabah, not far from the watershed. The great line of the depression of the Wady Arabah and the Jordan Valley has been traced to a distance of more than a hundred miles. The materials for working out a complete theory of the origin of this remarkable depression are stated to be now available. Prof. Hull has in hand, besides his scientific report, a popular account of his journey, which will first appear in the *Transactions* of the society.
From *Nature* **29**, 389, 21 February 1884.

0028-0836/84/080690-01$01.00

extreme, X factors disappear from the population and female heterogamety (WY females, YY males) describes the system (point A). At the other extreme the W factor disappears, leaving a system of male heterogamety (XX females, XY males; point B). Platyfish populations can thus be viewed as in transitional states between systems of either male or female heterogamety. Environmental change that differently affects the fitnesses of the various genotypes within each sex could trigger transitions between the two modes of sex determination.

Hermaphroditism to heterogamety

Just why it is that male heterogamety is common and female heterogamety rare[18–20] among dioecious plants (those having separate sexes) has been explained elegantly by Charlesworth and Charlesworth[21]. Their argument necessarily supposes that dioecious plants evolved from hermaphrodite ancestors through an intermediate stage, of either gynodioecy (females and hermaphrodites) or androdioecy (males and hermaphrodites). A transition from hermaphroditism to either gynodioecy or androdioecy may be favoured if loss of one sexual function can be balanced by increased resource allocation to the other, and if the single-sex individuals avoid self-pollination with its attendant inbreeding depression. But pollen from the male plant of an androdioecious species may be at a disadvantage in gaining access to the ovules of the hermaphrodites in competition with the hermaphrodites' own pollen. By contrast, females in a gynodioecious species do not have this problem because hermaphrodites produce more than enough pollen to fertilize all available ovules. Gynodioecy may therefore evolve more readily, and this may be one reason why androdioecy is much rarer in nature than gynodioecy[22–24].

From gynodioecy one further evolutionary step can be taken to male heterogamety. It is assumed that females of gynodioecious species are homozygous recessives — for they will have lost male function through mutation, and most mutants are recessive. This makes it possible for full dioecy to evolve, though it can only be achieved by way of a dominant mutation that produces female-sterile (that is, male) plants, replacing the hermaphrodites[21]. Mutations at loci that are genetically closely linked to the male-sterility locus will be favoured over those that are not because they will be less likely to allow male-sterile and female-sterile plants to segregate in the population. As a dominant mutation is required to produce males, it is male heterogamety that will evolve from gynodioecy; and as gynodioecy is more common than androdioecy, the higher frequency of male heterogamety is thus explained.

The processes that lead to transitions between different systems of sex determination have received erratic attention from evolutionary biologists in general and population geneticists in particular. Bull's book[1] provides a general analytical framework for studying these processes, a comprehensive review of a relevant but scattered literature and a focus on unanswered questions. It also makes an excellent complement to Charnov's recent book on sex allocation theory[25], which considers how individuals should partition resources into male and female function[26]. □

Paul H. Harvey is in the School of Biological Sciences at the University of Sussex, Brighton BN1 9QG, and Linda Partridge is in the Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT.

1. Bull, J.J. *Evolution of Sex Determining Mechanisms* (Benjamin/Cummings, Menlo Park, 1983).
2. White, M.J.D. *Animal Cytology and Evolution* 3rd edn (Cambridge University Press, 1973).
3. Hartl, D.L. & Brown, S.W. *Theor. Pop. Biol.* **1**, 165 (1970).
4. Bull, J.J. *Heredity* **43**, 361 (1979).
5. Williams, G.C. *Sex and Evolution* (Princeton University Press, 1975).
6. Maynard Smith, J. *The Evolution of Sex* (Cambridge University Press, 1978).
7. Bell, G. *The Masterpiece of Nature* (University of California Press, Los Angeles, 1982).
8. Charnov, E.L. & Bull, J.J. *Nature* **266**, 828 (1977).
9. Chitwood, B.G. & Chitwood, M.G. *An Introduction to Nematology* (University Park Press, Baltimore, 1974).
10. Christie, J.R. *J. exp. Zool.* **53**, 79 (1929).
11. Conover, D.O. *Am. Nat.* (in the press).
12. Bacci, G. *Sex Determination* (Pergamon, Oxford, 1965).
13. Crew, F.A.E. *Sex Determination* 4th edn (Methuen, London, 1965).
14. Fergusson, M.W.J. & Joanen, T. *Nature* **296**, 850 (1982).
15. Harvey, P.H. & Slatkin, M. *Nature* **296**, 807 (1982).
16. Kallman, K.D. in *Genetics and Mutagenesis of Fish* (ed. Schroder, J.H.) (Springer, Berlin, 1973).
17. Bull, J.J. & Charnov, E.L. *Heredity* **39**, 1 (1977).
18. Westergaard, M. *Adv. Genet.* **9**, 217 (1958).
19. Grewal, M.S. & Ellis, J.R. *Heredity* **29**, 359 (1972).
20. Lloyd, D.G. *Heredity* **32**, 11 (1974).
21. Charlesworth, B. & Charlesworth, D. *Am. Nat.* **112**, 975 (1978).
22. Darwin, C. *The Different Forms of Flowers on Plants of the Same Species* (Murray, London, 1877).
23. Lloyd, D.G. *Genetics* **45**, 325 (1975).
24. Charlesworth, D. *Heredity* **46**, 27 (1981).
25. Charnov, E.L. *The Theory of Sex Allocation* (Princeton University Press, 1982).
26. May, R.M. *Nature* **301**, 16 (1983).

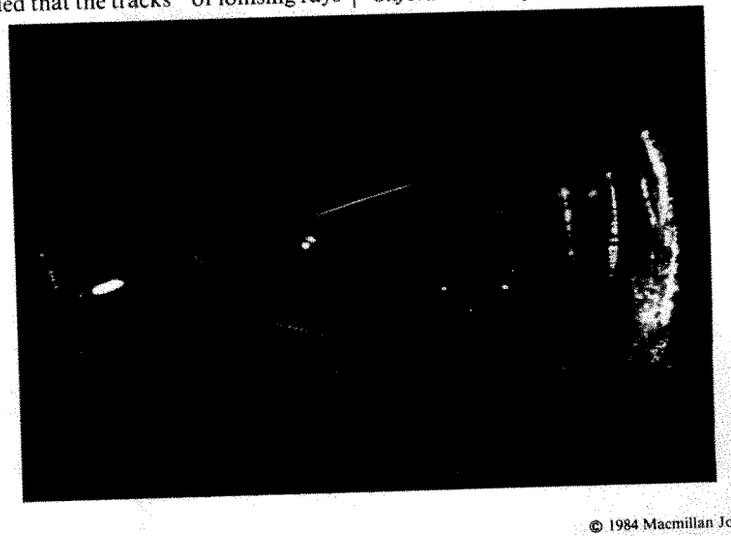
Ions caught in their tracks

THE cloud chamber, once described by Lord Rutherford as "the most original and wonderful instrument in scientific history", dominated nuclear physics for half a century. It was invented almost inadvertently by C.T.R. Wilson when he sought to duplicate in the laboratory the optical effects produced by the interplay of clouds and sunlight encountered on a climbing holiday in Scotland in 1894. To generate artificial mist, Wilson designed a chamber in which to expand moist air and found that mist formed above a critical value of the expansion. Experimenting with the X rays discovered by W.C. Röntgen in 1895, he discovered that below that value the passage of the rays was rendered visible by a condensation track formed from the supersaturated air. He had in effect constructed a particle detector.

The design was progressively modified and improved, and in 1911 Wilson was ready to record the transitory tracks photographically. With a small cylindrical chamber activated by a piston from below, it was possible to record clearly the droplets condensing along the tracks not only of X rays but also of α, β and γ rays. Wilson concluded that the tracks "of ionising rays of any kind through a moist gas may be made visible by condensing water upon the ions set free".

The accompanying photograph, taken at the Cavendish Laboratory in 1911 by the light of a mercury spark, was the first published in Wilson's historic paper 'On a method of making visible the paths of ionising particles through a gas' (*Proceedings of the Royal Society* **A85**, 285; 1911). A potential difference of 8 volts was applied between roof and floor of the chamber. The camera was fitted with a Beck 'isostigmar' lens at f/5.8 and loaded with an Ilford Monarch plate. The shower of tracks traces the passage of α particles — helium nuclei — from a dot of radium on the tongue of a spinthariscope (left). "The very beautiful sight of the clouds condensed along the tracks of the α particles was seen for the first time", recalled Wilson in his Nobel lecture of 1927. The photograph is reproduced by courtesy of the Science Museum, where the 1912 version of the Wilson cloud chamber is preserved. **Jon Darius**

Selected from Beyond Vision *by Jon Darius of the Science Museum (to be published in April by Oxford University Press).*



© 1984 Macmillan Journals Ltd

Chemistry

Chemically generated spatial structures

from Irving R. Epstein

IT might seem self-evident that a pattern formed by inhomogeneities in a liquid will eventually blur and disappear through the action of diffusion and that a liquid that is homogeneous will remain so. But recent work in nonlinear chemical dynamics is providing a set of extraordinary examples that do not conform to the intuitively attractive notion that spatial structures tend to decay rather than grow. These phenomena and the models proposed to describe them are beginning to have an impact not only in chemistry but in biology and geology as well, though some of the models are still highly controversial.

Probably the most venerable spatial structures seen in solutions are the Liesegang rings, known since the turn of the century[1]. Two solutions containing a pair of ions, such as silver and dichromate, which form a sparingly soluble compound, are placed at opposite ends of a tube containing a gel. As the ions diffuse towards one another, precipitate appears not, as one might expect, uniformly, but in a series of sharp nearly periodically spaced rings. Theories recently developed by Ross[2], Ortoleva[3] and co-workers challenge a 60-year old view[4] that the phenomenon results from the build-up and release of supersaturation. These new theories successfully explain Liesegang's as well as newer and more puzzling experiments by invoking the competitive autocatalytic growth of different sizes of colloid particles coupled to diffusion of the reactants. Ortoleva[5], by applying similar ideas, but on much larger scales of time and length, is able to explain the development of textural patterns in initially unbanded metamorphic rock — although the approach has met with some resistance from geologists.

Particularly exciting visually is the spontaneous development of trigger wave or bull's eye patterns (see the figure) in an initially homogeneous disc of reactant liquid. Winfree[6] has developed recipes and techniques for producing spirals and scroll waves as well as the simpler patterns, and Krinsky[7] has been able to generate multi-armed vortices (see the figure). A recent paper in *Nature* describes how to add a third dimension to these structures[8], while in this issue (p.717), Avnir and Kagan describe structures generated at membrane surfaces. Early studies of these phenomena were limited to a single chemical system, the Belousov-Zhabotinsky (BZ) oscillator, but De Kepper *et al.*[9] have shown that the same patterns may be observed in systems with quite different chemistry. The resemblance between these chemical structures and those seen in the aggregating slime mold *Dictyostelium discoideum*[10] are striking.

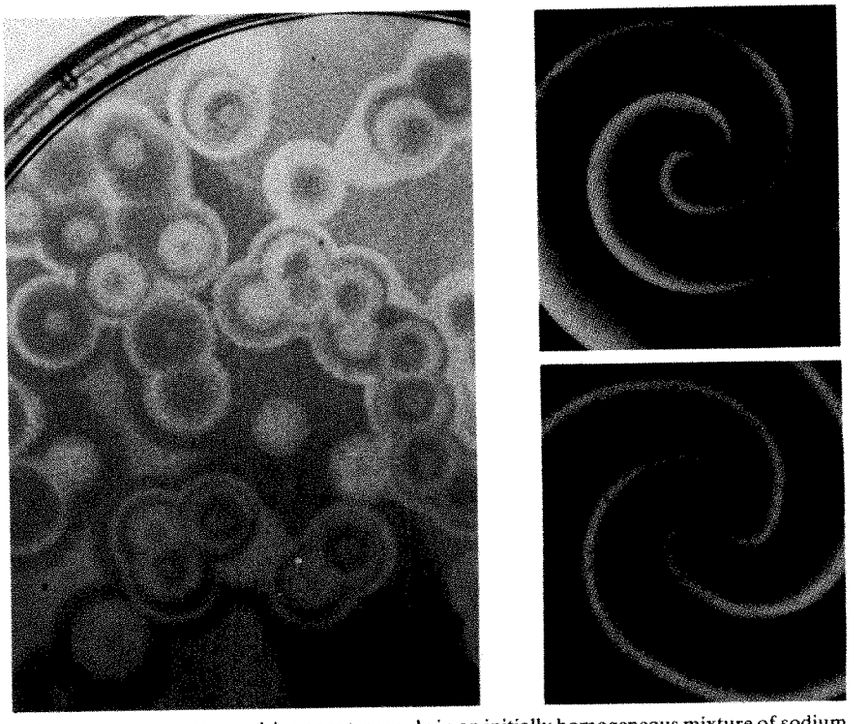
These patterns may be understood in a general sense as the result of a dynamic instability arising from the coupling of a chemically excitable system with diffusive transport and a recent computer simulation based on a two-parameter model[11] yields patterns remarkably like those found in the BZ reaction. However, a convincing calculation that predicts the wave pattern from a plausible chemical mechanism for a system as complicated as the BZ reaction has yet to appear. A first step in that direction has been taken by Showalter and colleagues[12], who treat a simpler phenomenon, the propagation of a single concentration wave in the arsenite-iodate reaction, by adding one-dimensional diffusion terms to a plausible model for the chemistry. Both the shape and the velocity of the wave are accurately predicted.

One rather controversial class of spatial phenomena is generated photochemically when initially homogeneous solutions are illuminated. Two recent papers[13,14] have refuted earlier claims of periodic temporal oscillation in the light emitted by certain photochemical systems subject to constant illumination. Instead, the observed behaviour is shown to result from spatial oscillations generated by convective motion of the fluid in response to temperature gradients set up by the illumination. (A related controversy involves the report published in *Nature* a few months ago[15] that previously published photochemically generated spatial structures[13,16], too, result from convection rather than photochemistry.)

Although the study of spatial pattern formation in chemical systems is still in its infancy, it is progressing rapidly. Answers to the questions raised by some of these beautiful and often counterintuitive phenomena promise important insights not only into chemical dynamics but into such biologically significant problems as morphogenesis as well. □

Irving R. Epstein is Professor and Chairman of Chemistry at Brandeis University, Waltham, Massachusetts 02254.

1. Liesegang, R.E. *Naturwiss. Wochenschr.* **11**, 353 (1896).
2. Kai, S., Müller, S.C. & Ross, J. *J. chem. Phys.* **76**, 1392 (1982).
3. Feeney, R., Schmidt, S.L., Strickholm, P., Chadam, J. & Ortoleva, P. *J. chem. Phys.* **78**, 1293 (1983).
4. Ostwald, W. *Kolloid Z.* **36**, 380 (1925).
5. Ortoleva, P., Merino, E. & Strickholm, P. *Am. J. Sci.* **282**, 617 (1982).
6. Winfree, A.T. *Science* **181**, 937 (1973).
7. Agladze, K.I. & Krinsky, V.I. *Nature* **296**, 424 (1982).
8. Welsh, B.J. Gomatam, J. & Burgess, A.E. *Nature* **304**, 61 (1983).
9. De Kepper, P., Epstein, I.R., Kustin, K. & Orbán, M. *J. phys. Chem.* **86**, 170 (1982).
10. Tomchik, K.J. & Devreotes, P.N. *Science* **212**, 443 (1981).
11. Madore, B.F. & Freedman, W.L. *Science* **222**, 615 (1983).
12. Hanna, A., Saul, A. & Showalter, K. *J. Am. chem. Soc.* **104**, 2828 (1982).
13. Laplante, J.P. & Pottier, R.M. *J. phys. Chem.* **86**, 4759 (1982).
14. Epstein, I.R., Morgan, M., Steel, C. & Valdes-Aguilera, O. *J. phys. Chem.* **87**, 3725 (1983).
15. Micheau, J.C., Gimenez, M., Borckmans, P. & Dewel, G. *Nature* **305**, 43 (1983).
16. Avnir, D., Kagan, M. & Levi, A. *Naturwiss.* **70**, 144 (1983).



Left, spatial wave pattern arising spontaneously in an initially homogeneous mixture of sodium iodide, sodium chlorite, malonic acid, sulphuric acid and starch indicator. Right, wave movement in two- and three-armed vortices (from ref. 7).

0028-0836/84/080692-01$01.00

© 1984 Macmillan

Calcium-activated potassium channels and their role in secretion

Ole H. Petersen & Yoshio Maruyama

MRC Secretory Control Research Group, The Physiological Laboratory, University of Liverpool, PO Box 147, Brownlow Hill, Liverpool L69 3BX, UK

Single-channel recording by the patch-clamp technique has now characterized three kinds of membrane potassium channels activated by intracellular calcium ions in animal cells. These play a crucial part in the regulation of membrane potential and of secretion.

THE concentration of potassium ions (K^+) in cells relative to extracellular space is normally maintained either directly by the ATP-dependent sodium-potassium (Na^+-K^+) pump in the plasma membrane or, indirectly, by co-transport processes ultimately driven by the same mechanism[1-3]. The exit of K^+ from cells, on the other hand, is mediated by trans-membrane K^+ channels of which there are several kinds[4]. The K^+ channels whose activity is regulated by intracellular calcium ion (Ca^{2+}) concentration[5-7] are of particular interest because they suggest explanations of several well-known phenomena in which the export of K^+ from cells appears to be linked with changes of cell metabolism and membrane potential. These include the adrenaline-induced increase in plasma K^+ concentration (due to K^+ exit from liver cells)[8,9], salivary gland K^+ transients (due to K^+ exit from acinar cells)[10,11] and the 'Gardos effect' in erythrocytes (K^+ release from red cells)[12,13]. In the nervous system these channels play a major role in regulating electrical activity[14].

The advent of the patch-clamp technique for studying the behaviour of single trans-membrane channels[15-18] has made possible the recognition of several categories of K^+ channels in the membranes of animal cells. In what follows, we shall be concerned only with the K^+ channels activated by Ca^{2+}, of which there appear to be three distinct types—two which are sensitive to membrane voltage but which differ in K^+ conductance, and which are known as the 'large' and 'small' voltage-activated channels, and a third which is insensitive to membrane voltage, unselective among cations but which nevertheless is activated by Ca^{2+} ions. The presence of K^+ channels activated by Ca^{2+} has already been demonstrated in a variety of tissues from several species by means of single-channel recording[5-7,19-29]. Individual cells may contain more than one type of Ca^{2+}-activated K^+ channel[19,20].

The large K^+ channel

The existence of K^+-channels which are sensitive to the internal free Ca^{2+} concentration ($[Ca^{2+}]_i$) within the cell has been demonstrated only in the past few years by means of the patch-clamp technique (Table 1). That some of these channels have very high conductance was first demonstrated by Marty[6] in the membranes of cultured chromaffin cells. Conductances as great as 200 pS or more have been found in various cell membranes, and channels of this type are activated by membrane depolarization. Marty noted two effects of Ca^{2+} at the inner surface of the membrane—increasing probability that high-conductance channels will open on membrane depolarization and a modest decrease of conductance in open channels. While the first effect has been amply confirmed[7,20-23], the reported decrease of channel conductance with increasing $[Ca^{2+}]_i$ has not always been found[20,21,23].

The sensitivity of high-conductance K^+ channels to $[Ca^{2+}]_i$ seems to vary considerably from one preparation to another. Marty's original observations with cultured chromaffin cell membranes suggest that depolarization provokes full channel opening at a Ca^{2+} concentration of 10^{-8} M. At the same time, however, depolarization of inside-out patches of membrane from mammalian salivary glands evokes marked activation of these channels when the inner surfaces are exposed to nominally Ca^{2+}-free solutions containing 1 mM EGTA (ref. 20). By contrast, in cultured rat muscle cells, depolarization from −50 to +50 mV has been shown not to evoke channel opening at 10^{-8} M Ca^{2+}, to have virtually no effect at 10^{-7} M but to require internal concentrations of 10^{-6} M for channel activation[21]. While it seems that the high-conductance channels of the rat muscle preparation are Ca^{2+}-dependent (see ref. 21), in general it may be more appropriate to use the term 'Ca^{2+}-activated', since depolarization can open the channels in the absence of Ca^{2+} in many preparations[20].

In secretory cells such as the chromaffin and the salivary and pancreatic acinar cells, it is clear that the K^+ channel is strictly under the dual control of membrane potential and $[Ca^{2+}]_i$[6,20,23]. Under physiological conditions, an increase in $[Ca^{2+}]_i$ will increase the open state probability (switch on the channel), which will increase the membrane potential and so, in turn, reduce the probability of channel opening. Thus, K^+ exit is kept in good control. The Ca^{2+} sensitivity appears appropriate for physiological regulation since a change in $[Ca^{2+}]_i$ from 10^{-8} to 10^{-7} M causes a marked increase in open state probability and an increase to 10^{-6} M causes near maximal opening of the K^+ conductance pathway[23].

The conductance of the K^+ channel is very high. In symmetrical high-K^+ solutions, values of 180 to 250 pS have been obtained[6,20,23] (Table 1). The variations of conductance between different tissues are, however, puzzling. In our laboratory we generally find that the K^+ channels in pig pancreatic acinar cells have a conductance of about 200 pS whereas in the salivary glands of mice and rats, the channels have a conductance of about 250 pS. The possibility of sub-states of the channel has been raised by observations of smaller channels of varying conductances when membrane patches are artificially exposed to symmetrical high K^+ solutions[20,23], but detailed investigations of these smaller current steps have not been undertaken.

In spite of the high unit conductance, the K^+ channel is very selective. The channel discriminates between the closely related alkali metal ions K^+ and Rb^+. With extracellular Rb^+ and intracellular K^+, K^+ current can easily be made to flow from the inside to the outside, but no inward Rb^+ current can be evoked. With the Rb^+/K^+ gradients reversed, inward K^+ current, but not outward Rb^+ current, can be observed (Y.M., D. V. Gallacher and O.H.P., unpublished data). It is important to be aware of this rather extreme selectivity as ^{86}Rb is frequently used as a tracer in flux experiments to assess K^+ permeability since it has a much longer half-life than ^{42}K (ref. 2).

The K^+ conductance of the plasma membrane depends not only on the single-channel conductance and the probability of

0028-0836/84/080693—04$01.00

© 1984 Macmillan Journals Ltd

Table 1 Properties of Ca^{2+}-activated channels permeable to K^+ in various preparations as determined by patch-clamp method

Tissue	Maximal single-channel conductance (pS)	Ion selectivity	Voltage dependent	Channel density	Refs
Neurones:					
Helix pomatia	19	K^+ channel	Yes	?	5
Bullfrog sympathetic ganglia	100	K^+ channel	Yes	?	70
Mouse neuroblastoma	22	Non-selective monovalent cation channel	No	?	19
Muscle:					
Rat myotubes	187,220	K^+ channel	Yes	?	7, 21
Rabbit T-tubule	226	K^+ channel	Yes	?	71
Rat heart	35	Non-selective monovalent cation channel	No	?	27
Endocrine glands:					
Bovine chromaffin cells	180	K^+ channel	Yes	?	6
Rat anterior pituitary cells	208	K^+ channel	Yes	?	22
Exocrine glands:					
Mouse parotid acini	250	K^+ channel	Yes	?	20
Mouse submandibular acini	250	K^+ channel	Yes	?	20
Rat parotid acini	250	K^+ channel	Yes	?	20
Pig pancreatic acini	200	K^+ channel	Yes	50 per cell	23
Mouse pancreatic acini	30–35	Non-selective monovalent cation channel	No	?	28, 29
Rat pancreatic acini	30–35	Non-selective monovalent channel	No	?	28, 29
Erythrocytes:					
Human red cells	18, 17	K^+ channel	?	?	24, 25
Frog red cells	18, 50	K^+ channel	No	?	26

channels being open at any time, but also on the channel density. The number of channels in a cell can be estimated by a combination of patch-clamp single-channel and whole-cell current recording if the K^+ channel is the only channel of quantitative importance for the conductance of the plasma membrane in the whole cell. This condition seems fulfilled in the pig pancreatic acinar cells. In the resting condition, where $[Ca^{2+}]_i$ lies between 10^{-8} and 10^{-7} M there are about 50 large K^+ channels per acinar cell[23].

The small K^+ channel

Very few patch-clamp investigations of this channel, which may or may not be related to the possible substates of the large K^+ channel mentioned above, have been carried out and relatively few details are therefore known. Lux *et al.*[5] described a 19 pS Ca^{2+}-activated K^+ channel in *Helix pomatia* neurone which is switched on much more slowly than the large 200 pS channel, described above, following depolarization. A K^+ channel with a similar conductance (18 pS) is found in human red cells[24,25] but only after their treatment with iodoacetic acid and adenosine (Gardos treatment[12]). It has recently been shown that the small K^+ channel as well as a somewhat larger K^+ channel (50 pS) in frog red cells can be activated by internal Ca^{2+} (refs 25, 26).

Non-selective cation channels

Not all Ca^{2+}-activated channels are selective. A Ca^{2+}-dependent non-discriminatory cation channel with a conductance of about 30 pS at room temperature (the conductance of this channel is very temperature sensitive) was first described by Colquhoun *et al.*[27]. In marked contrast with the large K^+ channel, the non-discriminatory channel is virtually voltage-insensitive. It is a truly Ca^{2+}-dependent channel in the sense that opening and closure of the channel cannot be observed in the absence of Ca^{2+} on the inside of the membrane. Such channels have subsequently been found in mouse neuroblastoma cells[19], mouse and rat pancreatic acinar cells[28,29] and rat thyroid follicular cells (D. Moor and Y.M., unpublished observation). The channel is virtually impermeable to Cl^- (refs 19, 27, 28) and is almost equally permeable to all the alkali metal ions[19]. Permeability for Ca^{2+} has not been directly demonstrated[19,30], is certainly low, but may not be entirely insignificant[31]. The precise relationship between the open state probability of the channels and $[Ca^{2+}]_i$ is not yet known. We have found that channel opening can be observed at $[Ca^{2+}]_i = 10^{-7}$ M immediately after excision of a pancreatic acinar membrane patch into the inside-out conformation but that after 10–20 s, higher (eventually micromolar) Ca^{2+} concentrations are required to evoke channel opening (Y.M. and O.H.P., unpublished data). The fact that the responsiveness of channels to $[Ca^{2+}]_i$ decreases with the passage of time suggests that the commonly reported observations[19,27–29] that the channels are insensitive to Ca^{2+} may not describe the behaviour of these unselective channels in intact cells. It is relevant that the pancreatic secretagogue cholecystokinin (CCK) has been shown indirectly to activate the non-discriminatory channel in intact pancreatic acinar cells via internal Ca^{2+} (ref. 29).

Role in secretion

From a biophysical point of view, two types of secretory cells can be distinguished: those with and those without the ability to conduct electrical action potentials. In general, epithelial cells seem not to fire action potentials whereas most endocrine cells do. (There are, however, exceptions to this rule[32].)

Endocrine cells: In the endocrine cells the K^+ channel acts as a link between $[Ca^{2+}]_i$ and the membrane potential. In typical endocrine cells, such as the chromaffin and the pancreatic β-cells as well as in nerve endings, Ca^{2+} needed in excitation-secretion coupling enters the cells through specific voltage-gated Ca^{2+} channels[33–37] which are opened by membrane depolarization and closed by hyperpolarization. Therefore, as the entry of Ca^{2+} produces an increase in $[Ca^{2+}]_i$, so the K^+ selective channels will be activated leading to membrane hyperpolarization and impedance of further Ca^{2+} influx. A particularly beautiful example of the interplay between Ca^{2+} influx, $[Ca^{2+}]_i$ and K^+ conductance resulting in an oscillating membrane potential pattern (depolarization—plateau with Ca action potentials—hyper-

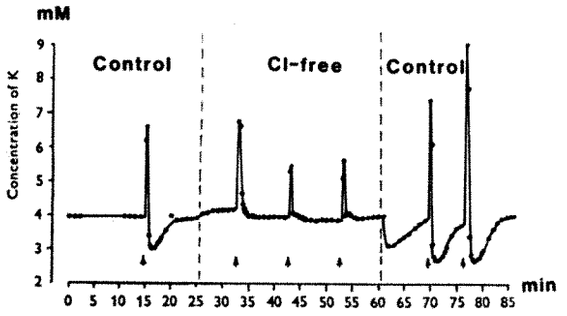


Fig. 1 Acetylcholine-evoked K^+ release and reuptake in perfused cat submandibular gland. The figure shows the concentrations of K^+ in the venous outflow from the gland in one typical experiment. The perfusion fluid flowing into the gland was a physiological saline solution containing 4 mM K^+. Arrows denote injection of 10 μg ACh into the arterial inflow to the gland. In the control periods the major salt component of the oxygenated perfusion solution was 140 mM NaCl whereas during the period labelled Cl-free it was 140 mM $NaNO_3$. Osmolarity, *p*H, K^+ and Ca^{2+} concentrations were kept constant throughout. Note that although the first ACh stimulation in the Cl^--free period evokes a normal K^+ release the K^+ reuptake is virtually abolished. Cl^- readmission evokes immediate K^+ reuptake. The fluid secretion responses to ACh during the Cl^--free perfusion were about 30% of the values obtained in the second control period (from ref. 47).

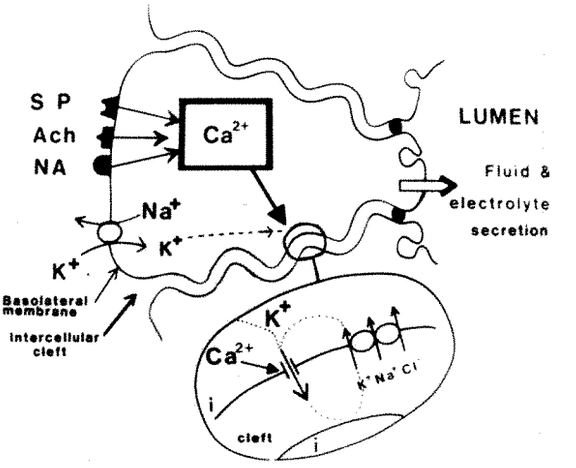


Fig. 2 Simplified scheme to account for control of electrolyte transport in baso-lateral plasma membrane of fluid secreting epithelia. The figure specifically represents part of a mammalian salivary gland acinus and its major secretagogues, substance P, acetylcholine and noradrenaline but with other secretagogues and/or various additions, the model could represent a wide range of secretory epithelia. In the proposed scheme, secretagogues first increase $[Ca^{2+}]_i$ (as suggested by a variety of evidence[32,34,35,72–74] including a recent direct demonstration in mouse acinar cells [75]). The increased $[Ca^{2+}]_i$ would open Ca^{2+}-activated K^+ channels in the baso-lateral membranes of the cell (such channels are well known[20,23], there is some evidence of their localization in the lateral plasma membranes[65] and a decrease in intracellular and increase in extracellular K^+ activity following stimulation have been demonstrated[45,46]). K^+ released through the channels into the narrow intercellular clefts would be taken up again, primarily through a K^+-Na^+-Cl^- co-transport system. The rate of transport in the cycle of K^+ release and reuptake would be regulated at the K^+ channel by internal Ca^{2+} and would be directly linked to the rate of NaCl uptake by the cotransport system. In the salivary glands it is likely that part of the K^+ release occurs via the Ca^{2+}-activated non-selective cation channel[20] explaining why the intracellular Na^+ concentration rises during stimulation[47,48] and part of the K^+ uptake could occur via the Na^+-K^+ pump (but intracellular Na^+ activity measurements have not been carried out). The operation of the Na^+-K^+-Cl^- co-transport system is of course dependent on the Na^+ gradient and therefore ultimately on the operation of the Na^+-K^+ pump. (Note that there is no evidence in regard to specific localizations of Na^+-K^+ pump and co-transport system to basal and lateral membranes, respectively and the two transport proteins probably co-exist both in the lateral and basal plasma membranes.) The Na^+-K^+-Cl^- co-transport is probably electroneutral as the most likely stoichiometry is 1 Na^+, 1 K^+ and 2 Cl^- (ref. 58).

polarization—gradual slow depolarization—depolarization . . .) was demonstrated several years ago by Gorman and Thomas in *Aplysia* neurones[38]. Interestingly, as previously pointed out[32], this pattern resembles very much that seen in mammalian pancreatic β-cells[39]. Ca^{2+}-activated K^+ channels[40] can indeed be regarded as the crucial regulatory link between $[Ca^{2+}]_i$, and therefore insulin secretion, and Ca^{2+} influx[32,41,42]. The insulin secretagogue glucose, according to one hypothesis, acts by decreasing the Ca^{2+} sensitivity of the K^+ channels, prolonging the plateau phase with the Ca^{2+} action potentials and thereby enhancing $[Ca^{2+}]_i$ (refs 32, 41, 42).

Exocrine cells: The role of Ca^{2+}-activated K^+ channels in the epithelial cells from which action (spike) potentials are absent must clearly be a very different one. The fluid-secreting epithelia of several tissues (salivary glands, sweat glands, lacrimal glands, the exocrine pancreas, the gastric mucosa and the tracheal epithelium) are under strict nervous and/or hormonal control. In many of these tissues it has been clearly shown that nervous or hormonal activation of secretion is associated with release of cellular K^+ (refs 32, 43). The salivary glands, which have been investigated in considerable detail, will be treated here as a model for other fluid-secreting epithelia.

Salivary gland acini secrete an isotonic plasma-like fluid at a low rate (if at all) in the resting condition but at an extraordinarily high rate during nerve stimulation[43]. Secretion is Ca^{2+} dependent[44]. Nerve stimulation or direct stimulation with acetylcholine, noradrenaline or substance P evokes loss of cellular K^+ (refs 32, 43). Both a marked decrease in intracellular K^+ and a marked increase in extracellular K^+ have been measured[45,46]. The stimulant-evoked K^+ loss, which is due to electrodiffusion[47,48], can be evoked in the absence of neurotransmitter stimulation by the Ca^{2+} ionophore A23187 (ref. 49). The direct demonstration of the large Ca^{2+}-activated K^+ channel in the baso-lateral plasma membranes of several mammalian salivary glands explains the stimulant-evoked K^+ loss and the Ca^{2+}-involvement[20]. Following release of K^+ from stimulated salivary glands, there is reuptake of K^+. This has generally been assumed to be due to operation of the ouabain-sensitive Na^+-K^+ pump and certainly K^+ reuptake (but not stimulant-evoked K^+ release) can be blocked in the presence of ouabain[47]. However, as seen in Fig. 1, K^+ reuptake is also acutely and reversibly abolished when the Cl^- component of the perfusion fluid is replaced by NO_3^- (ref. 47) (similar results were obtained using sulphate replacement[50]). Stimulant-evoked secretion is markedly reduced under these conditions as first shown by Lundberg[51]. The K^+ reuptake and secretion are also acutely sensitive to reductions in extracellular Na^+ concentration[47]. These findings could be explained by postulating linked K^+, Na^+ and Cl^- inward movement through a carrier system (Fig. 2) of the type now characterized in red cells[52–56], kidney cells[57–58] and the intestine[59], but not known to exist at the time of the original observations[47,51]. The involvement of a K^+-Na^+-Cl-co-transport system in secretion from the salivary gland is also supported by Lundberg's observation that replacement of Cl^- in the perfusion fluid by NO_3^-, I^- or SCN^- but not by Br^-, reduced the secretory responses by 85–90% (ref. 60). This resembles the situation in erythrocytes where only Br^- can replace Cl^- in the operation of the co-transport system although all the other anions can be equilibrated across the membrane by the anion (Cl^--HCO_3) exchanger[52,55].

The novel feature of the model proposed in Fig. 2 is that cycling of K^+ through channel and co-transport system allows NaCl uptake through the basolateral membrane of the cell and that the rate of NaCl uptake is directly linked to the rate of transport in the cycle of K^+ release and uptake. The point of

regulation has been clearly identified at the molecular level as the Ca^{2+}-activated K^+ channel[20]. The ability of ouabain slowly to block the K^+ reuptake[47] is of course entirely consistent with the hypothesis proposed, since uptake through the co-transport system will be dependent on the presence of the Na^+ gradient. The model shown in Fig. 2 does not deal with the processes in the luminal membrane, but our hypothesis is not incompatible with the experimental results obtained on *Calliphora* salivary glands and many other tissues suggesting the presence of a luminal Ca^{2+}-activated Cl^- conductance controlling salt exit from the epithelium[61–63]. Indeed it would seem desirable, perhaps even necessary, as suggested by Burgen[64], to have control of transport at both baso-lateral and luminal plasma membranes.

It may be questioned whether K^+ release through Ca^{2+}-activated K^+ channels is large enough to cause a marked change in the transmembrane K^+ gradient but in several salivary glands the changes have been shown directly to be very substantial (Fig. 1) (see also refs 11, 45, 46). The measurements of extracellular K^+ activity in cat and dog submandibular glands show maximal increases after nerve stimulation of 15–18 mM (ref. 45). The changes in K^+ activity along the lateral plasma membrane (narrow extracellular clefts between the cells[32]) are, however, likely to be even larger. A recent voltage clamp study on mouse pancreatic acini indicates that the ion channels controlled by secretagogues mainly reside in the lateral membranes bordering on the narrow extracellular spaces[65]. If this is generally true then nearly all the K^+ will be released directly into the intercellular spaces and the K^+ activity obtained at this site will clearly be higher than in the interstitial fluid at large.

The initial stimulant-evoked change in the transmembrane K^+ gradient is likely to provide a major stimulus for salt uptake via the co-transport system (explaining the very high initial secretory rates[43]), but there is evidence that it is only short-lived[45]. However, even in the steady state situation of sustained secretion, it is essential to maintain the open state of the K^+ channels as continued K^+ exit must balance the K^+ uptake via the co-transport processes since the primary acinar secretion has a low (plasma-like) K^+ concentration[43].

Future perspectives

Although detailed investigations of the properties of the Ca^{2+}-activated K^+ channels have been carried out on several different cell types, little is yet known of the mechanisms involved in modulation of the channels by hormones and neurotransmitters. Recent evidence from our laboratory suggests that the pancreatic secretagogue CCK, acting via an increase in $[Ca^{2+}]_i$, not only increases the open state probability of the large K^+ channel in pig pancreatic acinar cells but also rapidly (and reversibly) increases the number of K^+ channels per cell, (2–3-fold increase with 2×10^{-10} M CCK). The mechanism is unknown but the observations are reminiscent of those in which ADH and aldosterone have been shown rapidly to increase the density of amiloride-sensitive Na^+ channels in the toad urinary bladder[66,67]. The role of protein phosphorylation in controlling K^+ conductance is also likely to demand further attention. Voltage-clamp studies of the Ca^{2+}-activated K^+ conductance in internally perfused snail neurones show an enhancement of the outward K^+ current after addition of the catalytic sub-unit of cyclic AMP-dependent protein kinase to the internal perfusing medium[68], but single-channel current studies are not yet available. The role of the Ca^{2+}-activated K^+ channels in secretion, in particular in epithelial cells, also needs to be further clarified. The availability of specific blockers for the different channels would be an enormous help, but satisfactory compounds have not been found. The possible physiological regulatory role of internal Na^+ blockade of the large Ca^{2+}-activated K^+ channel[69] should be investigated. The finding that the large Ca^{2+}-activated K^+ channel has a very low conductance for Rb^+ should allow interesting experiments on the role of this particular channel to be carried out in various tissues.

1. Skou, J. C. *Physiol. Rev.* **45,** 596–617 (1965).
2. Ussing, H. H. *The Alkali Metal Ions in Biology* (Springer, Berlin, 1960).
3. Geck, P., Pietrzyk, C., Burckhardt, B.-C., Pfeiffer, B. & Heinz, E. *Biochim. biophys. Acta* **600,** 432–447 (1980).
4. Latorre, R. & Miller, C. *J. Membrane Biol.* **71,** 11–30 (1983).
5. Lux, H. D., Neher, E. & Marty, A. *Pflügers Arch. ges. Physiol.* **389,** 293–295 (1981).
6. Marty, A. *Nature* **291,** 497–500 (1981).
7. Pallotta, B. S., Magleby, K. L. & Barrett, J. N. *Nature* **293,** 471–474 (1981).
8. D'Silva, J. L. *J. Physiol., Lond.* **82,** 393–398 (1934).
9. Haylett, D. G. & Jenkinson, D. H. *J. Physiol., Lond.* **225,** 721–750 (1972).
10. Keszтyus, L. & Martin, J. *Pflügers Arch ges. Physiol.* **239,** 408–418 (1937).
11. Burgen, A. S. V. *J. Physiol., Lond.* **132,** 20–39 (1956).
12. Gardos, G. *Biochim. biophys. Acta* **30,** 653–654 (1958).
13. Lew, V. L. & Ferreira, H. G. *Nature* **263,** 336–338 (1976).
14. Meech, R. W. *A. Rev. Biophys. Bioengng* **7,** 1–18 (1978).
15. Neher, E. & Sakmann, B. *Nature* **260,** 799–802 (1976).
16. Sigworth, F. J. & Neher, E. *Nature* **287,** 447–449 (1980).
17. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391,** 85–100 (1981).
18. Neher, E. in *Techniques in Cellular Physiology* PII (ed. Baker, P. F.) P121, 1–16 (Elsevier, Amsterdam, 1982).
19. Yellen, G. *Nature* **296,** 357–359 (1982).
20. Maruyama, Y., Gallacher, D. V. & Petersen, O. H. *Nature* **302,** 827–829 (1983).
21. Barrett, J. N., Magleby, K. L. & Pallotta, B. S. *J. Physiol., Lond.* **331,** 211–230 (1982).
22. Wong, B. S., Lecar, H. & Adler, M. *Biophys. J.* **39,** 313–317 (1982).
23. Maruyama, Y., Petersen, O. H., Flanagan, P. & Pearson, G. T. *Nature* **305,** 228–232 (1983).
24. Hamill, O. P. *J. Physiol., Lond.* **319,** 97–98P (1981).
25. Grygorczyk, R., Schwarz, W. & Passow, H. *Biophys. J.* (in the press).
26. Hamil, O. P. in *Single-Channel Recording* (eds Sakmann, B. & Neher, E.) 451–471 (Plenum, New York, 1983).
27. Colquhoun, D., Neher, E., Reuter, H. & Stevens, C. F. *Nature* **294,** 752–754 (1981).
28. Maruyama, Y. & Petersen, O. H. *Nature* **299,** 159–161 (1982).
29. Maruyama, Y. & Petersen, O. H. *Nature* **300,** 61–63 (1982).
30. Petersen, O. H. & Maruyama, Y. in *Single-Channel Recording* (eds Sakmann, B. & Neher, E.) 425–435 (Plenum, New York, 1983).
31. Petersen, O. H. & Maruyama, Y. *Pflügers Arch ges. Physiol.* **396,** 82–84 (1983).
32. Petersen, O. H. *The Electrophysiology of Gland Cells* (Academic, London, 1980).
33. Baker, P. F., Hodgkin, A. L. & Ridgway, E. G. *J. Physiol., Lond.* **218,** 709–755 (1971).
34. Baker, P. F. & Knight, D. E. *Phil. Trans. R. Soc. Lond.* **B296,** 83–103 (1981).
35. Douglas, W. W. *Br. J. Pharm.* **34,** 451–474 (1968).
36. Lux, H. D. in *Single-Channel Recording* (eds Sakmann, B. & Neher, E.) 437–449 (Plenum, New York, 1983).
37. Reuter, H. *Nature* **301,** 569–574 (1983).
38. Gorman, A. L. F. & Thomas, M. V. *J. Physiol., Lond.* **275,** 357–376 (1978).
39. Meissner, H. P. *Nature* **262,** 502–504 (1976).
40. Marty, A. & Neher, E. *J. Physiol., Lond.* **326,** 36P–37P (1982).
41. Atwater, I., Ribalet, B. & Rojas, E. *J. Physiol., Lond.* **278,** 117–139 (1978).
42. Henquin, J. C. *Nature* **280,** 66–68 (1979).
43. Young, J. A. in *Membrane Transport in Biology*, vol. IV (ed. Giebisch, G.) 563–692 (Springer, Berlin, 1979).
44. Douglas, W. W. & Poisner, A. M. *J. Physiol., Lond.* **165,** 528–541 (1963).
45. Poulsen, J. H. & Bledsoe, S. W. *Am. J. Physiol.* **234,** E79–E83 (1978).
46. Poulsen, J. H. & Oakley, B. II. *Proc. R. Soc.* **B204,** 99–104 (1979).
47. Petersen, O. H. *J. Physiol., Lond.* **208,** 431–447 (1970).
48. Petersen, O. H. *J. Physiol., Lond.* **210,** 205–215 (1970).
49. Selinger, Z., Eimerl, S. & Schramm, M. *Proc. natn. Acad. Sci. U.S.A.* **71,** 128–131 (1974).
50. Petersen, O. H. & Poulsen, J. H. *Experientia* **24,** 919–920 (1968).
51. Lundberg, A. *Nature* **177,** 1080–1081 (1956).
52. Kregenow, F. M. & Caryk, T. *Physiologist* **22,** 73 (1979).
53. Chipperfield, A. R. *Nature* **286,** 281–282 (1980).
54. Dunham, P. B., Stewart, G. W. & Ellory, J. C. *Proc. natn. Acad. Sci. U.S.A.* **77,** 1711–1715 (1980).
55. Chipperfield, A. R. *J. Physiol., Lond.* **312,** 435–444 (1981).
56. Haas, M., Schmidt, W. F. & McManus, T. J. *J. gen. Physiol.* **80,** 125–147 (1982).
57. Greger, R. & Schlatter, E. *Pflügers Arch. ges. Physiol.* **392,** 92–94 (1981).
58. Greger, R. & Schlatter, E. *Pflügers Arch. ges. Physiol.* **396,** 325–334 (1983).
59. Musch, M. W. *et al. Nature* **300,** 351–353 (1982).
60. Lundberg, A. *Acta physiol. Scand.* **40,** 101–112 (1957).
61. Berridge, M. J., Lindley, B. D. & Prince, W. T. *J. Physiol., Lond.* **244,** 549–567 (1975).
62. Frizzell, R. A., Field, M. & Schultz, S. G. *Am. J. Physiol.* **236,** F1–F8 (1979).
63. Shorofsky, S. R., Field, M. & Fozzard, H. A. *Phil. Trans. R. Soc.* **B299,** 597–607 (1982).
64. Burgen, A. S. V. *Phil. Trans. R. Soc.* **B296,** 3 (1981).
65. Maruyama, Y. & Petersen, O. H. *Pflügers Arch. ges. Physiol.* **399,** 54–62 (1983).
66. Li, J. H.-Y., Palmer, L. G., Edelman, I. S. & Lindemann, B. *J. Membrane Biol.* **64,** 77–89 (1982).
67. Palmer, L. G., Li, J. H.-Y., Lindemann, B. & Edelman, I. S. *J. Membrane Biol.* **64,** 91–102 (1982).
68. De Peyer, J. E., Cachelin, A. B., Levitan, I. B. & Reuter, H. *Proc. natn. Acad. Sci. U.S.A.* **79,** 4207–4211 (1982).
69. Marty, A. *Pflügers Arch. ges. Physiol.* **396,** 179–181 (1983).
70. Adams, P. R., Constanti, A., Brown, D. A. & Clark, R. B. *Nature* **296,** 746–749 (1982).
71. Latorre, R., Vergara, C. & Hidalgo, C. *Proc. natn. Acad. Sci. U.S.A.* **79,** 805–809 (1982).
72. Putney, J. W. *Pharmac. Rev.* **30,** 209–245 (1978).
73. Schulz, I. & Stolze, H. H. *A. Rev. Physiol.* **42,** 127–156 (1980).
74. Ginsborg, B. & House, C. R. *A. Rev. Biophys. Bioengng* **9,** 55–80 (1980).
75. O'Doherty, J., Stark, R. J., Crane, S. J. & Brugge, K. L. *Pflügers Arch. ges. Physiol.* **398,** 241–246 (1983).

Revised Sm–Nd systematics of Kambalda greenstones, Western Australia

J. C. Claoué-Long*, M. F. Thirlwall† & R. W. Nesbitt*

* Department of Geology, Southampton University, Southampton SO9 5NH, UK
† Department of Earth Sciences, Leeds University, Leeds LS2 9JT, UK

Sm–Nd isotope systematics in mafic–ultramafic lavas from the Norseman–Wiluna greenstone belt, Western Australia, have been reinvestigated. A previous age based on mixing lavas and granites is not confirmed: instead, a Sm–Nd age of 3,262 ± 44 Myr is indicated for the lavas, 400 Myr older than intruding granites, and predating gneisses that have been proposed as basement rocks.

OPPOSING models for the development of the early crust preserved in the Yilgarn Archaean craton suggest either that gneiss terrains represent basement on which later greenstones were deposited[1,2], or that greenstones formed the early crust (at least in part) and granitoids were produced by partial melting of greenstones infolded to deep crustal levels[3]. Field relationships and geochronology are among the keys to settling this debate, but information is scarce and somewhat equivocal. Sm–Nd studies may prove useful for determining ages in the greenstone belts, but the method is hampered by a lack of preservation of original igneous minerals and the normally small range of whole-rock Sm/Nd ratios found in co-genetic suites of lavas. A previous attempt to apply Nd isotopes to the Kambalda area combined lavas with felsic intrusive rocks to generate a wide range of parent/daughter ratios, and produced an age for these samples of 2,790 ± 30 Myr (ref. 4). This approach is open to the danger that the samples are probably neither co-genetic nor of the same age. In acknowledging this, the previous paper notes that data for Kambalda lavas alone yield a poorly-constrained older date. Against this background, the present study discusses new Nd data for basic–ultrabasic lavas in the Kambalda region.

Regional geology

Correlations within the 800 km long Norseman–Wiluna greenstone belt are complicated by extensive strike-faulting and poor exposure, but in the Kambalda area there is sufficient continuity to permit recognition of two sequences of volcanic and sedimentary rocks, referred to as the Kambalda (lower) and Bluebush (upper) sequences[5,6] (Fig. 1). Although both horizons exhibit similar ranges of lithologies, nickel sulphides are preferentially concentrated in the Kambalda sequence. The area has been affected by at least four deformational events, as well as metamorphism (up to low amphibolite facies) and intrusion of Archaean granitoids, felsic–intermediate porphyries, minor mafic dykes and sills, and a Proterozoic dolerite dyke suite[6]. Thus all rocks are now metamorphic, but good preservation of igneous textures and chemistry merits consideration of the metalavas as igneous rocks.

Mafic–ultramafic lavas in the Kambalda sequence consist of >2,000 m of pillowed, or massive, fine-grained foot-wall basalts with 6–9% MgO, overlain by 0–1,000 m of ultrabasic lavas (olivine–spinifex textured komatiites, liquid composition 22–31% MgO), and subsequently by >600 m of hanging-wall basalts with <15% MgO (ref. 6). These are commonly pillowed and variolitic, but can be massive or occasionally bear pyroxene-spinifex. At the base of the ultrabasic formation occur one or more very thick flows, hosts to Ni–Cu sulphides, which are layered and contain differentiation zones of basaltic pyroxene-spinifex with <15% MgO. Lavas at the Kambalda Dome are intruded by a sodic granite and a series of felsic porphyries.

The Bluebush sequence is less well understood, but also consists of an ultrabasic formation sandwiched between basaltic foot–wall and hanging-wall formations. Attention here is confined to the ultrabasic formation, which is best known from good surface exposures at Ultramafic Hill and One Boob Hill, and consists of well-preserved interlayered flows of olivine-spinifex textured komatiite and pyroxene-spinifex textured basalt[6,7].

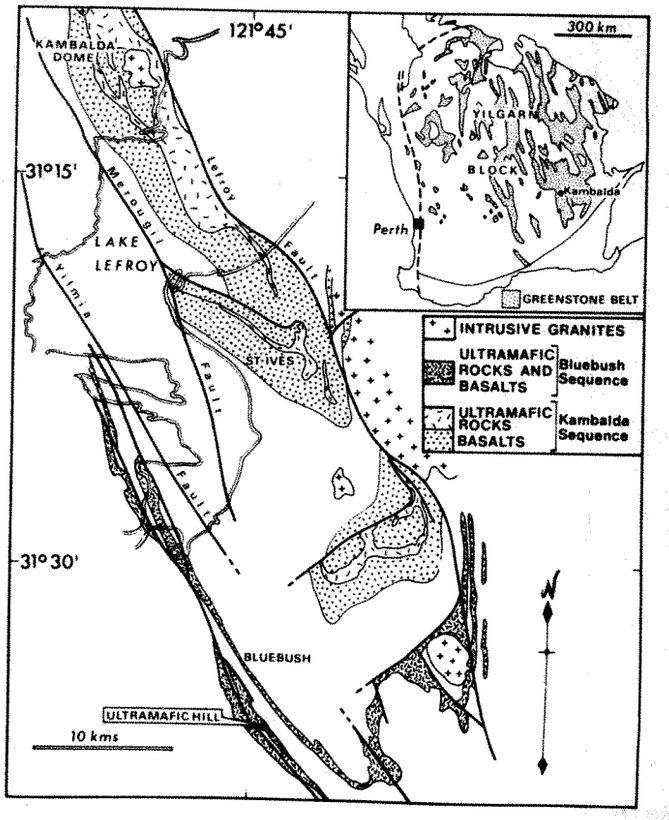


Fig. 1 Distribution of mafic and ultramafic lavas in the Kambalda–Bluebush area of the Norseman–Wiluna belt (adapted from ref. 6). Inset shows location of Kambalda in the Yilgarn block.

0028–0836/84/080697—05$01.00

© 1984 Macmillan Journals Ltd

Table 1 Sm–Nd isotopic data

Sample	Description	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}Sm/^{144}Nd$	$^{143}Nd/^{144}Nd$	$^{148}Nd/^{144}Nd$
Kambalda						
1020A/158.2	Massive, mafic HWB	2.136	8.993	0.1436	0.511610±08	0.241566±08
1020A/268.6	Granular, mafic HWB	1.816	6.089	0.1804	0.512413±08	
1020A/473.2	Mafic HWB with acicular texture	3.069	12.203	0.1520	0.511832±41	
1020A/507.3	Massive, mafic HWB	2.229	7.007	0.1923	0.512634±12	0.241586±10
6033/222.8	Random olivine-spinifex, thin komatiite	0.914	2.453	0.2254	0.513375±14	0.241610±18
6033/479.7	Random olivine-spinifex, top of thick komatiite flow	0.826	2.125	0.2352	0.513601±34	
29/456.0	Random olivine-spinifex, top of thick komatiite flow	0.636	1.484	0.2592	0.514110±25	
29/501.5	Pyroxene-spinifex zone, same flow as 29/456	1.164	3.462	0.2034	0.512997±14	0.241587±11
1025/624	Massive, mafic FWB	2.112	6.222	0.2053	0.512942±22	
1029/3366	Massive, mafic FWB	0.794	2.429	0.1976	0.512762±16	0.241625±19
1029/4780	Massive, mafic FWB	1.982	5.986	0.2002	0.512843±12	0.241600±11
Bluebush						
UMH62	Devitrified glass, basalt flowtop	0.756	2.563	0.1782	0.512594±32	
UMH72	Random pyroxene-spinifex, basalt	2.557	8.055	0.1920	0.512857±09	
UMH74	'String beef' texture, basalt flow	2.767	8.398	0.1993	0.512943±12	
UMH81	Random pyroxene-spinifex, basalt flow	2.568	7.863	0.1975	0.512879±14	
UMH97	Random olivine-spinifex, komatiite	0.859	2.221	0.2340	0.513705±24	0.241648±22

HWB, hanging-wall basalt; FWB, foot-wall basalt. Errors quoted are 2 standard errors on the mean. $^{147}Sm/^{144}Nd$ and $^{143}Nd/^{144}Nd$ determined on aliquots of the same sample solution. Total Nd blank is 0.2 ng and is therefore negligible for this study. $^{143}Nd/^{144}Nd$ normalized to $^{146}Nd/^{144}Nd = 0.7219$, the value determined for BCR1 = 0.512657 ± 12. A revised calibration of the Leeds rare-earth spike using standard solutions prepared from metal ingots gives Sm/Nd = 0.3249 for the California Institute of Technology Sm/Nd Standard[25], and reproducibility of $^{147}Sm/^{144}Nd$ is 0.1% 2σ.

Methods and results

Much of the ensuing discussion revolves around the choice of samples. Original igneous minerals are not preserved in the Kambalda and Bluebush lavas, and whole-rocks have been used. In contrast to the previous study, which combined mafic–ultramafic volcanics with felsic plutonic rocks to produce a wide range of Sm/Nd ratios[4], the approach adopted here is to use only mafic–ultramafic lavas. Samples from the Kambalda sequence are all from deep diamond-drill holes through the Kambalda Dome. Care was taken to choose rocks with good preservation of igneous textures, and to avoid visible forms of alteration (apart from the pervasive amphibolite facies metamorphism) such as biotite-rich zones, variolitic forms of basalt, and carbonation. Only surface samples are available from the Bluebush sequence: those used are all from the 400 m Ultramafic Hill section, where field relationships are best exposed, and were chosen for quality of textural preservation from portions of very large specimens free from obvious surficial weathering.

Analytical methods used for rare earth elements (REE) and Nd isotopes are described elsewhere[8,9]. Results for Kambalda and Bluebush are listed in Tables 1 and 2, and the data are plotted on an isochron diagram in Fig. 2*a*. A very wide range of Sm/Nd ratios has been found in the mafic–ultramafic lavas alone, and, with one sample excluded, the new data for Kam-

Table 2 Isochron results

Data set	Age (Myr)	$(^{143}Nd/^{144}Nd)^{i}$	ε^{i}_{Nd}	MSWD	n
Kambalda					
All lavas					
(i) New data, except 29/501.5	3,262±44	0.50851±6	2.3±0.2	3.2	10
(ii) As for (i) combined with previous data for footwall basalts & komatiites only[4]	3,274±57	0.50851±7	2.4±0.2	5.6	14
Footwall basalts only					
(iii) New data	3,533±1102	0.50815±148	2.2±0.7	3.7	3
(iv) Combined with ref. 4	2,930±1096	0.50897±146	2.7±0.6	8.5	6
Komatiites only					
(v) New data, except 29/501.5	3,290±140	0.50848±22	2.2±0.7	0.5	3
(vi) Combined with ref. 4	3,276±131	0.50850±20	2.3±0.7	0.4	4
Hanging-wall basalts only					
(vii) New data (HWB data in ref. 4 omitted, see discussion)	3,226±136	0.50855±15	2.1±0.6	8.6	4
Bluebush					
All lavas	3,045±554	0.50896±73	5.4±0.9	37.0	5

Regressions by the method of York[26]. All errors are 2σ, and are multiplied by √MSWD when MSWD > 1. ε^{i}_{Nd} is a measure of the deviation in $^{143}Nd/^{144}Nd$ of a suite of samples from a reference composition CHUR (chondritic uniform reservoir)[27], and is calculated by comparing the present day composition, $(^{143}Nd/^{144}Nd)^{0}_{Sa}$, of a hypothetical sample having $(^{147}Sm/^{144}Nd)^{0}_{Sa} = (^{147}Sm/^{144}Nd)^{0}_{CHUR}$, with the present day composition, $(^{143}Nd/^{144}Nd)^{0}_{CHUR}$, of CHUR according to the definition of ref. 21:

$$\varepsilon^{i}_{Nd} = \left[\frac{(^{143}Nd/^{144}Nd)^{0}_{Sa}}{(^{143}Nd/^{144}Nd)^{0}_{CHUR}} - 1\right] \times 10^{4}.$$

Present day reference values for CHUR[28] are:

$$(^{143}Nd/^{144}Nd)^{0}_{CHUR} = 0.51264 \quad \text{and} \quad (^{147}Sm/^{144}Nd)^{0}_{CHUR} = 0.1967.$$

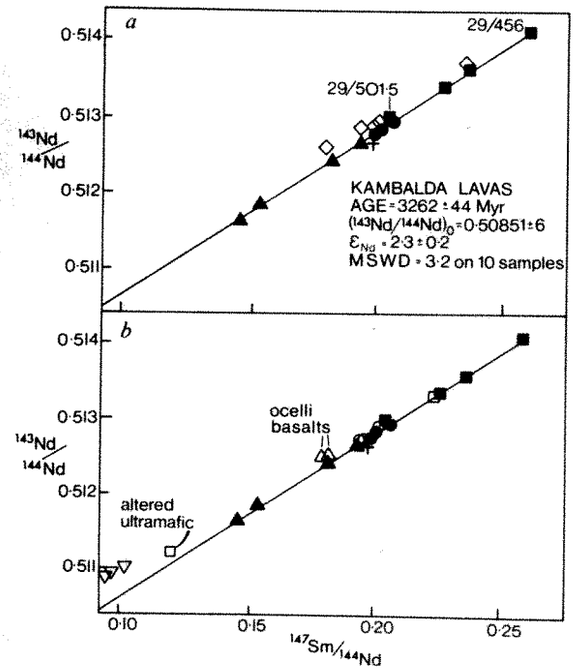


Fig. 2 *a*, Sm–Nd evolution diagram for Kambalda and Bluebush data in Table 1. Isochron plotted is for Kambalda only (omitting sample 29/501.5, see discussion). *b*, Data and isochron for Kambalda plotted as in *a* (solid symbols) compared with data from ref. 4 (open symbols). Previous data for ocelli-basalts do not coincide with purely mafic lavas in the hanging-wall formation (new data), and the tightly-constrained lavas isochron does not extrapolate to points representing intrusive granites. Kambalda: ●, footwall basalts; ■, komatiites; ▲, hanging-wall basalts; ▽, granites. ◇, Bluebush lavas. + Represents the present-day chondritic value.

balda yield a tightly constrained isochron (MSWD = 3.2) of age 3,262 ± 44 Myr with an initial $^{143}Nd/^{144}Nd$ of 0.50851 ± 6 and $\varepsilon^{i}_{Nd} = 2.3 \pm 0.2$. Data for Bluebush do not form an isochron, but plot separately from the Kambalda line and clearly have a much higher ε^{i}_{Nd} value in the region of 5–6.

The sample excluded (29/501.5) from the otherwise coherent new data set for Kambalda is from the basaltic differentiation zone of a layered komatiite flow whose flowtop is also analysed (sample 29/456, see Fig. 2*a*). It is not possible to ascribe the severe divergence in Sm/Nd ratio (22%) between this sample and the flowtop composition to simple crystal fractionation within the flow, and it is unique in departing from the otherwise regular and predictable variation found in the rest of the data set. 29/501.5 lies close to a minor basic intrusion, and its unusual Sm–Nd composition suggests that a process of exchange of light-REE (LREE) with the younger intrusion may have occurred.

The new results for the Kambalda lavas are not colinear on the isochron diagram with re-normalized data from a previous study[4] (Fig. 2*b*). Unfortunately there are no sample descriptions in the previous paper, but the two basalts most divergent from the new data are referred to as ocelli-bearing. This type has been avoided in the present study in the belief that purely mafic varieties are more likely to preserve original igneous geochemistry. An anomalous ultramafic sample is also reported by the previous authors, who believe it unlikely to represent original igneous geochemistry (for reasons similar to those given above with respect to sample 29/501.5), and they report that it came from an alteration zone at the margin of a felsic porphyry.

A total of 18 samples of lavas were selected by this and the previous study as *a priori* candidates for analysis, and definition of an isochron. Of these, it is apparent that ocelli-basalts and an altered ultramafic rock from the previous paper, and a further ultramafic sample from this study, no longer possess original igneous Sm–Nd compositions, and they are excluded from further discussion. Rejection of these samples does not invalidate the regression defined by the remaining lavas, but does make it less easy to justify. However, it is notable that the four other data points for lavas used in the previous study (one ultramafic, three foot-wall basalts) plot together with the new data. Combining these four data points with the new analyses yields a line within error of that based on the new results alone, and a comparable age (3,274 ± 57 Myr), initial $^{143}Nd/^{144}Nd$ (0.50851 ± 7), and ε^{i}_{Nd} (2.4 ± 0.2).

The poor coherence of data for the Bluebush lavas is most unlikely to reflect variation in ages of the flows that were sampled. The scatter could represent orginal variations in initial $^{143}Nd/^{144}Nd$, but it is not possible to rule out disturbance of the Sm–Nd isotopic system by weathering of the surface samples.

Whole-rock 'isochrons'

To obtain a wide spread of parent/daughter ratios, the previous Sm–Nd study of Kambalda enlisted a mixture of whole-rock samples ranging from komatiite to granite, on the assumption that such diverse samples may have the same age and essentially homogeneous initial $^{143}Nd/^{144}Nd$ (ref. 4). This assumption is the main weakness of whole-rock dating applied in the absence of mineral data. Justification for such studies can come from the internal consistency of results so obtained, and indeed the previous interpretation of the komatiite–granite correlation as a real age for Kambalda was based on the tightness of the regression through all the samples used. However, the slope of this regression was largely controlled by the samples of granite, and the large variation in Sm/Nd now found in the mafic–ultramafic lavas alone produces a tightly-constrained line that does not extrapolate through the granite data (Fig. 2*b*). This strongly suggests that the lavas and granites were derived from isotopically distinct sources, and that the age derived from mixing the plutonic and volcanic samples is spurious. However, it remains to be shown that the correlation within the lavas is not a mixing line between magmas from an isotopically heterogeneous mantle.

Reference to Fig. 3 shows that, regardless of Sm/Nd ratios, all lavas in the Kambalda sequence have essentially flat heavy-REEs (HREEs), whereas Bluebush lavas show HREE depletion. This difference corresponds to a worldwide division of komatiite types previously thought to reflect temporal changes in komatiite genesis[10]: early HREE-depleted komatiites (for example, in the Barberton belt) are thought to result from garnet control in the mantle, whereas younger lavas so far studied have chondritic ratios of HREE and do not show the influence of garnet separation. The temporal sequence of source regions is reversed with the Kambalda and Bluebush lavas, and their close spatial association alone is evidence that mantle heterogeneity existed below the Kambalda region during the Archaean.

The foot-wall, komatiite and hanging-wall formations at Kambalda each represent co-magmatic lava batches. Within each formation, variations in Sm/Nd ratio (34% for the hanging-wall basalts, 27% for the komatiites) may be ascribed to either the combined effects of differing degrees of partial melting of, and fractionation in, magmas from a homogeneous source, or the possibility that Sm/Nd in each lava directly reflects variations in Sm/Nd within the source area. Continuity of source composition between formations is not easy to justify because the variation in Sm/Nd ratio between lava batches is large (Fig. 3), and a very high degree of LREE fractionation is indicated if the same source is involved.

Taking the worst case, it is possible that the corrrelation on the isochron diagram is a mixing line between lavas derived from two, perhaps more, mantle sources possessing different Sm/Nd ratios. This leaves two main alternatives: (1) the lavas were erupted 3,200–3,300 Myr ago as the lavas correlation suggests. This requires that any Sm/Nd heterogeneity between the two (or more) mantle sources existed for a very short time before eruption of the three batches of magma. (2) The lavas

were erupted at 2,700–2,900 Myr as has previously been supposed. In this case the correlation in Fig. 2 represents mixing of data from a mantle that possessed long-lived heterogeneities in Sm/Nd.

These alternatives may be investigated by examining how data points for the individual lava batches fit the isochron. If lavas are co-genetic within each of the Kambalda formations, then individual true isochrons are defined by the limited numbers of points in each group. Errors on the ages yielded by these domains within the isochron are large, because of the limited spread of Sm/Nd ratios found in any one formation, but they are equal within error and constrain the age to older than 3,100 Myr (see Table 2). An additional constraint arises from the fact that the age deduced from the whole data set lies within the errors of the ages deduced from the individual formations. If mantle heterogeneity in Sm/Nd had existed for a significant time before eruption, then each source would have evolved $^{143}Nd/^{144}Nd$ separately to generate distinct initial ratios. As a consequence, isochrons through the domains would be parallel, but offset relative to one another by an amount proportional to the degree, and duration, of the heterogeneity. The regression

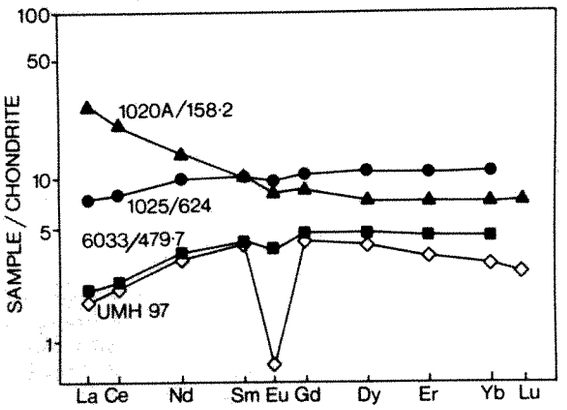


Fig. 3 Chondrite normalized REE abundances for selected Kambalda and Bluebush samples (symbols as for Fig. 2). Kambalda lavas have essentially flat HREE patterns, and show pronounced variation in LREE slopes. The Bluebush komatiite has identical LREE depletion to its Kambalda counterpart, but is depleted in HREE also. The large negative Eu anomaly may be due to alteration in the Bluebush surface sample.

through all the data would give an older age than isochrons through the individual magma batches. This 'tram line' effect is not observed in the Kambalda data, a point which is also illustrated by the homogeneous ε^{i}_{Nd} of the subsets.

Another explanation of Sm–Nd variations in the Kambalda samples is that the Sm/Nd ratio of each lava directly reflects Sm/Nd heterogeneities within the source region. This implies that essentially no fractionation of LREE occurred during partial melting and eruption and, therefore, the 3,262 ± 44 Myr age given by the lavas isochron would refer to the date of differentiation of sources within the mantle, not necessarily the date of eruption. If the lavas were subsequently erupted at 2,800 Myr, this model requires that the hanging-wall basalts were partially melted from a source which had been LREE-enriched for 400 Myr. Thus sample 1020A/158.2 would have to come from mantle source with $\varepsilon^{i}_{Nd} = -1.0$ at 2,790 Myr. However, available evidence suggests that the Archaean mantle was characterized by long-lived depletion of large-ion lithophile (LIL) elements[11–13] and it seems probable that if a LREE-enriched region existed in the mantle below Kambalda, it was created a short time before, or during, the partial melting event.

These arguments suggest that, while variations in Sm/Nd ratio almost certainly existed in the source of the Kambalda lavas, they were short-lived. Any heterogeneity of initial $^{143}Nd/^{144}Nd$ was therefore insignificant in terms of the error of the Nd age, and it is concluded that the data yield a true age for the lavas, which were erupted >3,200 Myr ago.

Archaean mantle

In the compositions of the Kambalda and Bluebush lavas there lies evidence for the existence of a wide variety of source regions. The revised Kambalda ε^{i}_{Nd} value of 2.3 ± 0.2 is 30% lower than that obtained from the komatiite–granite regression[4]. However, it confirms the depleted source for the greenstones found by the previous authors, is consistent with $\varepsilon_{Nd,t}$ values obtained for Archaean mafic–ultramafic volcanics of various ages in other cratons[11–13], and is further evidence for worldwide distribution of long-lived LIL-element depletion in at least part of the Archaean mantle. It is argued above that this already-depleted mantle may have undergone a separation, shortly before 3,200–3,300 Myr ago, into compositions ranging from LREE depleted to LREE enriched, and within a short time span these became sources for the three batches of magma erupted at Kambalda.

Another window into the mantle is provided by the stratigraphically-younger Bluebush sequence. Despite the scatter of data for these lavas, it is apparent that they derive from a very radiogenic source ($\varepsilon^{i}_{Nd} = 5.4 \pm 0.9$). The high ε^{i}_{Nd} may be an artefact of systematic alteration of Sm–Nd isotopes in the surface samples, but it corresponds with differences in HREE (Fig. 3), which suggest a garnet residue in the Bluebush source but not in the Kambalda source(s). It is most unlikely that the source of even the most depleted samples from the Kambalda sequence could have evolved ^{143}Nd sufficiently rapidly to be the source for Bluebush also, and it is inferred that yet another mantle composition was available at the time that the Bluebush lavas were erupted.

The Kambalda granite

The sodic granite used in the previously-published Sm–Nd isochron for Kambalda has been independently dated at 2,820 ± 15 Myr by U–Pb on zircons[14]. To produce a Sm–Nd isochron by combining this granite with the mafic–ultramafic lavas it intrudes requires the assumption that the felsic samples have the same initial $^{143}Nd/^{144}Nd$ as, and are essentially the same age as, the mafic–ultramafic lavas[4]. However, it is most unlikely that the granite is directly derived from the mantle source of the lavas, and an origin by extreme fractional crystallization from the lavas is also highly unlikely because of the large LREE fractionation required. Thus it is not surprising that the new Sm–Nd isochron for Kambalda, based on lavas alone, does not extrapolate through the granite data (Fig. 2*b*).

Other possible origins for the Kambalda granite are that it represents re-melting of old granite–gneiss basement, or that it derives from a partial melt of older greenstone rocks infolded to great depths. It is possible to choose between these options because the $\varepsilon^{i}_{Nd,t}$ of the granite is independently set at 3.9 ± 0.6 using the zircon age[4,14]. The high ε^{i}_{Nd} raises the paradox that the granite (which is very LREE enriched[4]) was derived from LREE depleted (high Sm/Nd) source, and this does not correspond to known compositions of granite–gneisses in the area[15]. This leaves the possibility that the 2,800 Myr-old granite was melted from pre-existing greenstones. Suitably light-REE enriched liquids would result if such material, in the form of garnet-amphibolite or garnet-granulite, was partially melted at depth[4], and basic–ultrabasic lavas comprising the bulk of this, and other, greenstone belts do have the radiogenic Nd compositions required to form a source for the granite[11–13]. For instance, it could be produced by melting of Kambalda greenstones with average $^{147}Sm/^{144}Nd = 0.2262$, which is close to the mean measured value at Kambalda. This is independent, if indirect, evidence that greenstones much older than the 2,800 Myr-old granite exist in the Yilgarn block.

Crustal evolution

Detailed geochronological studies in the Yilgarn block are sparse, but reported zircon ages of 2,560 ± 30 Myr for some minor felsic dykes, and 2,820 ± 15 Myr for the granite, both at Kambalda[14], are significantly younger than the 3,262 ± 44 Myr-old lavas that they intrude. It has been suggested that

the felsic intrusives are a subvolcanic expression of felsic volcanism higher in the greenstone stratigraphy[16], and a whole-rock Sm–Nd date of 2,780 ± 70 Myr has been reported for felsic volcanics at Kanowna, 60 km north of Kambalda[13]. If all these ages are correct, this implies that development of the greenstone belt extended over a period in excess of 380 Myr and highlights a remarkable lack of recognized unconformities—a paradox that applies to many, if not most, greenstone belts[17].

The regionally extensive banded gneisses, which have been considered as representing the basement to the greenstones[18], have yielded a whole-rock Sm–Nd age of 2,800 ± 100 Myr for localities close to Kambalda[15], and a whole-rock Pb–Pb age of 2,700 ± 97 Myr for samples from widespread localities in the Yilgarn granite–greenstone terrain[19]. These are younger than both the age reported here for the Kambalda greenstones, and a further whole-rock Sm–Nd age of 3,050 ± 100 Myr reported for greenstones in the Diemals–Marda area[13]. This observation does not correspond with simpler models of craton development whereby greenstones are late features deposited on granite–gneiss basement.

Older dates are found in gneisses of the Western Gneiss Terrain, from where zircon dates of $3{,}267^{+48}_{-25}$ Myr for Gabidine Springs and $3{,}341^{+133}_{-73}$ Myr for Windmill Hill, comparable in age to the Kambalda volcanics, have been reported[20]. Attention has recently focused on gneisses near Mount Narryer which have produced whole-rock Rb–Sr and Sm–Nd ages of 3,300–3,600 Myr[21], and in which have been found four detrital zircons yielding near-concordant U–Pb ages between 4,100 and 4,200 Myr (refs 22, 23). Isotopic dates in the Yilgarn block thus cover a period of over 1,500 Myr. Together with the great age of the Kambalda greenstones in relation to associated banded gneisses, this suggests that formation of the early crust in the Yilgarn was a multi-stage process, and more complex models must be sought.

Conclusions

Sm–Nd systematics in rocks from the Kambalda region of the Yilgarn Craton, Western Australia, yield important constraints on the evolution of the Archaean mantle and crust. The paradox of low Sm/Nd but high ε^i_{Nd} in a 2,800 Myr-old granite suggests that it represents a partial melt of older greenstone material rather than granitic gneisses. This indirect clue to the existence of very old greenstones in the Yilgarn is confirmed by the 3,262 ± 44 Myr Sm–Nd age of lavas hosting the Kambalda nickel deposits, which predate gneisses previously proposed as basement rocks. The known development of this section of the Norseman–Wiluna belt is extended to a period of more than 380 Myr.

The lavas at Kambalda may have been derived from at least two sources, with distinct Sm/Nd ratios, that separated from a mantle with $\varepsilon^i_{Nd} = 2.3 \pm 0.2$ immediately before partial melting and eruption. Scatter of data in volcanics from an overlying sequence at Bluebush may reflect disturbance of the Sm–Nd system. However, ε^i_{Nd} is systematically very high for all samples from Ultramafic Hill, and this combines with other characteristics to suggest that a separate part of the mantle was tapped to generate the younger volcanics. A previously-suggested evolution of komatiites with time, from HREE-depleted types to lavas with chondritic ratios of these elements is not confirmed, and it is inferred that a wide variety of mantle compositions was available under the Kambalda region during development of the greenstone belt.

That the Kambalda lavas are apparently 400 Myr older than previous dates from the same greenstone belt[24] is perhaps an artefact of the severe lack of Sm–Nd studies in the area, and the fact that existing chronologies depend on decay schemes such as Rb–Sr, K–Ar and U–Th–Pb, which either have not recorded primary crystallization ages or have only been applied to younger intrusive rocks. However, this study shows that confidence placed in whole-rock isochrons is clouded by little-discussed problems of source heterogeneity and choice of samples. Further detailed applications of Sm–Nd isotopes are required to substantiate this presently anomalous result.

We thank P. Guise and R. Paver for technical assistance, and A. Dunkley for drafting the diagrams. Constructive reviews of an earlier draft were given by A. Cattell, J. Gresham, J. Hallberg, D. Harley, A. Larking, two anonymous reviewers, and by D. Groves who also helped with selection of some samples. We also thank Western Mining Corporation for access to the area, many useful discussions, and permission to publish the data. Isotope and REE research at Leeds is funded by NERC and the Royal Society; J.C-L. and R.W.N. acknowledge support from NERC grants GT4/80/GS/102 and GR3/4437 respectively.

Received 7 September; accepted 1 December 1983.

1. Gee, R. D., Baxter, J. L., Wilde, S. A. & Williams, I. R. *Geol. Soc. Aust. Spec. Publ.* No. 7, 43–56 (1981).
2. Archibald, N. J., Bettenay, L. F., Binns, R. A., Groves, D. I. & Gunthorpe, R. J. *Precambr. Res.* **6**, 103–131 (1978).
3. Glickson, A. Y. & Lambert, I. B. *Tectonophysics* **30**, 55–89 (1976).
4. McCulloch, M. T. & Compston, W. *Nature* **294**, 322–327 (1981).
5. Gemuts, I. & Theron, A. in *Economic Geology of Australia and Papua New Guinea* Vol. 1 (ed. Knight, C. L.) 100–121 (Australian Institute of Mining and Metallurgy, Victoria, 1975).
6. Gresham, J. J. & Loftus-Hills, G. D. *Econ. Geol.* **76**, 1373–1416 (1981).
7. Levy, I. W. thesis, Australian Natn. Univ. (1975).
8. Thirlwall, M. F. *Chem. Geol.* **35**, 155–166 (1982).
9. Thirlwall, M. F. *Earth planet. Sci. Lett.* **58**, 27–50 (1982).
10. Nesbitt, R. W., Jahn, B. & Purvis, A. C. *J. Volcan. geotherm. Res.* **14**, 31–45 (1982).
11. Zindler, A. H. in *Komatiites* (eds Arndt, N. T. & Nisbet, E. G.) 399–420 (Allen & Unwin, London, 1982).
12. Fletcher, I. R. & Rosman, K. J. R. *Geochim. cosmochim. Acta* **46**, 1983–1987 (1982).
13. Fletcher, I. R., Rosman, K. J. R., Trendall, A. F. & de Laeter, J. R. *5th International Conference on Geochronology, Cosmochronology, Isotope Geology (Abstr.)* (Nikko, Japan, 1982).
14. Compston, W. *History of the Crust and Mantle at Kamblada using Isotopic Age Determinations* (Western Australian Institute of Technology, Perth, 1980).
15. McCulloch, M. T., Compston, W. & Froude, D. *J. geol. Soc. Aust.* **30**, 149–155 (1983).
16. Barrett, F. M., Binns, R. A., Groves, D. I., Marston, R. J. & McQueen, K. G. *Econ. Geol.* **72**, 1195–1223 (1977).
17. McCall, G. J. H. *Geol. Soc. Aust. Spec. Publ.* No. 7, 3–18 (1981).
18. Archibald, N. J. & Bettenay, L. F. *Earth planet. Sci. Lett.* **33**, 370–378 (1977).
19. Bickle, M. J., Chapman, H. J., Bettenay, L. F., Groves, D. I. & de Laeter, J. R. *Geochim. cosmochim. Acta* **47**, 907–914 (1983).
20. Nieuwland, D. A. & Compston, W. *Geol. Soc. Austr. Publ.* No. 7, 159–171 (1981).
21. de Laeter, J. R. *et al. Nature* **292**, 322–324 (1981).
22. Froude, D. O. *et al. Nature* **304**, 616–618 (1983).
23. Moorbath, S. *Nature* **304**, 585–586 (1983).
24. Groves, D. I. & Lesher, C. M. *Regional Geology and Nickel Deposits of the Norseman-Wiluna Belt, Western Australia* (University of Western Australia Extension Service, Perth, 1982).
25. Wasserburg, G. J., Jacobsen, S. B., De Paulo, D. J., McCulloch, M. T. & Wen, T. *Geochim. cosmochim. Acta* **45**, 2311–2323 (1981).
26. York, D. *Earth planet. Sci. Lett.* **5**, 320–324 (1969).
27. De Paulo, D. J. & Wasserburg, G. J. *Geophys. Res. Lett.* **3**, 249–252 (1976).
28. Jacobson, S. B. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **50**, 139–155 (1980).

Alteration of c-*myc* chromatin structure by avian leukosis virus integration

William Schubach* **& Mark Groudine†**

Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98014, USA
† Department of Radiation Oncology, University of Washington Hospital, Seattle, Washington 98015, USA

*The most common sites of integration of the leukosis virus (ALV) long terminal repeat (LTR) in bursal lymphomas and derivative cell lines correspond to a region encompassed by two major hypersensitive sites in the 5′ flanking region of the pre-integration, unrearranged c-*myc *gene. After integration of the ALV LTR, the major hypersensitive site within the avian c-*myc *oncogene region is within the proviral LTR, and the major hypersensitive sites normally found in uninfected cells 5′ to the first c-*myc *coding exon are no longer detectable.*

DIFFERENTIAL sensitivity to DNase I distinguishes the chromatin structure of genes that are actively transcribed from those that are inactive[1]. In addition, specific DNase I hypersensitive sites have been found in a variety of systems, predominantly near the 5′ ends of actively transcribed genes (refs 2–8; see also ref. 9 for a review). In some cases, these sites appear with the developmentally regulated transcriptional activation of specific genes[4–6], and in other systems, their appearance precedes the activation of gene expression[7,8]. Hypersensitive sites have also been detected within certain regulatory sequences in DNA, such as the long terminal repeats (LTRs) of transcribed avian endogenous provirus[10,11], the 72 base pair (bp) repeat involved in enhancement of simian virus 40 (SV40) gene expression[12,13], and an enhancer complex located upstream from an actively transcribed glue protein gene in *Drosophila*[14,15]. It is possible that the local alteration in chromatin structure revealed by DNase I hypersensitivity is related to the apparent enhancer function of these elements.

There is also indirect evidence that DNase I hypersensitivity characterizes regions involved in targeted recombination. In yeast mating-type interconversion, a specific and genetically regulated DNase I hypersensitive site corresponds to the site of a double-strand endonucleolytic cleavage that is destined to be the recipient site in the active cassette[16]. Also, in T lymphocytes, a cluster of DNase I hypersensitive sites occurs within a span of several kilobases between joining (J) and heavy chain (C_H) genes in the region involved in heavy chain switching in B lymphocytes[17].

To understand the possible relationships among hypersensitive sites, 'enhancement' and recombination, we studied the DNase I hypersensitivity pattern upstream from the cellular oncogene of chickens, c-*myc*. c-*myc* is the endogenous cellular homologue of the transforming gene (v-*myc*) of the avian myelocytomatosis virus, MC29[18–20]. In bursal lymphomas induced by the avian leukosis virus (ALV), integration of the ALV provirus in the region of c-*myc* has been shown to enhance significantly transcription of the c-*myc* gene[21]. In addition, rearrangements around c-*myc* in B-cell tumours of other species have been described. For example, in mouse myeloma[22,23] and in Burkitt's lymphoma[24–27] cell lines translocations involving immunoglobulin genes and c-*myc* are associated with altered c-*myc* transcription. Thus, we felt a study of the chromatin structure around the c-*myc* oncogene prior to and after LTR integration into this locus might provide insight into amplified or altered gene expression in bursal lymphomas. This was facilitated by the isolation of bursal lymphoma cell lines haploid for c-*myc*, containing only a single c-*myc* allele associated with an upstream ALV proviral LTR. Thus, the chromatin structure of the normal, unrearranged c-*myc* locus does not interfere with the analysis of the structure of the LTR-associated c-*myc* chromosomal domain. Our results show that after the integration of the ALV LTR, the major hypersensitive site within the c-*myc* region usually occurs within the proviral LTR, and the major hypersensitive sites normally found in uninfected cells 5′ to the first c-*myc* exon are no longer detectable. Also, we find that the most common sites of integration of the ALV LTR in bursal lymphomas and derivative cell lines correspond to a region encompassed by two major hypersensitive sites in the 5′ flanking region of the unrearranged c-*myc* gene.

Mapping hypersensitive sites

The presence and location of hypersensitive sites in the c-*myc* locus were determined by treating nuclei of various tissues obtained from embryonic and young chicks with increasing concentrations of DNase I for brief periods of time. DNA isolated from these nuclei was subsequently digested with restriction endonuclease, electrophoresed on agarose gels and blot hybridized to nick-translated probes derived from a genomic clone of the c-*myc* region. In this analysis, hypersensitive sites are manifest as DNase I generated sub-bands, one end of which is defined by the restriction enzyme recognition site and the other by the DNase I hypersensitive site.

Figure 1 shows the results of this type of analysis of nuclei derived from 14-day embryonic red blood cells (RBC) and a bursa from a 4-week post-hatching chicken. In Fig. 1*a*, RBC nuclei were digested with DNase I, and the DNA was isolated and digested with *Sst*I, 'Southern' blotted and probed with the 9.2-kilobase (kb) λ-*myc* probe depicted at the bottom of the figure. This probe includes the coding and 5′ flanking sequences of the c-*myc* region. In addition to the 7.3-kb *Sst*I parent fragment, two additional bands of 4.8 and 5.8 kb are generated by DNase I. Extensive mapping of these sub-bands has revealed two hypersensitive sites (designated II and III in Fig. 1*a*) upstream from c-*myc*. The 3.2-kb *Sst*I band, which encompasses the c-*myc* coding region, is very sensitive to digestion by DNase I since it is smaller and disappears before the sub-bands.

Because *Sst*I cleaves 190 bp downstream from the identified 5′ coding sequences of c-*myc*[28], it is not a suitable restriction enzyme for defining hypersensitive sites close to the 5′ end of this coding region. Thus, we used the indirect end-labelling approach of Wu[2], in which a probe is used that hybridizes to that end of the sub-band defined by the restriction endonuclease recognition site. With this approach, the location of DNase I hypersensitive sites can be inferred directly from the length of the sub-bands generated. Nuclei from a 4-week bursa were digested with increasing concentrations of DNase I, restricted with *Cla*I and *Bam*HI, Southern blotted, and hybridized to a 1,050-bp probe the 3′ end of which is defined by the *Cla*I site and which spans the c-*myc* intron indicated in the map below

* Present address: Department of Medicine, University of Minnesota School of Medicine, Minneapolis, Minnesota 55455, USA.

0028-0836/84/080702—07$01.00

© 1984 Macmillan Journals Ltd

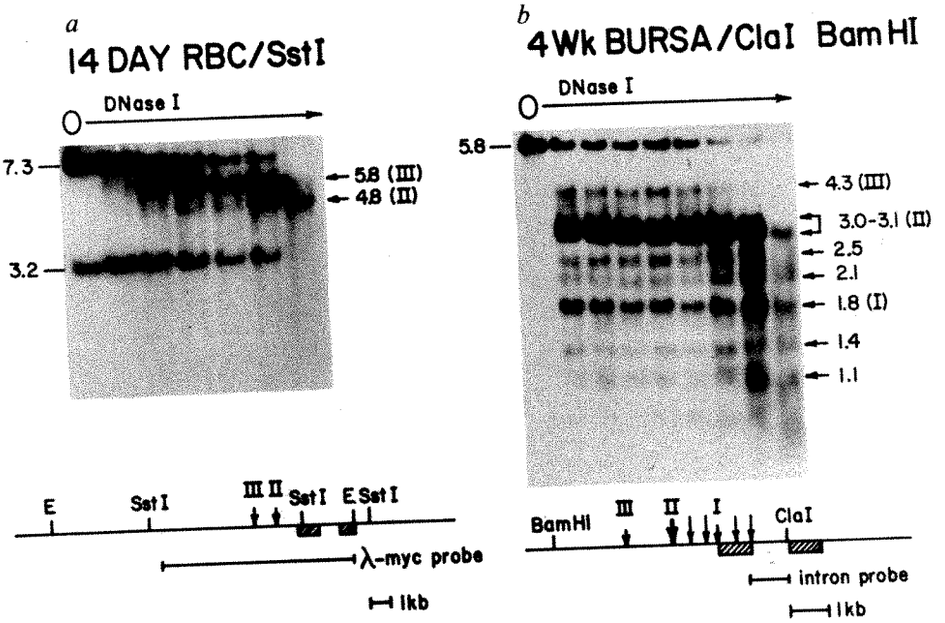


Fig. 1 Endogenous DNase I hypersensitive sites upstream from c-*myc*. Nuclei from designated tissues were digested with increasing concentrations of DNase I (as designated by the direction of the arrow), DNA isolated, digested with the enzymes denoted, and subjected to blot hybridization following electrophoresis in 1% agarose gels. The blots were probed with the nick-translated probes designated below the autoradiograms. Nuclear isolation, DNase I digestion of nuclei, DNA purification, restriction enzyme digestion and blot hybridization were performed as described previously[4,5,8]. All probes described in this and subsequent figures containing c-*myc* sequences were derived from a λ clone containing a 9.2-kb insert with sequence homology to v-*myc*, isolated from a chicken genomic library[47] by conventional screening methods[48]. Plasmid subclones were prepared using the pBR322 analogue pSV2 (obtained from B. Seed). Published data[28,30,49] have been used to correlate specific fragments with intron and exon positions. λ-*myc* is a 9.2-kb *Eco*RI fragment with an artificial R1 site at the 5′ end and a 3′ end that is the *Eco*RI site at the 3′ side of the second c-*myc* exon. 'Intron' is a 1-kb *Sal*I–*Cla*I fragment that includes primarily the intron of c-*myc*. Fragments were isolated from their host-vector sequences by restriction enzyme digestion, agarose gel electrophoresis, isolation in hydroxyapatite troughs[50] and nick-translated[51]. Restriction sites are marked; E, *Eco*RI. *a*, Four week RBC were digested with *Sst*I and hybridized to the λ-*myc* probe. A 3-kb *Sst*I band detected by this probe is very sensitive to DNase I. A parent band of 7.3 kb and sub-bands of 5.8 and 4.8 kb are seen corresponding to the hypersensitive sites III and II in the diagram at the bottom of the figure. *b*, Normal 4-week bursa DNA digested with *Cla*I plus *Bam*HI and analysed using the 1-kb intron probe depicted at the bottom of the figure. A parent fragment of 5.8 kb and sub-bands corresponding to the arrows are seen. See text for details.

Fig. 1*b*. Figure 1*b* shows that *Cla*I/*Bam*HI digestion of normal bursa DNA reveals a parent band of 5.8 kb and DNase I-generated sub-bands of 4.3, 3.0–3.1, 2.5, 2.1, 1.8, 1.4 and 1.1 kb; arrows show positions of the specific DNase I hypersensitive sites in c-*myc* from which these sub-bands are generated. Since the *Cla*I site is 1.8 kb from the 5′ side of the first c-*myc* exon[28], and the 3′ end of the probe is defined by this *Cla*I site, we can determine fairly accurately the positions of these hypersensitive sites relative to the 5′ side of the first c-*myc* exon. A prominent hypersensitive site lies 1.2–1.3 kb upstream from the first exon (site II). Less prominent, but distinct sub-bands lie at the end of the first exon (site I) and 2.5 kb upstream from the first exon (site III). Minor DNase I hypersensitive sites lying between I and II, at the 3′ end of the first exon, and within the intron are also designated in Fig. 2*b* by arrows. These latter sites vary in intensity and appearance in different nuclear preparations.

Results comparable with those in Fig. 2*b* were obtained for 14-day embryonic RBC and yolk sac cells, and bursa, thymus, liver, brain, bone marrow, muscle and spleen from a 4-week-old chick, as well as cultured chick embryonic fibroblasts (CEF), Rous sarcoma virus (RSV)-transformed CEF and MSB cells[29]. In addition, when the same blots are probed with sequences from a gene known to be inactive in these tissues (for example, a probe for sequences flanking the endogenous retroviral locus *ev*-1), no hypersensitive sites are seen, and the *ev*-1-containing sequences are less sensitive to DNase I than c-*myc* coding sequences (data not shown). Thus three prominent DNase I hypersensitive sites, denoted I, II and III, lie upstream from c-*myc* in the chromatin from the majority of chicken tissues studied at these stages of development, and the c-*myc* sequences appear to be in an active chromatin structure in all of these cells.

Cell lines: restriction mapping

We have analysed the restriction sites and mapped the location and orientation of the LTR for five bursal lymphoma cell lines. The data and results of this analysis are presented in Fig. 2, and for comparison the restriction map of the c-*myc* region of normal chicken DNA[30] is included in Fig. 2*b*.

Figure 2*a* shows the analysis of DNA from a diploid bursal lymphoma cell line, BK3A. When DNA from these cells is digested with *Eco*RI and probed with the c-*myc* probe (shown at the bottom of Fig. 2*b*), three bands are generated: the 14- and 20-kb bands correspond to fragments that flank the *Eco*RI site in the 'normal' c-*myc* allele. A third 3.7-kb fragment is generated by the introduction in an ALV LTR upstream from c-*myc* due to the presence of an *Eco*RI site in the U3 region of the LTR[31]. Since this band is detected by a probe for sequences of the U5 region of the LTR, but not by a probe for U3 sequences (Fig. 2*a*), this 3.7-kb tumour-specific junction fragment demonstrates that an LTR is located 1.0 kb upstream from the first major c-*myc* exon in the same transcriptional orientation as c-*myc*. This arrangement has been confirmed by digestion with *Bam*HI, *Sst*I and *Sal*I and probing with defined segments of c-*myc* and the ALV genome (not shown). Double digestion of BK3A with *Bam*HI plus *Sal*I and probing with the 5′ exon of c-*myc* confirms the arrangement of LTR and c-*myc* sequences in BK3A cells (Fig. 2*b*).

The same analyses were performed for the biologically cloned cell lines BK25 and 1104HI, and these results are illustrated in Fig. 2*a*. In both cases digestion with *Eco*RI generates only one band that is detected by probes for both c-*myc* and 5′ LTR sequences. The higher molecular weight *myc* bands are not seen, and *Bam*HI/*Sal*I double digestion detects only one band containing 5′ exon sequences. Thus, these cells contain only one c-*myc* allele, the one associated with a nearby LTR, the 'normal' c-*myc* allele having been lost. In BK25 cells, the LTR lies within 0.1–0.2 kb of the first c-*myc* exon, and in 1104HI the LTR lies 1.2 kb upstream from the first c-*myc* exon. In both cases, the LTR is in the same transcriptional orientation as c-*myc*, as depicted in Fig. 2*b*.

A similar analysis of a subclone from the 1104B1 cell line, 1104BI S13, is depicted in Fig. 2*a*. In this case, *Eco*RI yields a single band of 5.4 kb that hybridizes to c-*myc* and 3′ LTR, but not 5′ LTR probes. Thus, in this cell line, the LTR and contiguous c-*myc* sequences lie in opposite transcriptional orientations, and only the LTR-associated c-*myc* locus is present.

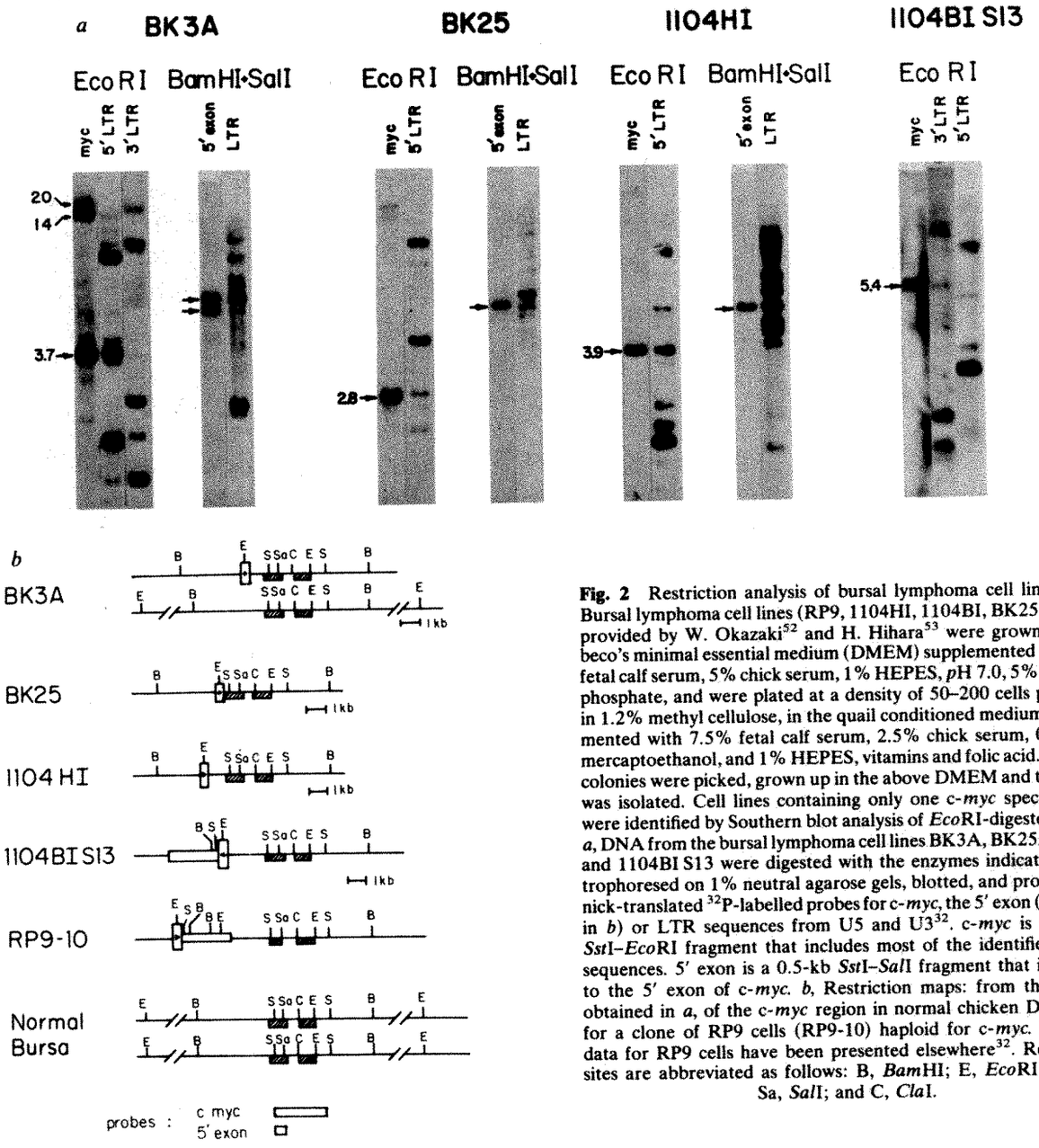


Fig. 2 Restriction analysis of bursal lymphoma cell line DNA. Bursal lymphoma cell lines (RP9, 1104HI, 1104BI, BK25, BK3A) provided by W. Okazaki[52] and H. Hihara[53] were grown in Dulbecco's minimal essential medium (DMEM) supplemented with 5% fetal calf serum, 5% chick serum, 1% HEPES, *p*H 7.0, 5% tryptose phosphate, and were plated at a density of 50–200 cells per plate in 1.2% methyl cellulose, in the quail conditioned medium supplemented with 7.5% fetal calf serum, 2.5% chick serum, 0.15 mM mercaptoethanol, and 1% HEPES, vitamins and folic acid. Isolated colonies were picked, grown up in the above DMEM and the DNA was isolated. Cell lines containing only one c-*myc* specific band were identified by Southern blot analysis of *Eco*RI-digested DNA. *a*, DNA from the bursal lymphoma cell lines BK3A, BK25, 1104HI and 1104BI S13 were digested with the enzymes indicated, electrophoresed on 1% neutral agarose gels, blotted, and probed with nick-translated ^{32}P-labelled probes for c-*myc*, the 5′ exon (depicted in *b*) or LTR sequences from U5 and U3[32]. c-*myc* is a 2.4-kb *Sst*I–*Eco*RI fragment that includes most of the identified c-*myc* sequences. 5′ exon is a 0.5-kb *Sst*I–*Sal*I fragment that is unique to the 5′ exon of c-*myc*. *b*, Restriction maps: from the results obtained in *a*, of the c-*myc* region in normal chicken DNA, and for a clone of RP9 cells (RP9-10) haploid for c-*myc*. Mapping data for RP9 cells have been presented elsewhere[32]. Restriction sites are abbreviated as follows: B, *Bam*HI; E, *Eco*RI; S, *Sst*I, Sa, *Sal*I; and C, *Cla*I.

Preliminary restriction analysis (in collaboration with M. Linial) of the *myc* region in this subclone shows that there are approximately 2.5 kb of proviral sequences inserted 2.6–2.7 kb upstream from the first c-*myc* exon (Fig. 2*b*).

When RP-9 cells are biologically cloned, clones containing only the c-*myc* allele and upstream proviral sequences are obtained. The restriction map of one such clone, RP9-10, is shown in Fig. 2*b*. Detailed mapping of c-*myc* in RP-9 cells has been presented elsewhere[32]. Essentially, this clone contains approximately 2.5 kb of proviral information consisting of a complete LTR and *gag* gene and part of *pol* gene, inserted 2.5–2.6 kb upstream from the first c-*myc* exon. The proviral sequences and c-*myc* are in the same transcriptional orientation (Fig. 2*b*).

Cell lines: chromatin structure

In most bursal lymphomas induced by ALV, the proviral LTR is integrated upstream from c-*myc* such that regulatory sequences in the LTR act directly to promote or otherwise enhance transcription of c-*myc*[21,33–35]. In addition to these types of integrations which are consistent with a downstream promoter model for the LTR activation of c-*myc*[21], enhanced c-*myc* expression has been reported in bursal lymphomas in which the LTR is located downstream from c-*myc* or upstream from c-*myc* in the opposite transcriptional orientation[36]. Since retroviral LTRs or analogous elements have been found to have an enhancer function distinct from that associated with downstream promotion[37–39], and because DNase I hypersensitive sites are associated with enhancer sequences in SV40 chromatin[12,13] and the *Drosophila Sgs*4 gene[14], we asked whether the introduction of an LTR upstream from c-*myc* altered the pattern of DNase I sensitivity that we observed in uninfected tissues. Such an analysis is difficult in tumours where c-*myc* is diploid. One locus contains a nearby LTR and one does not. Thus, there is both a 'normal' allele containing the family of endogenous hypersensitive sites, and an 'activated' allele containing an LTR and any alteration of this endogenous pattern of chromatin structure that the LTR might introduce. Our analysis has been facilitated by the isolation of the biologically cloned bursal lymphoma cell lines described above (Fig. 2*b*) which contain only the LTR-associated c-*myc* allele.

The distribution of DNase I hypersensitive sites in these cell lines compared with that in normal bursa is shown in Fig. 3. Normal bursal DNA taken from DNase I-treated nuclei (as used

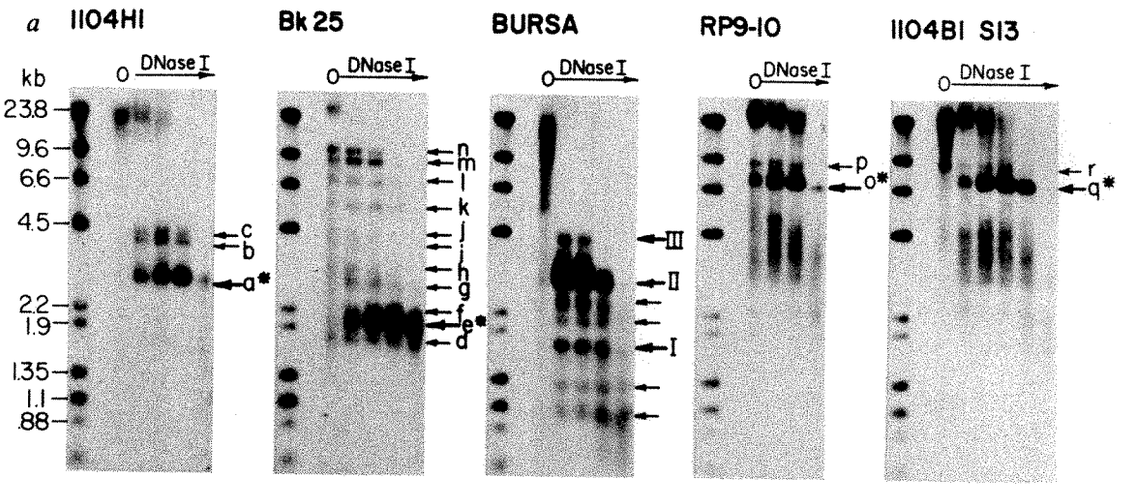
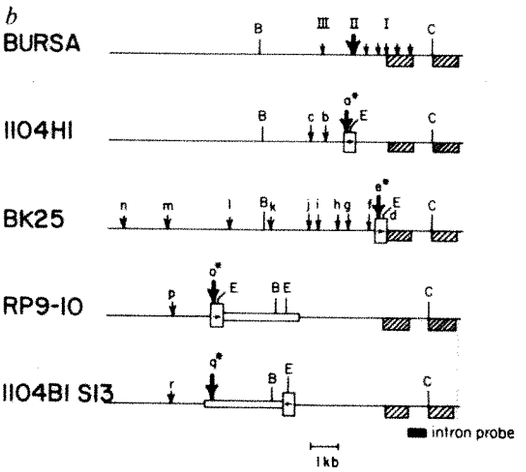
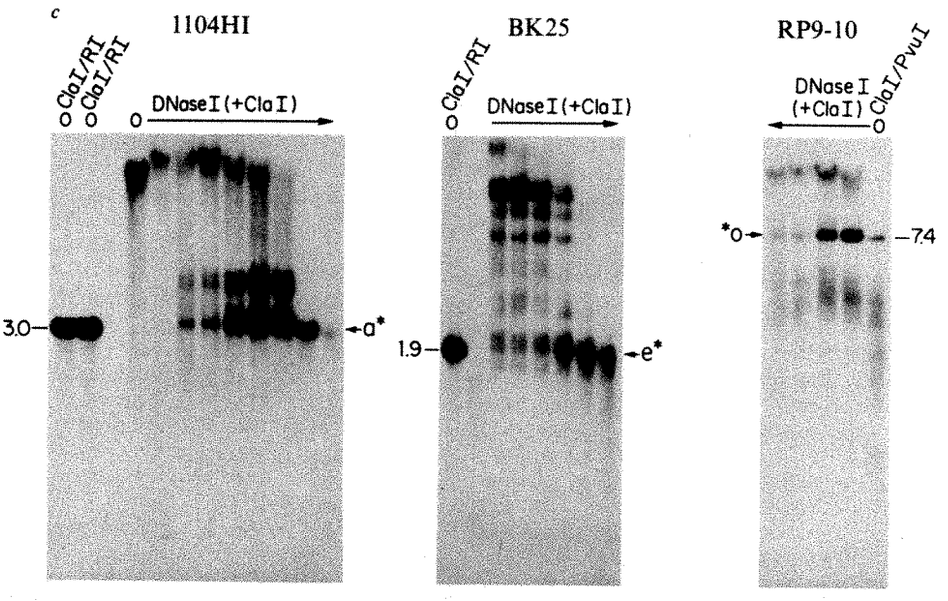


Fig. 3 Effect of LTR integration on chromatin structure upstream from c-*myc*. ***a***, Nuclei from normal bursa or the c-*myc* haploid cell lines indicated were digested with DNase I, DNA isolated, restricted with *Cla*I, blotted, and probed with the intron probe. All data presented in this figure were derived from a single blot of a large gel that contained all the samples. This was done to facilitate comparison of the locations of sub-bands in the various DNase I 'series'. Sub-bands are designated by arrows and letters to the right of each series. The major sub-band in each series is indicated by a bold arrow and an asterisk. Note that some sub-bands are present in the '0' (undigested with DNase I) DNA, a variable finding reported by several investigators[7]. The molecular weight markers are a combination of ^{32}P-labelled *Hin*dIII-digested DNA and *Hae*III-digested ϕXRF DNA. In the normal bursa series, the major sub-bands are indicated by roman numerals, as in Fig. 1. In the case of BK25, we have indicated all sub-bands seen in any of the multiple DNase I series we have performed, even though 'g' to 'j' sub-bands are rarely observed to contain significant signal. ***b***, Locations of the hypersensitive sites detectable by the analysis presented in Fig. 3*a* are superimposed upon the restriction maps of these cell lines and normal bursa DNA. As in Fig. 3*a*, the hypersensitive sites observed in the cell lines are indicated by arrows and letters, with the major hypersensitive sites indicated by bold arrows and asterisks. The locations of the normal bursa hypersensitive sites are included for comparison. ***c***, Locations of the major hypersensitive sites present in 1104HI, BK25 and RP9-10 are compared with the location of specific restriction enzyme recognition sites in the upstream LTR. For 1104HI and BK25, the *Cla*I digestions of DNase I series are compared with *Cla*I/*Eco*RI doublet digests of DNA, whereas for RP9-10, a *Cla*I-digested DNase I series is compared with a *Pvu*I/*Cla*I double digest of DNA. See text for rationale and details of this analysis. All blots are hybridized by the intron probe.





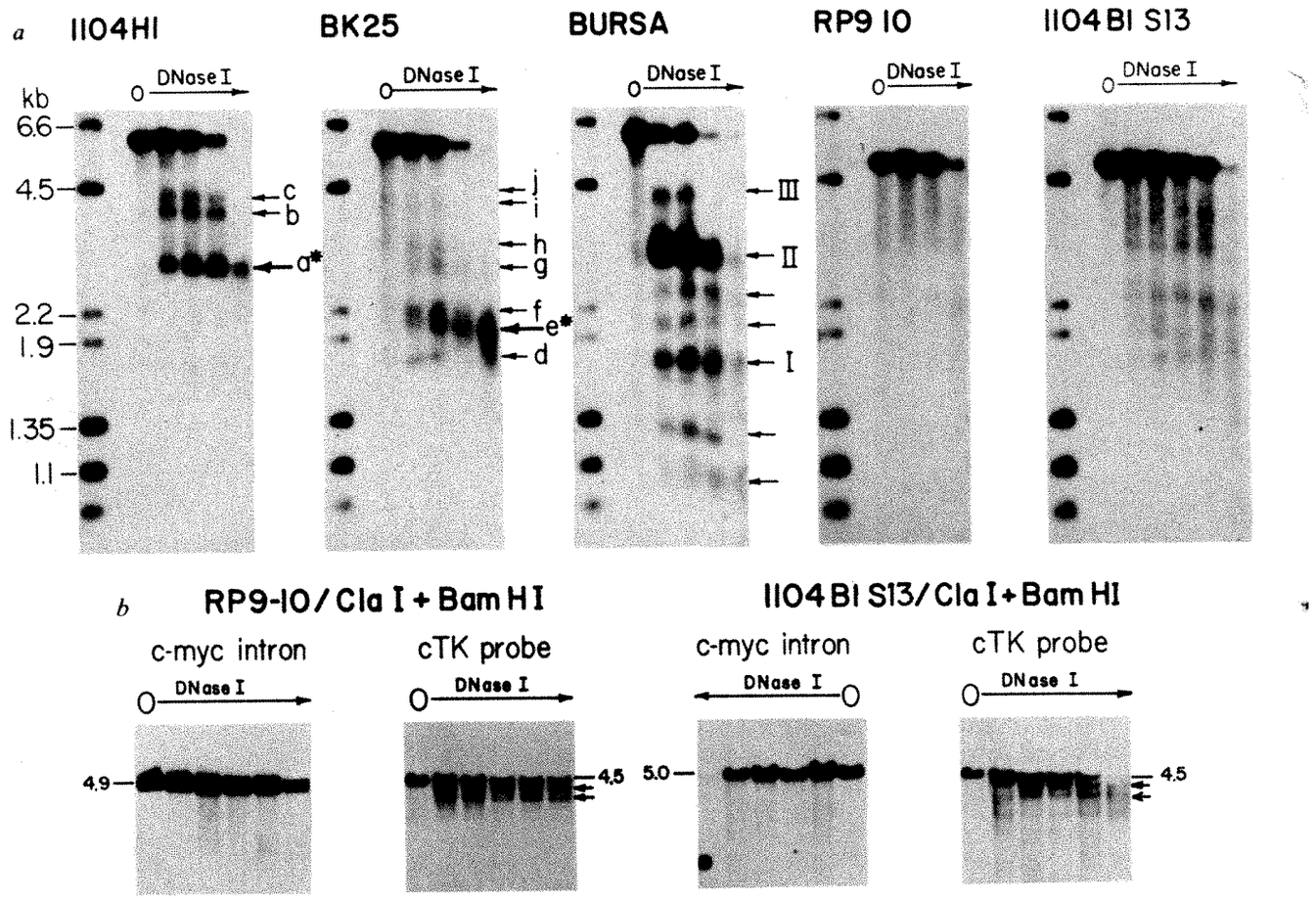


Fig. 4 Suppression of normal c-*myc* hypersensitive sites is associated with the upstream insertion of proviral sequences in different transcriptional orientations. *a*, DNA from the DNase I 'series' described in Fig. 3*a* was digested with *Cla*I plus *Bam*HI, blotted and hybridized to the intron probe. As described in Fig. 3*a* legend, all of these samples were loaded on the same gel and transferred to a single blot, to facilitate comparison. Arrows, letters, roman numerals and molecular weight markers are as for Fig. 3*a*. *b*, DNA derived from DNase I-digested nuclei of RP9-10 (a different preparation from that illustrated in Figs 3*a* and 4*a*) and 1104B1 S13 were restricted with *Cla*I and *Bam*HI, blotted and hybridized to either the c-*myc* intron probe or a probe for the chicken thymidine kinase (*tk*) gene (a generous gift of M. Wigler). While no hypersensitive sites are apparent in these digests with the intron probe, two prominent hypersensitive sites (arrows to the right of the blots) are seen with the *tk* probe.

in Fig. 1) was digested with *Cla*I, Southern blotted, and probed with the intron probe (Fig. 3*a*). The results of such digests confirm the observations described above concerning the presence and location of hypersensitive sites upstream from c-*myc*. DNAs from DNase I-treated nuclei isolated from 1104HI and BK25 were digested with *Cla*I and probed with the intron probe. Applying the same reasoning used in the analysis of normal bursa in Fig. 2, this will define DNase I-hypersensitive sites upstream from c-*myc* in these cell lines. As shown in Figs 3*a* and schematically in 3*b*, prominent DNase I hypersensitive sites occur near the *Eco*RI site in the LTR of these cell lines (sites a* and e* in Fig. 3*a* and 3*b*). That these hypersensitive sites reside in the LTR is also suggested by the results presented in Fig. 3*c*. In this figure the sub-bands, generated by *Cla*I digestion of the DNA from DNase I-treated nuclei of these cell lines, are compared to control DNA from these cell lines that has been digested with *Cla*I and *Eco*RI. Since *Eco*RI cleaves within the LTR, the *Cla*I/*Eco*RI band defines the location of the LTR relative to c-*myc* in these cells. By this analysis, the hypersensitive site lies about 0.1 kb upstream from the *Eco*RI site, near position 100 of the LTR (±60 bp)[31,40]. Rehybridization of the blot with probes specific for the U5 and U3 regions of the LTR has confirmed the location of this site within the LTR (not shown).

A similar analysis was performed for another haploid cell line RP9-10. These cells are unusual in that they contain a large amount of proviral DNA upstream from c-*myc*, including *gag* sequences that contain an *Eco*RI site. Thus, in this analysis, the *Pvu*I site unique to LTR sequences[40] was used to define the location of the LTR. Upon *Cla*I digestion of a DNase series from RP9-10 cells, a sub-band of 7.5 kb (site o* in Fig. 3*a*, *b*) is seen that corresponds rather closely to the location of the LTR in these cells, as defined by *Pvu*I/*Cla*I double digest of control RP9-10 DNA. This analysis and extensive mapping, using a variety of restriction endonucleases and cellular and proviral probes (not shown), places the hypersensitive site approximately 0.1 kb upstream from position 39 of the LTR.

In 1104BI S13 cell line, in which the LTR has inserted in the opposite transcriptional orientation, 2.7 kb upstream from the 5′ end of c-*myc*, *Cla*I digestion of DNase I-treated nuclear DNA and hybridization to the intron probe demonstrates a sub-band of approximately 7.5 kb (Fig. 3*a*, sub-band q*, Fig. 3*b*, site q*). After *Cla*I digestion, this same 7.5-kb sub-band is detected when the blot is rehybridized to several proviral sequence-specific probes, but not by a probe for cellular sequences 5′ to the integrated provirus (not shown), indicating that the site resides in proviral sequences. Thus, although no hypersensitive site is present in the c-*myc* associated LTR in these cells, a new proviral-associated hypersensitive site has been introduced into this locus by the insertion of proviral sequences. Hypersensitive sites located in proviral sequences other than LTRs have been observed previously in avian retroviruses[11]. Interestingly, as can

be seen in Fig. 3*a* and *b*, the provirus-associated hypersensitive sites in RP9-10 and 1104BI S13 (o* and q*, respectively) are in similar locations relative to c-*myc*, even though the proviruses are integrated in opposite orientations. Similarly, sites 'p' in RP9-10 and 'r' in 1104BI S13 which are absent in normal bursa (Fig. 3*b*), are located in comparable regions upstream from the provirus in these cell lines.

Newly integrated proviral sequences appear to affect the local structure of chromatin in surrounding cellular sequences. For example, none of the hypersensitive sites characteristic of the c-*myc* region in normal cells is observed in the RP9-10 cell line upon digestion of DNase I-treated nuclear DNA with *Cla*I (Fig. 3*a*). Since the LTR hypersensitive site is apparent in the RP9-10 chromatin, the lack of the normal c-*myc* sites is not a consequence of our failure to digest these nuclei in a fashion sufficient to detect these sites. Digestion of these same DNA samples with *Cla*I and *Bam*HI (*Bam*HI cleaves within the *gag* region of the provirus, downstream of the LTR; see Fig. 2*b*), and hybridization to the c-*myc* intron probe, confirms the absence of hypersensitive sites in the c-*myc* region between the newly integrated proviral DNA sequences and the first c-*myc* exon (Fig. 4*a*). In contrast, multiple hypersensitive sites are seen in bursa, 1104HI and BK25 when DNA from DNase I-digested nuclei from these cells is digested with *Cla*I and *Bam*HI and blot hybridized to the intron probe (Fig. 4*a*). The absence of these sites in RP9-10 is also seen in a *Cla*I/*Bam*HI digestion of an expanded DNase I series from another preparation of these cells (Fig. 4*b*). As a control, multiple hypersensitive sites around the chicken thymidine kinase (*tk*) gene are detected when this blot is rehybridized to a TK probe (Fig. 4*b*). A similar loss of the normal c-*myc* hypersensitive sites is observed in the 1104B1 S13 cell line when DNA from the DNase I-treated nuclei of these cells is digested with *Cla*I (Fig. 3*a*) or *Cla*I and *Bam*HI (Fig. 4*a* and *b*) and the subsequent blots are hybridized to the c-*myc* intron probe. As described above, in 1104B1 S13 the proviral sequences are inserted in the 'opposite' orientation; thus, the *Bam*HI site is in the *gag* gene, downstream from the newly introduced hypersensitive site in this cell line, and defines the 5′ end of the *Bam*HI/*Cla*I parent fragment (see Fig. 2*b*). As in the case of the RP9-10 samples, when the 1104BI S13 blot is rehybridized to the *tk* probe, multiple hypersensitive sites are detected (Fig. 4*b*). Thus, in both of these cell lines, the integration of proviral sequences appears to alter the endogenous structure of the c-*myc* locus, as shown by the loss of DNase I-hypersensitive sites downstream from the newly integrated proviral sequences, and the introduction of new hypersensitive sites into this chromosomal domain.

The analysis of the effect of proviral insertion on the endogenous c-*myc* chromatin structure in the BK25 and 1104HI cell lines is more complex than that for RP9-10 and 1104BI S13, due to the position of insertion of the LTRs in two former cell lines. As described above, both of these cell lines contain hypersensitive sites in the LTR upstream from the first c-*myc* exon (sites a* and e*). In 1104HI, the LTR hypersensitive site is approximately 1.2 kb upstream from the 5′ end of c-*myc* and in BK25, the LTR site is approximately 100 bases upstream from c-*myc*. Since in both cell lines, approximately 350 bp of proviral information has been inserted, the major endogenous site found in normal cells, which usually resides at 1.2 kbp upstream from the 5′ end of c-*myc*, would be displaced to approximately a position 1.55 kbp upstream of c-*myc*, and appear as a sub-band in a *Cla*I or *Cla*I/*Bam*HI digest of BK25 and 1104HI of 3.3–3.4 kbp (1.55 kbp+1.8 kbp from the *Cla*I site to the 5′ end of the first c-*myc* exon). As is evident in Fig. 3*b* and *c*, no such sub-band is observed in either cell line. In 1104HI, two additional hypersensitive sites located upstream from the LTR are revealed. The sizes of these sub-bands localize them to 2.3 and 1.8 kbp 5′ to c-*myc*. While it is possible that the latter sub-band could correspond to the normal, major site (if our measurements are in error by ±300 bp), we feel this is unlikely. Clearly, however, the complexity of sites seen in the normal c-*myc* chromosomal domain are absent in the 1104HI cells. In BK25, multiple hypersensitive sites are observed upstream from the LTR insertion (Fig. 3*a*, *b*, *c*). As in 1104HI, however, none of these map to the locations of the major sites observed prior to the LTR insertion. Thus, in both of these cell lines, insertion of a proviral LTR is associated with the introduction of a new hypersensitive site within the proviral sequences, as well as the suppression of some endogenous c-*myc* hypersensitive sites and the uncovering of other 'cryptic' sites in the 5′ flanking region of the c-*myc* gene.

Discussion

We have described the normal pattern of DNase I hypersensitive sites that lie upstream from c-*myc* in normal chicken cells. Major DNase I hypersensitive sites lie near the beginning of the coding region (site I), and 1.2 (site II) and 2.5 kb (site III) upstream from the coding region in every tissue from the various stages of chicken development we have thus far investigated. Interestingly, several investigators have observed a nonrandom distribution of integration sites of the LTR upstream from c-*myc* (refs 32–36, and W. Hayward, personal communication), most lying within an interval of 1.2 kb upstream from the coding region, a region flanked by the DNase I-hypersensitive sites I and II found in normal bursa. These findings raise the possibility that the chromatin in this local region may assume a conformation (as determined by DNase I sensitivity) preferentially targeted for integration within the c-*myc* domain. Clearly, however, we cannot eliminate the possibility that integration occurs in a random fashion in the c-*myc* region, with those insertions at specific sites (for example, upstream from splice donor sequences[30]) giving these cells a selective growth advantage.

The identification of bursal lymphoma cell lines that are haploid at the c-*myc* locus has allowed us to examine the effects of integration of an ALV proviral LTR in this region. In all of these lines a DNase I hypersensitive site can be identified within the proviral sequences integrated upstream from c-*myc*. In three cases (RP9-10, BK25, 1104HI) the hypersensitive site is located between positions 25 and 150 of the LTR, a region associated with enhancement of gene expression in injected and transfected *in vitro* constructs[38,39]. In RP9 cells, a transcript containing proviral and c-*myc* sequences has been described[32], indicating that the LTR is acting as a downstream promoter in these cells. However, in 1104HI and BK25, the amplified c-*myc* transcripts lack LTR sequences[32]; thus, in these cases, the observed enhancement of c-*myc* expression does not appear to be the result of downstream promotion, and probably reflects an enhancing effect of the LTR. These results suggest a correlation between those sequences which have enhancer function and the location of the DNase I hypersensitive site in the LTR.

In 1104BI S13 cells, the LTR lies 2.7 kb upstream from c-*myc* in the opposite transcriptional orientation and does not contain a prominent DNase I hypersensitive site as seen in the other cell lines. Despite this, c-*myc* expression is enhanced to high levels and the transcript is identical in size to the normal 2.5-kb transcript identified in fibroblasts[19] and does not contain proviral sequences (M. Linial, personal communication). Thus, in these cells either the LTR or some other proviral sequence is acting as an enhancer over a remote distance[38,41–43]. Are enhancer sequences and hypersensitive sites, in fact, related? One interpretation of the 1104BI S13 data would be that the enhancer resides in the LTR upstream from c-*myc*, but it lacks a hypersensitive site, possibly due to minor sequence changes. Alternatively, the enhancer in these cells may reside in the proviral sequences defined by the hypersensitive site upstream from the LTR. The latter interpretation would suggest that proviral sequences outside the LTR may contain an enhancer that can be functionally recruited when the enhancer in the LTR is defective, or which is 'dominant' to the LTR enhancer sequences. These possibilities can be tested in various ways using cloned fragments encompassing c-*myc* and proviral sequences from these cell lines.

The data in Figs 3 and 4 suggest that the introduction of proviral sequences upstream from c-*myc* alters the pattern of

endogenous DNase I hypersensitive sites normally found in the upstream region of c-*myc* in uninfected cells. The prominent hypersensitive site 1.2 kb upstream from the coding region (Site II), as well as the other 'minor' sites, are much reduced or absent in RP9-10 and 1104BI S13 cells. In BK25 and 1104HI, the LTR is inserted between the major endogenous c-*myc* hypersensitive site (1.2 kbp upstream from the 5′ exon) and the 5′ exon; this insertion is associated with the loss of this major endogenous hypersensitive site, as well as the acquisition of new sites within the LTR, and the uncovering of some additional sites in cellular sequences upstream to the insertion sites. The above results raise the possibility that the local alterations of chromatin structure associated with the insertion of the LTR or other proviral sequences may affect the transcriptional activity of c-*myc*. For example, one possibility is that specific sequences within the LTR act as dominant 'sinks' of superhelical torsional stress in the DNA of the c-*myc* locus (see refs 44–46 for discussion of this concept), assayable as DNase I hypersensitive sites. In this view, the loss of the major endogenous c-*myc* hypersensitive site would be the consequence of the reversion of these cellular sequences to the 'normal' B form. Within the context of transcriptional enhancement, the LTR-associated hypersensitive site may represent a conformation of DNA which provides a more efficient entry site than the endogenous c-*myc* hypersensitive site for various transcriptional factors, or may provide a novel nucleation site for the positioning of nucleosomes in this region. This latter possibility could also result in the alteration of the pattern of hypersensitive sites in the c-*myc* locus.

We thank Mary Peretz and Angela Hein for technical assistance, Helen Devitt for typing the manuscript, and our colleagues for their comments, especially M. Linial who isolated and helped characterize one cell line and P. Neiman for his support. M.G. is a Scholar of the Leukaemia Society of America. This work was supported by grants from the NSF (PCM-8204696) and NCI (CA 20068; CA 28151).

Received 16 August; accepted 2 November 1983.

1. Weintraub, H. & Groudine, M. *Science* **93,** 848–853 (1976).
2. Wu, C. *Nature* **284,** 854–860 (1980).
3. Kuo, T., Mandel, J. & Chambon, P. *Nucleic. Acids Res.* **7,** 2105–2113 (1979).
4. Stalder, J. *et al. Cell* **20,** 451–460 (1980).
5. Weintraub, H., Larsen, A. & Groudine, M. *Cell* **24,** 333–344 (1981).
6. Groudine, M. & Weintraub, H. *Cell* **24,** 393–401 (1981).
7. Burch, J. & Weintraub, H. *Cell* **33,** 65–76 (1983).
8. Weintraub, H., Beug, H., Groudine, M. & Graf, T. *Cell* **28,** 931–940 (1982).
9. Elgin, S. C. R. *Cell* **27,** 413–415 (1981).
10. Groudine, M., Eisenman, R. & Weintraub, H. *Nature* **292,** 311–317 (1981).
11. Conklin, K. F., Coffin, J. M., Robinson, H. L., Groudine, M. & Eisenman, R. *Molec. cell. Biol.* **2,** 638–652 (1982).
12. Cremisi, C. *Nucl. Acids Res.* **9,** 5949–5964 (1981).
13. Cereghini, S. *et al. Cold Spring Harb. Symp. quant. Biol.* **47,** 935–944 (1983).
14. McGinnis, W., Shermoen, A. W., Heemskerk, J. & Beckendorf, S. K. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1063–1067 (1983).
15. Shermoen, A. W. & Beckendorf, S. K. *Cell* **29,** 601–607 (1982).
16. Nasmyth, K. A. *Cell* **30,** 567–578 (1982).
17. Storb, U., Arp, B. & Wilson, R. *Nature* **294,** 90–92 (1979).
18. Roussel, M. *et al. Nature* **281,** 452–455 (1979).
19. Sheiness, D. *et al.* **33,** 962–968 (1980).
20. Sheiness, D. K., Hughes, S. H., Stubblefield, E. & Bishop, J. M. *Virology* **105,** 415–424 (1980).
21. Haywood, W. S., Neel, B. G. & Astrin, S. M. *Nature* **290,** 475–480 (1981).
22. Shen-Ong, G. L. C., Keath, E. J., Piccoli, S. P. & Cole, M. D. *Cell* **31,** 443–452 (1982).
23. Marcu, K. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 519–523 (1983).
24. Della-Favera, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7824–7827 (1982).
25. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7837–7841 (1982).
26. Della-Favera, R., Martinotti, S., Gallo, R. C., Erikson, J. & Croce, C. M. *Science* **219,** 963–967 (1983).
27. Erikson, J., Ar-Rushdi, A., Drwinga, H. L., Nowell, P. C. & Croce, C. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 820–824 (1983).
28. Watson, D. K., Reddy, E. P., Duesberg, P. H. & Papas, T. S. *Proc. natn. Acad. Sci. U.S.A.* **80,** 2146–2150 (1983).
29. Akiyama, Y. & Katos, S. *Biken. J.* **17,** 105–116 (1974).
30. Neel, B. G. *et al. J. Virol.* **44,** 158–166 (1982).
31. Ju, G. & Skalka, A. M. *Cell* **22,** 379–386 (1980).
32. Pachl, C., Schubach, W. H., Eisenman, R. & Linial, M. *Cell* **33,** 335–344 (1983).
33. Fung, T. Y-K., Crittenden, L. B. & Kung, H.-J. *J. Virol.* **44,** 742–746 (1982).
34. Cooper, G. M. & Neiman, P. E. *Nature* **292,** 857–858 (1981).
35. Rovigatti, V., Royler, C., Neel, B., Hayward, W. & Astrin, S. in *Tumor Cell Heterogeneity,* 319–330 (Academic, New York, 1982).
36. Payne, G. S., Bishop, J. M. & Varmus, H. E. *Nature* **295,** 209–214 (1982).
37. Rechavi, G., Givol, D. & Canaani, E. *Nature* **300,** 607–611 (1982).
38. Luciw, P. A., Bishop, J. M., Varmus, H. E. & Capecchi, M. R. *Cell* **33,** 705–716 (1983).
39. Jolly, D., Esty, A., Subramani, S., Freidmann, T. & Verma, I. *Nucleic Acid Res.* **11,** 1855–1872 (1983).
40. Schwartz, D. E., Tizard, R. & Gilbert, W. *Cell* **32,** 853–869 (1983).
41. Fromm, M. & Berg, P. *J. molec. appl. Genet.* **1,** 457–481 (1982).
42. Wasylyk, B., Wasylyk, C., Augereau, P. & Chambon, P. *Cell* **32,** 503–514 (1983).
43. Mareau, P. *et al. Nucleic Acids Res.* **9,** 6047–6068 (1981).
44. Nordheim, A. & Rich, A. *Nature* **303,** 674–679 (1983).
45. Cantor, C. R. *Cell* **25,** 293–295 (1981).
46. Weintraub, H. *Cell* **32,** 1191–1203 (1983).
47. Dodgson, J. B., Strommer, J. & Engel, J. D. *Cell* **17,** 879–888 (1979).
48. Benton, W. D. & Davis, R. W. *Science* **196,** 180–182 (1977).
49. Robins, T., Bister, K., Garon, C., Papas, T. & Duesberg, P. *J. Virol.* **41,** 635–642 (1982).
50. Tabak, H. F. & Flavell, R. A. *Nucleic Acids Res.* **5,** 2321–2332 (1978).
51. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72,** 1184–1188 (1975).
52. Okazaki, W. *et al. Avian Path.* **98,** 311–329 (1980).
53. Hihara, H., Shimizu, T. & Yamamoto, H. *Natn. Inst. Anim. Hlth Qt.* **14,** 163–173 (1974).

Induction of altered chromatin structures by simian virus 40 enhancer and promoter elements

Jan Jongstra, Tim L. Reudelhuber, Pierre Oudet, Christophe Benoist, Chi-Bom Chae, Jean-Marc Jeltsch, Diane J. Mathis & Pierre Chambon

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cedex, France

Two simian virus 40 transcriptional promoter elements, the 72-base pair (bp) repeat and the 21-bp repeat region, induce chromatin structures of increased DNase I sensitivity when transposed elsewhere in the viral genome. The induction of a sufficiently long stretch of DNase I-sensitive chromatin leads to the appearance of a visible nucleosome-free region.

LATE in infection, simian virus 40 (SV40) DNA is found in the nucleus of infected cells in the form of a minichromosome (see ref. 1 for references). When nuclei of SV40-infected cells or isolated SV40 minichromosomes are treated with various endonucleases (refs 2–4 and references therein) a region extending for ~350 base pairs (bp) from the unique *Bgl*I site towards the late side is preferentially cut (the ori region; Fig. 1, ORI A). Detailed studies of the products of DNase I digestion early and late in infection have revealed the existence of several discrete hypersensitive sites within this DNA fragment[5,6] and electron microscopy has shown that ~25% of the minichromosomes extracted from nuclei late in infection contain a nucleosome-free region (the nucleosome gap) of ~350 bp located in the same position[3,7]. Studies with viral mutants in which parts of the ori region are duplicated indicate that this region contains the information required for the generation of the nuclease-

0028-0836/84/080708—07$01.00

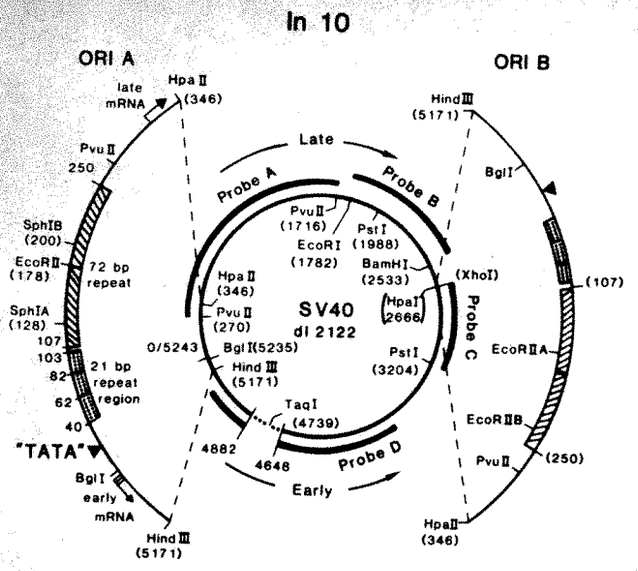


Fig. 1 Schematic representation and construction of the SV40 mutant In10. The inner circle represents the genome of SV40 d12122 which has a 234-bp deletion around the *Taq*I site (dotted line). The position and coordinates of some relevant restriction sites and landmarks are indicated and numbered according to the BBB system[1]. The SV40 ori region (ORI A) which contains the origin of replication (around the *Bgl*I site) and the early and late gene promoter sequences are shown enlarged at the left-hand side. The three early gene promoter elements (TATA box, 21-bp repeat region and 72-bp repeat) are indicated. A similar ori region from *Hin*dIII to *Hpa*II (5,171–346) was inserted at position 2,666 in the same orientation with respect to early gene transcription, as shown enlarged on the right-hand side (ori B). The ori fragment was taken from plasmid pSV1 and contained an 8-bp deletion from position 173 to 181, deleting the *Eco*RII site at position 178 (ref. 12). The solid bars indicate the restriction fragments used as hybridization probes to map regions of increased DNase I sensitivity at ori A and ori B. To generate the SV40 insertion mutant In10, the following plasmids were first constructed: pSV22, a recombinant between the DNA of SV40 d12122 and a *Pst*I site-deleted pBR322 joined at their unique *Eco*RI sites; pSV10, the small SV40 *Pst*I fragment (1,988–3,204) was inserted in the *Pst*I site of pBR322; pSV10IN10, constructed by blunt-end ligation (after DNA polymerase I treatment) of the *Hin*dIII–*Hpa*II ORI fragment (5,171–346) in the unique *Hpa*I site present in the SV40 *Pst*I fragment of pSV10. The small *Pst*I fragment of pSV10IN10 was purified on a sucrose gradient and ligated in pSV22 in place of the original SV40 *Pst*I fragment to give pIN10. Virus stocks of SV40 d12122 and In10 were prepared from pSV22 and pIN10 as follows: DNA from plasmids pSV22 and pIN10 was digested with *Eco*RI to separate the SV40 moiety from the pBR322 sequences, ligated at low DNA concentrations (5–10 $\mu g\ ml^{-1}$) to circularize the DNA, and transfected into CV-1 cells in the presence of 0.5 $mg\ ml^{-1}$ DEAE-dextran[12] using 50 ng of ligated DNA per 6 cm Petri dish. Virus was collected by repeated freezing and thawing when 90% of the cells had lysed. Virus stocks were then prepared by infections at low multiplicity with plaque-purified virus and used for subsequent experiments. Recombinant constructions were verified in plasmids by partial DNA sequencing and in virus stocks by detailed restriction analysis of Hirt extracts.

sensitive chromatin structure[8,9] and the nucleosome gap[8,10].

These findings are of particular interest because the ori region contains the DNA sequences involved in initiation of transcription of early and late SV40 genes (see ref. 1) in addition to the viral replication origin[11]. Recent studies have defined three DNA regions which have a role in promotion of early gene transcription *in vivo*. These are (Fig. 1) the 'TATA box', which dictates the proper initiation site for transcription[12–14], the 21-bp repeat region containing six G+C-rich repeated sequence motifs, the deletion of which decreases early gene expression[12,15,16], and the enhancer element, the 72-bp repeat. Deletion of one of the two 72-bp sequences has very little effect on early gene expression but deletion of all, or part, of the remaining repeated sequences reduces early transcription considerably[12,14,17–21].

Therefore, the early promoter sequences are localized within the same fragment of the SV40 genome that displays an altered chromatin structure. Similarly, a variety of cellular genes show alterations in chromatin structure upstream from the mRNA cap site, which correlate with transcriptional activity (refs 22–25 and references therein). Thus, alterations in chromatin structure, as detected by increased sensitivity to endonucleases and the presence of a nucleosome gap, might be induced by transcriptional promoter elements.

Here we describe experiments which were designed to identify the sequences in the ori region necessary to induce chromatin structures of increased DNase I sensitivity and a nucleosome gap. Our approach was based on the observation that SV40 mutants having deletions in the large-T intron can accommodate extra DNA sequences in the *Hpa*I site at position 2,666 (Fig. 1) and still grow normally[26]. Different parts of the ori region were inserted at this *Hpa*I site using the mutant d12122, which has a deletion between positions 4,648 and 4,882 (ref. 27) to create mutants carrying two ori regions: the wild type ori A, and a second wild type or mutated origin region, ori B (Fig. 1).

Induction of DNase I sensitivity

Figure 1 describes the double-origin mutant In10 which has the 418-bp *Hin*dIII–*Hpa*II ori fragment (5,171–346; Fig. 1) inserted as a second ori (ori B). To measure the increased DNase I sensitivity at ori A and B, CV-1 cells were infected with an In10 virus stock, nuclei were prepared 40–42 h later, and regions of increased DNase I sensitivity were mapped using an indirect end-labelling technique[28]. The pattern of DNase I digestion at ori A and B was revealed after hydridization of the blotted DNA to probes A and B, respectively (see Fig. 1 and Fig. 2 legend).

An example of a resulting autoradiogram using probe A is shown in Fig. 2*a* (ORI A). The heavy band (L) corresponds to In10 DNA which was not digested with DNase I, but only linearized with *Eco*RI. In10 chromatin DNA digested randomly with DNase I gave rise to a continuous smear, the intensity of which increased with increasing time of DNase I digestion. Regions of increased DNase I sensitivity at ori A produced two sets of DNA fragments, labelled I and II. DNA purified from In10-infected cells (or from cells infected with other In mutants described in this study) and digested with DNase I and *Eco*RI showed only the background smear (not shown). The early border of the DNase I-sensitive region II mapped at position 109 ± 25 and thus coincided well with the early border of the 72-bp repeat (Fig. 2*c*). The late border of region II mapped at position 323 ± 20. Occasionally, rather than a continuous smear, region II in ori A was resolved into two or three separate sets of fragments of unequal intensity, suggesting the existence of several subregions. The most prominent subregion (arrow in Fig. 2*a*) was always proximal to the early side of region II. Figure 2*a* also shows that using probe A, the DNase I-sensitive region I was ~30 bp long and centred over position 5,220. However, the presence of the highly DNaseI-sensitive region II between region I and the *Eco*RI site used as a reference point for the mapping experiments, might have led to an underestimation of the length and intensity of region I. When the *Mbo*I site at position 4,099 was used as a reference point using a *Mbo*I–*Ava*II fragment (4,099–5,118) as probe (probe D, Fig. 1) region I was shown to include the TATA box and extended from position 5,210 to 35. The pattern of DNase I sensitivity determined over ori A in In10 was similar to that found in wild-type SV40, d12122 and all double-origin mutants tested (results summarized in Fig. 2*c*, 'ORI A'). Similar results were reported by Saragosti *et al.*[3] for wild-type SV40.

Figure 2*a* (ORI B) shows that when probe B was used instead of probe A, a pattern of DNase I sensitivity similar to that seen at ori A was revealed at ori B. Region II at ori B mapped from position 110 ± 25 to 310 ± 20, while region I mapped from position $5{,}220 \pm 20$ to 45 ± 20 (Fig. 2*c*). These locations were

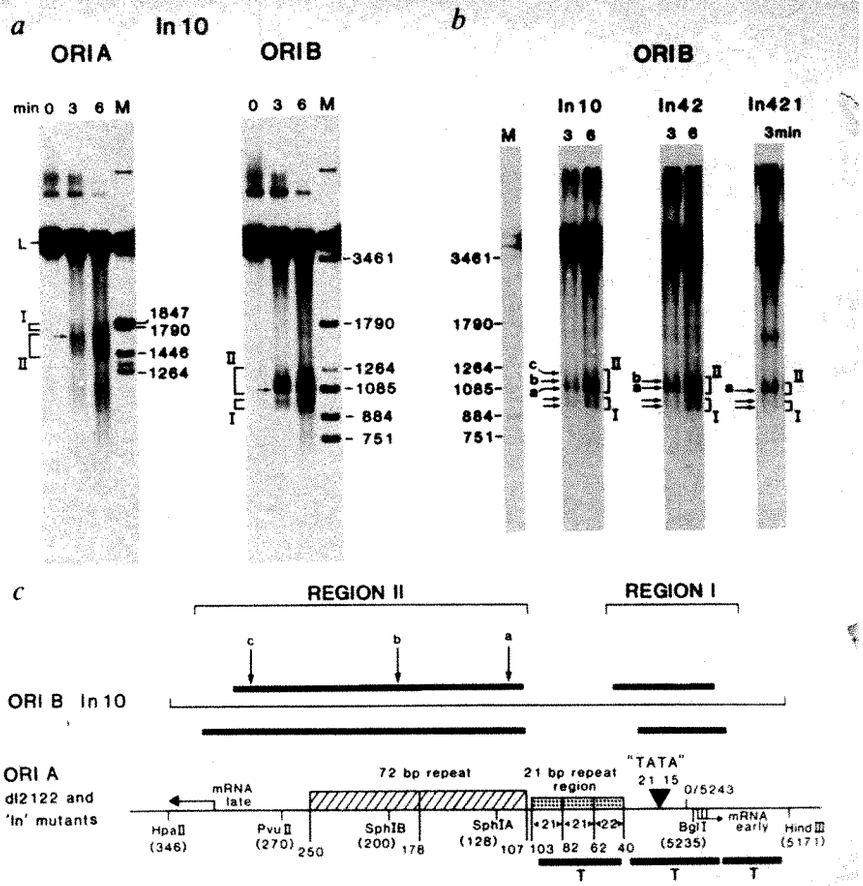


Fig. 2 Mapping of the regions of increased DNase I sensitivity at ori A and ori B of In10, In42 and In421. ***a***, Mapping of DNase I-sensitive regions at ori A and ori B was done as follows. CV-1 cells were infected with the appropriate virus stock and collected 40–42 h after infection by scraping them into a small volume of ice-cold buffer A (75 mM KCl, 5 mM PIPES *p*H 7.0, 150 mM sucrose) containing 1 mM EDTA, then washed twice with the same buffer by centrifugation for 5 min at 3,000 r.p.m. in a Sorvall HB-4 rotor at 4 °C. The cells were lysed in buffer A containing 0.5% Nonidet P-40 for 5 min on ice and the nuclei were washed once with buffer A by centrifugation as described above. The nuclear pellet was resuspended in buffer A at a DNA concentration of 0.5–1.0 mg ml^{-1}. Nuclease digestions were carried out at room temperature by adding 0.1 vol. 10 mM $CaCl_2$ and DNase I (Worthington; 2,624 U mg^{-1}) to a final concentration of 120 U ml^{-1} in a total volume of 550 μl. Samples (90 μl) were removed at various times and immediately mixed with 110 μl of 20 mM Tris-HCl *p*H 7.4, 10 mM EDTA, 0.2% SDS, containing 20 μg proteinase-K and incubated at 37 °C for 2–3 h. The samples were extracted once with 1 vol. phenol–chloroform (1:1) and once with 3 vol. ether. The aqueous phase was digested with RNase A at 10 μg ml^{-1} for 1 h at 37 °C and extracted with phenol–chloroform and ether, then the DNA was digested with a twofold excess of *Eco*RI (BRL) and layered onto a 2% agarose gel for electrophoresis. DNA was transferred from the agarose gel to a nitrocellulose filter and hybridized according to Wahl *et al.*[42], but with omission of the dextran sulphate. To map the regions of increased DNase I sensitivity at ori B, the filter was hybridized to probe B (Fig. 1) labelled with ^{32}P by nick-translation. After dehybridization at 65 °C for 2 h as described by Thomas[43], regions of increased DNase I sensitivity at ori A were mapped using probe A (Fig. 1). The time of digestion with DNase I is indicated (in min) at the top of each lane. Lane M contains marker fragments prepared by digesting SV40 DNA with the restriction enzymes: *Eco*RI + *Bgl*I, *Eco*RI + *Bam*HI, *Pvu*II, *Hin*fI and *Mbo*I. Lane M contains 50 ng of each digest and the numbers to the right of each panel represent the length (in bp) of the restriction fragments detected by the hybridizing probe. The 884-bp marker fragment visualized by probe B (in ORI B) is the *Eco*RI–*Hpa*I fragment (1,782–2,666), which marks the extremity of the inserted fragment proximal to probe B. The DNase I-sensitive regions I and II described in the text are indicated by brackets and a prominent band visible in region II at ori A and ori B is indicated by an arrow. L, linearized viral DNA. In the particular experiment shown here dehybridization was incomplete; this resulted in the appearance of a signal corresponding to ORI B in the panel labelled ORI A below the 1,264-bp marker fragment. ***b***, Fine structure in region II at ori B. Samples of In10, In42 and In421 were standardized as described in the text to allow a valid comparison of the DNase I digests. The 3-min digestion points of the three mutants were digested to the same degree at ORI A. Similarly, the 6-min time points for In10 and In42 were directly comparable. The extent of the DNase I-sensitive regions is indicated by brackets labelled I and II and arrows indicate the fine structure within these regions. Lane M contains marker fragments as for ***a***. The construction of pIN42 and pIN421 is described in Fig. 5 legend. ***c***, Diagrammatic comparison of regions of increased DNase I sensitivity at ori B in In10 and ori A in wild-type and mutant SV40. The lower line (ori A) represents the SV40 ori region with the TATA box, the 21-bp repeat region and the 72-bp repeat. The three solid bars below the line indicate the three T-antigen-binding sites[32,33]. The solid bars above the lines ori A and ori B In10 indicate the extent of regions of increased DNase I sensitivity I and II, and are a summary of several mapping experiments using probes A and D to analyse ori A, and probes B and C to analyse ori B. The vertical arrows indicate the approximate location of the bands a, b and c shown in *b*.

confirmed using probe C and the *Pst*I site (3,204; Fig. 1) as a reference point (not shown). Several similarities in the digestion patterns at ori A and ori B in In10 are noteworthy. First, both regions of DNase I sensitivity at ori B mapped over the same DNA sequences as at ori A. Second, region II at ori B was more sensitive to digestion with DNase I than region I; this confirmed that the increased DNase I sensitivity of region II compared with region I at ori A (see above), was not due to the use of a probe from the late side which might have led to a preferential mapping of DNase I cut sites proximal to the *Eco*RI site. Third, region II at ori B also showed a fine structure with a prominent band mapping close to the early side of the 72-bp repeat (arrow in Fig. 2*a*, ORI B). Figure 2*b* gives the results of an experiment showing more clearly the presence of five bands at ori B in In10 (arrows), two of which mapped within region I and three within region II.

To investigate which sequences were responsible for the induction of region II and the prominent cuts contained within it, we constructed mutants In42 and In421, which contain *Hin*dIII–*Pvu*II ori fragments (5,171–270) with an intact 72-bp repeat or only one 72-bp sequence, respectively (Fig. 5*a*). To validly compare the DNase I digestion patterns at ori B for the different mutants, we first ensured that all samples were equilibrated for viral yield and degree of digestion. To this end, different amounts of total DNA extracted from infected cells were loaded on the gel and then only those samples which showed identical signals for the linear SV40 DNA (band L in Fig. 2*a*) and an equal degree of digestion at ori A as determined by hybridization with probe A, were compared at ori B using probe B (the same standardization procedure was used for all the experiments described below). The absence of the *Pvu*II–*Hpa*II fragment (270–346) at ori B in In42 resulted in the appearance of a shorter region II lacking band c (Fig. 2*b*). Additional deletion of one 72-bp sequence (In421) shortened region II even further and eliminated band b (Figs 2*b*, 5*a*). From these results, together with the data of several mapping experiments, we conclude that bands a and b fall on the early side of each 72-bp sequence and band c on the late side of the

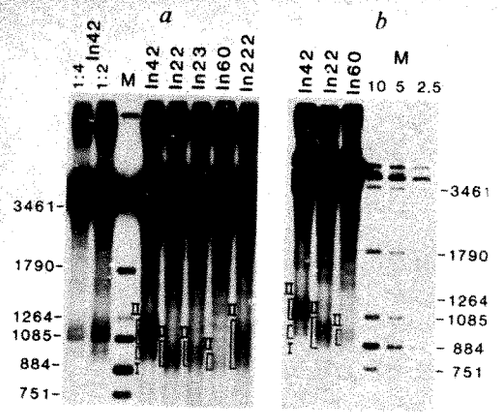


Fig. 3 The 72-bp repeat induces an altered chromatin structure over itself. *a*, The DNase I digestion patterns at ori B of different In mutants as indicated at the top of each lane, were visualized using probe B (see Fig. 1) and the extent of regions of increased DNase I sensitivity are indicated by brackets. Samples were standardized as described in the text. To estimate the magnitude of the differences in intensity of region II in In42, In22 and In23, twofold and fourfold dilutions of the In42 sample shown in the central panel were included (In42 diluted 1:2 and 1:4). Lane M contains markers as described for Fig. 2*a*. The samples were digested at ori A to ~5% (as determined by the ratio of the signals corresponding to linear SV40 and to DNase I cuts at ori A). *b*, A different set of standardized samples digested at ori A to ~25% was run on a separate gel in the presence of different amounts of marker fragments (lanes M containing 10, 5 or 2.5 μl of the same marker mix). The construction of the In mutants is described in Fig. 5 legend.

*Pvu*II (270) restriction site. In all three mutants In10, In42 and In421, region II was of equal intensity and was always more sensitive than region I.

The relationship between bands a, b and c and the specific cuts made at the wild-type SV40 ori region at positions 370, 270 and 190 as reported by Cremisi[5] is unclear, but the differences in position may be related to the use of rather long fragments starting from the *Eco*RI site to probe the structure of the ori region. The use of shorter probes localized nearer to the ori region of wild-type SV40 has allowed the identification of as many as five specific cuts within region II[6], three of which were mapped at positions 110, 185 and 290 and thus may correspond to the bands a, b and c described here. The two additional cuts which were mapped by Cereghini *et al.*[6] within the 'late' moiety of each 72-bp sequence probably reflect the higher degree of resolution obtained in their assay. As specific bands within regions of increased DNase I sensitivity result from the cuts made at hypersensitive sites at an early stage of DNase I digestion, it is possible that the exact number and location of such cuts differ with experimental conditions and may depend on various parameters such as temperature, digestion buffer, amount of DNase I or method of preparation of nuclei.

Induction of DNase I sensitivity by enhancer

To further define the sequences necessary to induce the increased DNase I sensitivity over region II, we inserted in both orientations at ori B a DNA fragment from position 113 to 270, containing mainly the 72-bp repeat (In22 and In23; Fig. 5*a*). Both mutants displayed a DNase I-sensitive region of similar intensity over the inserted fragment (see Fig. 3*a*, region II). This sensitive region differed from the region II in In42 in several ways. First, regions II in In22 and In23 seemed to be less intense than region II in In42. To estimate the magnitude of this difference, several dilutions of the In42 sample were included on the same gel. From a comparison of these samples, we estimated the intensity of regions II in In22 and In23 to be 2–3-fold less than in In42 (Fig. 3*a*, In42 diluted 1:2 and 1:4). Second, regions II in In22 and In23 appeared more diffuse, and third, although some fine structure was visible in region II in In22 and In23, no prominent band close to the early border of the inserted 72-bp repeat was observed. The decreased DNase I sensitivity of the isolated 72-bp repeat at ori B in In22 and In23 (compared with region II at ori B in In42) could be due to the absence of certain ori sequences on the early side of this repeat or could be related in some way to the smaller size of the insert. To test the effect of the size of the insert, we took advantage of a uniquely reconstructed *Xho*I site at one end of ori B in In22 to insert a second 72-bp repeat (from position 113 to 270), thus constructing In222 carrying an insert of approximately the same length as In42 (Fig. 5*a*). Figure 3*a* shows that a duplication of the DNase I-sensitive region seen in In22 was present at ori B of In222, but of the same intensity as in In22 or In23. This suggested that sequences between the *Hin*dIII site (5,171) and position 113 affected the intensity of the DNase I sensitivity generated over the 72-bp repeat.

We also tested whether a single 72-bp sequence was able to induce an altered chromatin structure by deleting one of the two 72-bp sequences from ori B of In22 (In60, Fig. 5*a*). The remaining region of increased DNase I sensitivity at ori B was shorter than in In22 or In23 and also seemed less intense (Fig. 3*a*). To ensure that these differences in intensity were not due to different digestion kinetics, we prepared another set of standardized samples from In42, In22 and In60 (Fig. 3*b*) which were more heavily digested with DNase I than the set shown in Fig. 3*a*. Using the marker lanes as standards, we again estimated that region II in In22 was 2–3-fold less intense than in In42 and that the DNase I sensitivity induced at ori B in In60 by a single 72-bp sequence was reduced ~2–3-fold compared with the intact 72-bp repeat (In22) or a dimer of this repeat (In222). This latter decrease might be due to the small size of the insert in In60 rather than be related to the deletion of specific sequences. Also, note that the insert at ori B in In60 consists of slightly less than an intact 72-bp sequence since its early border is at position 113.

Two reports which appeared when this work was in progress have dealt with the effects of the 72-bp repeat on chromatin structure using SV40 mutants containing transposed ori fragments. Gerard *et al.*[9] concluded that this repeat was not in itself sufficient for the induction of increased nuclease sensitivity. These authors used an ori fragment containing a single 72-bp sequence and might, therefore, have been unable to detect the increase in nuclease sensitivity induced by this fragment (compare In22 and In60, Fig. 3*a*, *b*). The results given in Fig. 3*a* clearly demonstrate that the 72-bp repeat induces an increase in DNase I sensitivity independent of its orientation (In22 and In23). This is in contrast to the findings of Fromm and Berg[29] who inserted an ori fragment (from position 95 to 270) into the *Hpa*I site (2,666) of a SV40 genome in which a fragment from position 73 to 270 had been deleted from the wild-type ori region. They found that the 95–270 fragment induced an increase in DNase I sensitivity only when inserted in the orientation comparable to ori B in In23. This discrepancy could be due to the small difference in the sequences of the ori fragments tested in the two studies (base pairs 113 to 270 in our study) or to other differences in the recombinant constructions.

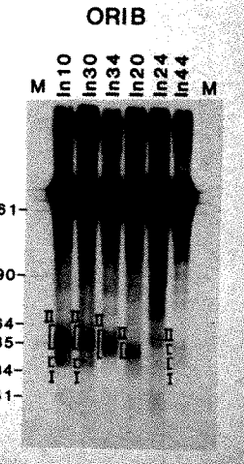


Fig. 4 Determination of the ori sequences needed to induce DNase I-sensitive region I. Samples were standardized as described in the text and the pattern of DNase I digestion was visualized as described in Fig. 3 legend. Extent of regions I and II are indicated by brackets. In24 was digested slightly more than the other samples to ensure that no region of increased DNase I sensitivity was present at ori B. Lanes M contain markers as in Fig. 2*a*. The construction of In mutants other than In10 is described in Fig. 5 legend.

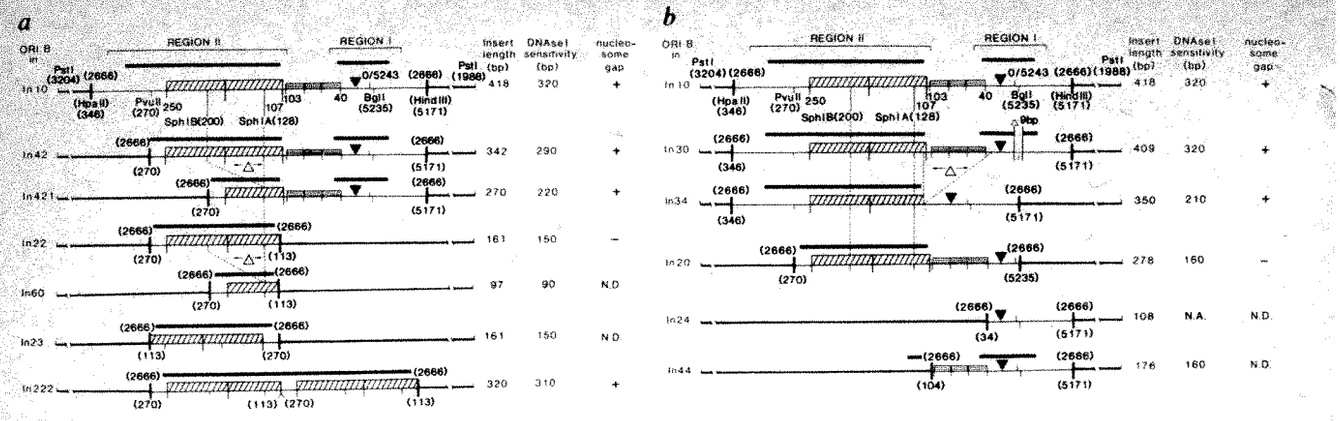


Fig. 5 Diagrammatic representation of the results obtained with the various In mutants. The small *Pst*I fragments containing ori B are shown (see Fig. 1). For each mutant the insert at ori B is represented by a thin horizontal line bounded by thick vertical bars. The extent of the regions of increased DNase I sensitivity (I and II) are indicated by black horizontal bars as in Fig. 2*c*. The column 'DNase I sensitivity' gives the distance (in bp) between the outer extremities of the domain of DNase I-sensitive chromatin. Approximately 25% of the minichromosomes extracted from cells infected with any of the In mutants exhibited at least one nucleosome gap. More than 150 minichromosomes containing at least one gap of those mutants for which + appears in the right-hand columns of *a* and *b* were examined to determine the percentages of minichromosomes exhibiting two gaps—these were as follows: for In10, 32%; In34, 30%; In30, 27%; In42, 22%; In421, 28% and for In222, 24%. When no minichromosomes containing two gaps were seen (indicated by −) more than 300 minichromosomes containing a single gap were examined. N.A., not applicable; N.D., not determined. ***a***, Summary of results defining ori sequences needed to induce region II. In42, In22 and In23 were constructed by directly inserting the appropriate ori fragments in pSV22X, a derivative of pSV22 (see Fig. 1 legend) in which the *Hpa*I site at position 2,666 was converted to a unique *Xho*I restriction site by inserting a *Xho*I linker. In421 was derived from In42 by deletion of one 72-bp sequence between *Sph*I A and *Sph*I B at ori B. The insert in In22 is the pHS102 fragment A (113–270) described by Moreau *et al.*[17]; the same insert is present at ori B in In23 in the opposite orientation. In60 is derived from In22 by deletion of the *Sph*I A–*Sph*I B fragment at ori B. In222 was constructed by inserting a second 72-bp repeat (113–270) in the *Xho*I site reconstituted at the 'late' side of ori B in In22. ***b***, Summary of results defining ori sequences necessary to induce region I. In20 was constructed in a way similar to In10 (as described in Fig. 1 legend) using a purified *Bgl*I–*Pvu*II fragment (5,235–270) derived from pSV1. The other In mutants were constructed as described above for In42 using the following ori fragments: the *Hin*dIII–*Hpa*II fragment (5,171–346) from pAS[12] which has a 9-bp deletion around the *Bgl*I site (In30) or from pMD102[15] which has a deletion between positions 34 and 113 (In34), or the *Hin*dIII–*Bam*HI fragments from pRE7 (5,171–34; In24) or from pMKD231 (5,171–104; In44) described by Everett *et al.*[15].

Importance of 21-bp repeat region

As DNase I-sensitive region I maps over the origin of replication proper[11], we investigated whether an intact replication origin at ori B was necessary for the generation of region I. ori B in In30 is similar to ori B in In10 (Fig. 1) but has a 9-bp deletion around the *Bgl*I site (see Fig. 5*b*), which is known to abolish SV40 replication[30]. Although this deletion had no effect on the induction of DNase I-sensitive region I or II (Figs 4, 5*b*), insertion at ori B of the *Bgl*I–*Pvu*II fragment (5,235–270), which lacks the early moiety of the sequences contained within region I (In20; Fig. 5*b*) but not the TATA box, did not generate a stretch of increased DNase I sensitivity corresponding to region I (Fig. 4). Comparing these results with those obtained using In42 (carrying the *Hin*dIII–*Pvu*II fragment at ori B; see Figs 2*b*, 5*a*) indicated that the generation of region I required the presence of sequences between the *Hin*dIII and *Bgl*I sites. In addition, the insert at ori B of In20 induced a region II which mapped from position 110 ± 10 to 265 ± 15 (Figs 4, 5*b*), thus confirming the above conclusion that sequences between *Pvu*II (270) and *Hpa*II (346) are important in defining the late border of region II.

To determine whether, as in the case of region II, the sequences within region I were able to induce DNase I sensitivity over themselves, we constructed In24 which carried at ori B the fragment from position 5,171 to 34 and contained essentially all the DNA sequences included in region I (In24; Fig. 5*b*). No increase in DNase I sensitivity was induced over this insert (Fig. 4). When we tested In44, which contained at ori B a fragment spanning positions 5,171 to 104, encompassing as in In24 all sequences contained within region I plus the 21-bp repeat region, we observed two regions of increased DNase I sensitivity (In44; Figs 4, 5*b*), one region mapped from position 5,230 ± 10 to 60 ± 10 and was therefore similar to region I at ori A, while a second narrow region mapped just outside the insert on its 'late' side. Both regions of increased DNase I sensitivity at ori B in In44 were of lower but equal intensity (Fig. 4), compared with the characteristic wild-type pattern at ori A in In44 or at ori B in In10, In30, In42 or In421. These results suggested that DNA sequences localized between positions 34 and 104 (that is, the 21-bp repeat region) were responsible for the induction of region I over the ori sequences located directly on its early side, and might also influence the pattern of increased DNase I sensitivity over the 72-bp repeat which in the wild-type ori is located directly on its late side. Both suggestions were confirmed by the results obtained at ori B in In34, which was similar to In10 but lacked the 21-bp repeat region (Fig. 5*b*). Not only was region I absent at ori B in In34, but inspection of several autoradiograms similar to that shown in Fig. 4 revealed also that band b of region II in In34 was more intense than band a, in contrast to the relative intensity of these bands in region II of In10 (Fig. 2*b*).

Induction of a nucleosome gap

About 25% of wild-type SV40 minichromosomes extracted from nuclei late in infection contain a nucleosome gap over their ori region. To investigate whether this gap could be observed at ori B, minichromosomes were extracted from CV-1 cells 40–42 h after infection with an In10 virus stock. Approximately one-quarter of the minichromosomes had a nucleosome gap (Fig. 6*a*) which appeared to map preferentially over ori A when using the single-cut restriction enzymes *Eco*RI (Fig. 6*b*) or *Msp*I (Fig. 6*c*). (Note that the *Hpa*II–*Msp*I site was lost at ori B.) In addition, two diametrically opposed gaps were observed in approximately 7% of the minichromosomes (Fig. 6*d*). These two gaps were mapped over ori A and ori B after digestion with *Eco*RI (Fig. 6*e*) or *Msp*I (Fig. 6*f*). To determine which ori sequences were responsible for the generation of the gap, we analysed most of the 'In' mutants described above for the presence of a gap at ori B. The results, summarized in Fig. 5*a*,*b* indicate that a similar fraction of the minichromosomes of the two mutants In 42 and In421 exhibited two gaps, mapping over ori A and ori B, while mutants In20 and In22 exhibited a gap only over ori A. The absence of a visible gap over ori B in In20

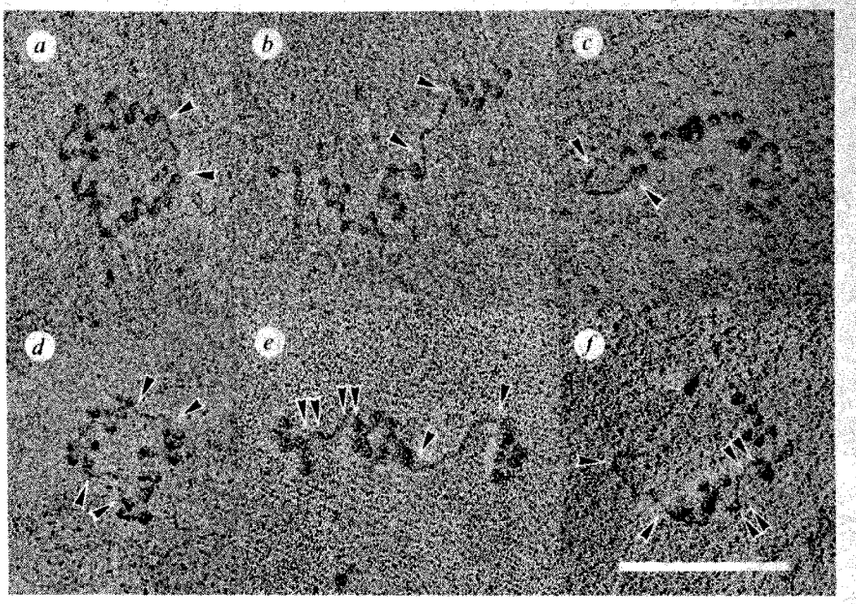


Fig. 6 Electron microscopic visualization of In10 minichromosome. Minichromosomes were extracted and purified from CV-1 cells 40–42 h after infection as described elsewhere[44]. Aliquots from the 75S peak fraction were digested with *Eco*RI (*b* and *e*) or *Msp*I (an isoschizomer of *Hpa*II; *c* and *f*) after addition of $MgCl_2$ to a final concentration of 5 mM. The digestion was stopped by adding 5 mM EDTA. Undigested minichromosomes (*a* and *d*) and the digested samples were diluted at least 10-fold in water and 10-μl aliquots were deposited on carbon-coated grids which had been positively charged by glow discharge in the presence of amylamine. After 1 min of adsorption, the grids were rinsed for 5 min with water then stained for 10 s in 0.1% aqueous uranyl acetate. The grids were then washed for 5 s with water, air-dried and shadowed as described previously[44]. Scale bar in *f* represents 0.25 μm. The positions of nucleosome gaps were mapped on minichromosomes cut with a restriction enzyme by counting the nucleosomes between the gap and the extremities of the linearized molecules. *a*, *b*, *c* Show minichromosomes containing one nucleosome gap which mapped over ori A. *d*, *e*, *f* Show minichromosomes containing two gaps. In *e* and *f* the gaps indicated by single arrowheads map over ori A and the gaps indicated by double arrowheads map over ori B.

was not due to the smaller size of the inserted fragment, as the insert at ori B in In421 was of a similar size. A possible means of accounting for these results is to postulate that the presence of DNase I-sensitive region I was a prerequisite for the observation of a gap. However, when we examined minichromosomes from In34 which showed no region I at ori B, we found a gap over ori B (Fig. 5*b*). Alternatively, the induction of a visible nucleosome gap might require the existence of a minimal length of altered chromatin structure which could be made up of either a complete region II as in In34 or a truncated region II plus an adjacent region I as in In42 or In421. To test this hypothesis, we examined minichromosomes extracted from In222-infected cells and found that a fraction of them contained two gaps (Fig. 5*a*). These results strongly support the conclusion that the total length of increased DNase I sensitivity at ori B is the crucial factor in allowing gap formation, and that the nuclease-sensitive structure and the nucleosome gap occur on the same minichromosomes. In fact, we have recently determined that the visible gap region of isolated wild-type minichromosomes is preferentially excised with *Bgl*I and *Msp*I restriction enzymes (E. Weiss *et al.*, in preparation).

Discussion

Our experiments, reported here, demonstrate that generation of the full pattern of increased DNase I sensitivity at the SV40 ori region depends on the presence of multiple ori sequences and on their precise arrangement. First, the generation of a full-length region II depends on both sequences within the 72-bp repeat and a separate set of sequences located between the *Pvu*II (270) and *Hpa*II (346) sites (compare In10, In42 and In44). Second, region I is induced only when the 21-bp region, which is itself resistant to DNase I digestion, is present together with sequences between the *Hin*dIII (5,171) and *Bgl*I (5,235) sites (compare In10, In34 and In20). Third, the 21-bp repeat region has a distinct qualitative effect on the generation of increased DNase I sensitivity over the 72-bp repeat, leading to a well defined 'early' border and a prominent subregion (band a; compare In10 and In34). Studies using mutated 21-bp repeat regions are required to investigate whether the effects of this region on chromatin structure are related to its promoter activity for early[12,14–16] or late[19] genes and whether its resistance to DNase I digestion is due to the binding of a transcription factor[31] or of T-antigen[32,33]. In any case, a large fraction of the increased DNase I sensitivity at ori is critically dependent on the presence of the 72-bp repeat (compare In10, In42, In421 and In44). Most important, an alteration of chromatin structure is generated at ori B by insertion, in either orientation, of the 72-bp repeat region, in the absence of other ori sequences (In22 and In23).

Our results also indicate that the formation of a visible nucleosome gap is correlated with the generation of a sufficiently long stretch of altered chromatin structure, the minimal length of which seems to be between 160 and 210 bp (see Fig. 5*a*,*b*). This can be achieved in various ways, but the major conclusion is that sequences mostly contained within the 72-bp repeat are sufficient to generate a visible nucleosome gap (see In222). It is possible that a single 72-bp repeat could generate a nucleosome gap which is not detectable because its length is too close to the maximal size of the variable internucleosomal linker length of spread SV40 minichromosomes[1,3].

The SV40 72-bp repeat has been characterized as a *cis*-acting bidirectional enhancer of transcription from potential promoter elements[21]. The observations that proximal potential promoter elements are preferentially activated and that activation of transcription from a given promoter element decreases as the length of interposed sequences increses, have suggested that the SV40 enhancer acts like a bidirectional 'entry site' for some element(s) of the class B transcription machinery[17,21]. Our present results suggest that the generation of an altered chromatin structure by the SV40 enhancer could lead to the formation of an 'open window' within the nucleosomal structure, thereby increasing the accessibility of the DNA within the enhancer for binding of macromolecules involved in transcription. Of course, this does not exclude the possibility that sequences within the 72-bp repeat could be specifically recognized by some component of the transcription machinery, as was recently suggested by the stimulatory effect of the 72-bp repeat on *in vitro* transcription from heterologous promoter elements in the absence of evidence for chromatin reconstruction[34]. One could speculate that the generation of an open chromatin structure and sequence-specific interaction between the 72-bp repeat and the transcription machinery act in concert to facilitate the activation of promoter elements embedded in the complex genome of higher eukaryotes.

The molecular mechanisms underlying the generation of altered chromatin structures over the 72-bp repeat are unknown. Several possibilities exist. *In vitro* reconstitution experiments showing that nucleosomes form less well on the ori region than on the rest of the SV40 genome suggest that some DNA sequences within the ori region cannot easily be folded around the histone octamer core[35,36]. We note that it has been demonstrated recently that some sequences within and on the late side

of the 72-bp repeat can exist in the Z-DNA conformation *in vitro*[37] and that nucleosomes cannot be formed over Z-DNA[38]. The alterations in chromatin structure may also be brought about by the binding of specific proteins, thereby inhibiting nucleosome formation. Interactions between enhancer sequences and specific cellular proteins have been suggested recently by studies demonstrating that the activity of some viral and cellular enhancers is cell- and/or species-specific (refs 39, 40 and references therein). Such proteins could be component(s) of the transcription machinery (see above) or belong to the class of proteins which specifically recognize Z-DNA[41]. Obviously, the identification of such putative proteins, the further development of *in vitro* systems, and the precise characterization of the sequences within the 72-bp repeat responsible for the enhancing activity and the generation of altered chromatin structures, are required to elucidate the molecular mechanisms underlying these important biological functions.

We thank Drs R. Everett and E. May for gifts of materials; K. Dott, M. Acker, B. Augsburger and E. Regnier for technical assistance; and C. Werlé, B. Boulay, E. Badzinski and C. Kutschis for help in preparing the manuscript. This work was supported by grants from the CNRS (ATP 3582), the INSERM (PRC 124026), the Ministère de la Recherche et de l'Industrie (82V1283), the Association pour le Développment de la Recherche sur le Cancer, the Fondation Médicale de la Recherche Française, and the Fondation Simone and Cino del Duca. J.J. was the recipient of a long-term EMBO fellowship and T.L.R. was supported by a fellowship from the University Louis Pasteur, Strasbourg. C.-B.C. was on sabbatical leave from the University of North Carolina.

Received 26 September; accepted 6 December 1983.

1. Tooze, J. *DNA Tumor Viruses* (Cold Spring Harbor Laboratory, New York, 1982).
2. Scott, W. A. & Wigmore, D. J. *Cell* **15,** 1511–1518 (1978).
3. Saragosti, S., Moyne, G. & Yaniv, M. *Cell* **20,** 65–73 (1980).
4. Shakhov, A. N., Nedospasov, S. A. & Georgiev, G. P. *Nucleic Acids Res.* **10,** 3951–3965 (1982).
5. Cremisi, C. *Nucleic Acids Res.* **9,** 5949–5963 (1981).
6. Cereghini, S. *et al. Cold Spring Harb. Symp. quant. Biol.* **47,** 935–944 (1983).
7. Jakobovits, E. B., Bratosin, S. & Aloni, Y. *Nature* **285,** 263–265 (1980).
8. Chae, C. B. *et al. J. cell. Biochem. Suppl.* **6,** 328 (1982).
9. Gerard, R. D., Woodworth-Gutai, M. & Scott, W. A. *Molec. cell. Biol.* **2,** 782–788 (1982).
10. Jakobovits, E. B., Bratosin, S. & Aloni, Y. *Virology* **120,** 340–348 (1982).
11. Bergsma, D. J., Olive, D. M., Hartzell, S. W. & Subramanian, K. N. *Proc. natn. Acad. Sci. U.S.A.* **79,** 381–385 (1982).
12. Benoist, C. & Chambon, P. *Nature* **290,** 304–310 (1981).
13. Mathis, D. J. & Chambon, P. *Nature* **290,** 310–315 (1981).
14. Wasylyk, B., Wasylyk, C., Matthes, H., Wintzerith, M. & Chambon, P. *EMBO J.* **2,** 1605–1611 (1983).
15. Everett, R. D., Baty, D. & Chambon, P. *Nucleic Acids Res.* **11,** 2447–2464 (1983).
16. Baty, D., Barrera-Saldana, H. A., Everett, R. D., Vigneron, M. & Chambon, P. *Nucleic Acids Res.* (in the press).
17. Moreau, P. *et al. Nucleic Acids Res.* **9,** 6047–6068 (1981).
18. Gruss, P., Dhar, R. & Khoury, G. *Proc. natn. Acad. Sci. U.S.A.* **78,** 943–947 (1981).
19. Fromm, M. & Berg, P. *J. molec. appl. Genet.* **1,** 457–481 (1982).
20. Banerji, J., Rusconi, S. & Schaffner, W. *Cell* **27,** 299–308 (1981).
21. Wasylyk, B., Wasylyk, C., Augereau, P. & Chambon, P. *Cell* **32,** 503–514 (1983).
22. Weisbrod, S. *Nature* **297,** 289–295 (1982).
23. Cartwright, I. L. *et al. CRC Crit. Rev. Biochem.* **13,** 1–86 (1982).
24. Bellard, M., Dretzen, G., Bellard, F., Oudet, P. & Chambon, P. *EMBO J.* **1,** 223–230 (1982).
25. McGinnis, W., Shermoen, A. W., Heemskerk, J. & Beckendorf, S. K. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1063–1067 (1983).
26. Shenk, T. *Cell* **13,** 791–798 (1978).
27. Volckaert, G., Feunteun, J., Crawford, L. V., Berg, P. & Fiers, W. *J. Virol.* **30,** 674–682 (1979).
28. Wu, C. *Nature* **286,** 854–860 (1980).
29. Fromm, M. & Berg, P. *Molec. cell. Biol.* **3,** 991–999 (1983).
30. Myers, R. M. & Tjian, R. *Proc. natn. Acad. Sci. U.S.A.* **77,** 6491–6495 (1980).
31. Dynan, W. S. & Tjian, R. *Cell* **35,** 79–87 (1983).
32. DeLucia, A. L., Lewton, B. A., Tjian, R. & Tegtmeyer, P. *J. Virol.* **46,** 143–150 (1983).
33. Tegtmeyer, P., Lewton, B. A., DeLucia, A. L., Wilson, V. G. & Ryder, K. *J. Virol.* **46,** 151–161 (1983).
34. Sassone-Corsi, P., Dougherty, J., Wasylyk, B. & Chambon, P. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
35. Wasylyk, B., Oudet, P. & Chambon, P. *Nucleic Acids Res.* **7,** 705–713 (1979).
36. Hiwasa, T., Segawa, M., Yamaguchi, N. & Oda, K. I. *J. Biochem.* **89,** 1375–1389 (1981).
37. Nordheim, A. & Rich, A. *Nature* **303,** 674–679 (1983).
38. Nickol, J., Behe, M. & Felsenfeld, G. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1771–1775 (1982).
39. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33,** 717–728 (1983).
40. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33,** 729–740 (1983).
41. Nordheim, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7729–7733 (1982).
42. Wahl, G. M., Stern, M. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76,** 3683–3687 (1979).
43. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5201–5205 (1980).
44. Ghose, D., Weiss, E., Homo, J. C., Van Regenmortel, M. H. V. & Oudet, P. *Expl Cell Res.* **147,** 135–142 (1983).

LETTERS TO NATURE

A new mine determination of the newtonian gravitational constant

S. C. Holding & G. J. Tuck

Physics Department, University of Queensland, Brisbane 4067, Australia

Recent theoretical interest in the possibility of observing a non-newtonian component of gravity arises from the lead that such observations will give to unification theories[1]. Absolute determinations of the newtonian constant *G* by geophysical methods, at mass separations several orders of magnitude greater than those available in the laboratory, provide information not obtainable in any other way. Stacey[2] drew attention to the fact that modern geophysical measurements were precise enough in principle to provide an important check on *G* and Stacey *et al.*[3] revived the mine method first used by G. B. Airy. Subsequently Stacey analysed a set of overlapping sea-floor and sea-surface gravity survey data made available by the Exxon Oil Co., to avoid the problem of density uncertainty that beset the mine measurements, and Stacey and Tuck[4] compared the results with values of *G* calculated from published mine and borehole data. The striking conclusion was that the values of *G* were all systematically high. Residual doubts, especially concerning the adequacy of density sampling and the possibility of bias by regional gravity anomalies, left the conclusion as a rather tentative one. We report here a new determination of *G*, which is also high, but in which the doubt about density is substantially reduced. This information makes it implausible to explain the high value of *G* in terms of an inadequate knowledge of density. The possible effect of regional gravity anomalies is not as securely discounted.

The measurements were made in the Hilton mine (24°34′ S, 139°28′ E), operated by Mount Isa Mines Ltd. We have made full use of the company's file of density data, from extensive coring of the area, and knowledge of geological structure by company staff. Over 2,300 core sample densities were used to establish the density pattern of the region. Gravity values were obtained at the surface and at 11 subsurface levels down to 993 m in stubs to one side of the P49 shaft (Fig. 1), using La Coste-Romberg meter G608. This was checked against the well calibrated G20 meter used previously and procedures for checking instrument drift, applying tidal corrections and so on, were the same as in the previously reported survey[3]. Data from the J53 shaft are not considered here as the density inhomogeneity immediately surrounding J53 and the shorter vertical extent of the shaft combine to give a much less accurate value of *G*. The equations for calculating *G* from the gravity profile and layer densities are given by Stacey *et al.*[3] (and confirmed by Dahlen[5]), but a slight variation was required in the present case to take full account of the documented inhomogeneities.

The method used by Stacey *et al.*[3] was to average all density values at depths between gravity stations to obtain mean layer densities between those levels. Our method was first to assume a regional average density (2,750 kg m^{-3}) and then to correct the individual gravity data for the localized departures from this average (using the laboratory value of *G*, 6,672 × 10^{-11} m^{3} kg^{-1} s^{-2}). This correction procedure was a fully three-dimensional one since we have a detailed picture of the geological structure and density of the region. The corrected gravity data were then used to calculate *G* (as in ref. 3). A small iterative adjustment was then applied by using the calculated value of *G* in repeating the corrections to the *g* values, but the effect on the estimate of *G* was not significant. Thus the density measurements were used to obtain representative densities of the iden-

0028–0836/84/080714—03$01.00

© 1984 Macmillan

Table 1 Vertical profile data for P49 shaft

Depth (m)	Gravity difference (10^{-3} m s^{-2})	Geological correction (10^{-6} m s^{-2})	Surface mass anomaly correction (10^{-6} m s^{-2})	$U(z)$ (10^{-3} m s^{-2})	$X(z)$ 10^{6} kg m^{-2})	Gravity residuals (10^{-6} m s^{-2})
0	0	0	0	0	0	0
183.62	0.19382	1.45	−56.57	0.5669	0.5050	−4.74
243.45	0.23759	1.79	−52.78	0.7516	0.6695	−3.64
363.55	0.32471	2.55	−48.89	1.1224	0.9998	−5.73
483.55	0.41367	3.40	−46.71	1.4929	1.3298	−7.57
603.65	0.50187	4.29	−45.46	1.8637	1.6602	−11.05
723.50	0.59163	5.08	−44.46	2.2338	1.9898	−13.25
783.55	0.63614	5.43	−44.05	2.4192	2.1550	−14.90
813.55	0.65821	5.59	−43.86	2.5119	2.2375	−15.96
843.55	0.68131	5.65	−43.67	2.6045	2.3200	−16.09
963.53	0.77117	6.20	−43.02	2.9751	2.6501	−18.78
993.54	0.79386	6.38	−42.88	3.0678	2.7326	−19.28

Hilton mine: $g_0 = 9.786$ m s^{-2}; $\phi_g = 20°34'$ S; height above sea level = 386 m. $X(z)$ values assume the reference density, 2,750 kg m^{-3}.

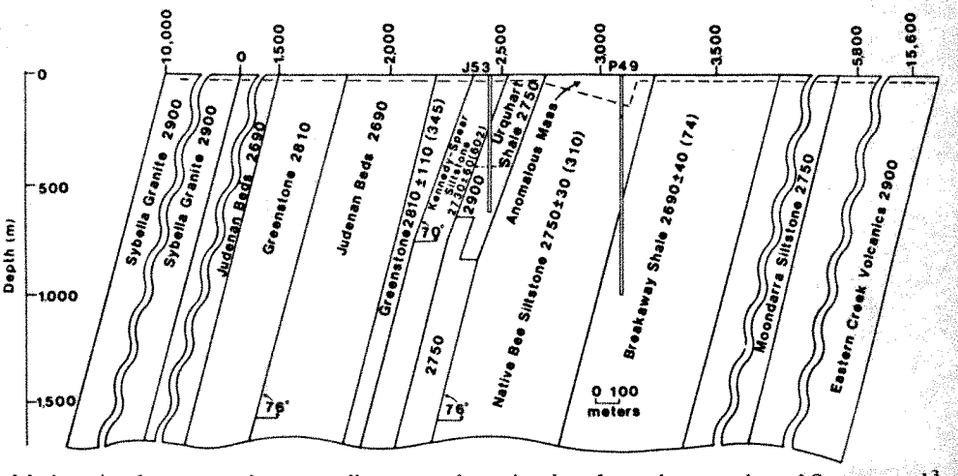


Fig. 1 Schematic cross-section of the rock formations of the Hilton mine (looking north). Structural trends are N–S, so that this cross-section is representative. Calculations assume the structure to extend to 4.5 km depth. Density values are given with standard deviations of individual samples about the means and the numbers of samples from each formation in parentheses. Gravity profiles were obtained for both shafts, but the greater homogeneity of rocks immediately surrounding the P49 shaft made this much more useful for calculating G.

tified geological formations instead of being simply averaged. The important difference between these procedures is that it avoids the bias of over-sampling of rocks that happen to be geologically interesting. Treatment of the density data for Urquhart shale required particular care, because this rock has some mineralization, but almost half of our density values were from it. The estimated mean density is a weighted average of the mineralized and unmineralized zones.

Special consideration is required for the density of the top 100 m or so of rock. This zone is weathered, porous and fractured, so that reliable and representative density data are impossible to obtain. We therefore chose to ignore the surface data point in calculating G and to consider the gravity gradient from 183 m (the first subsurface gravity station) to 993 m depth. This profile is unaffected by a uniform surface layer of unknown density. It is, however, influenced by inhomogeneity of the surface layer and a surface gravity survey was used to provide a correction for this. The surface survey was first corrected for the known deeper structure and then used to model a shallow mass to which we could attribute the residual gravity anomaly. This anomalous mass was then used to apply minor corrections to the subsurface g values; these corrections imply no knowledge of G, since they are based directly on comparisons of g.

All gravity data and corrections are listed in Table 1. The gravity values are corrected for instrumental drift and tides; topographic effects are insignificant. Excavation corrections are also included but these were made very small by mounting the gravity meter on a tripod near to the geometrical centre of a tunnel when a measurement is made. The first of the correction terms, as listed, was calculated assuming the laboratory value of G and was therefore re-weighted in the final calculation; the second is based on gravity measurements and so makes no assumption about G. $U(z)$ and $X(z)$ are depth-dependent parameters as defined by Stacey *et al.*[3].

The effectiveness of the density modelling is attested by the small scatter of gravity data from the equation of Stacey *et al.*[3] and consequently in the formal uncertainty of the calculated value of G:

$$G = (6.730 \pm 0.003) \times 10^{-11} \quad \text{m}^3\,\text{kg}^{-1}\,\text{s}^{-2}$$

The quoted uncertainty represents one standard deviation and is clearly smaller than the possible systematic error in the measurement of density; it is simply an indication of the internal consistency in the data. This is also demonstrated by a plot of gravity residuals (Table 1, Fig. 2), that is the departures of the gravity data, relative to the surface value, from the values calculated assuming the laboratory value of G. We interpret the intercept on the graph of residuals to be primarily a result of poor density values for the surface layers, although in principle it could be used to infer the range of a supposed short range repulsive force additional to normal gravity. We cannot explain the poor fit of the data point at 183 m. If this is omitted then we obtain

$$G = (6.734 \pm 0.002) \times 10^{-11} \quad \text{m}^3\,\text{kg}^{-1}\,\text{s}^{-2}$$

We are satisfied that inadequate knowledge of density must now be discounted as an explanation for the high values of G calculated here; this is probably true also for some of the earlier values and certainly for the marine measurement[4]. The remaining doubt arises from the possibility of a regional bias in the gravity gradient, as would be apparent if we could make a satisfactory direct measurement of the free-air gradient above the Earth's surface. The headframes at Hilton are not high enough to obtain satisfactory free-air gradient data. If we are to explain our observations in terms of conventional (newtonian) physics, we require a mass distribution at depth to give an anomalous vertical gradient of -2.1 mgal km^{-1} (1 gal = 10^{-2} m s^{-2}) (as in Fig. 2). For this gradient to be consistent to 10% over the top kilometre, as is apparent, the depth of the mass must be not less than 30 km. (It could be shallow and

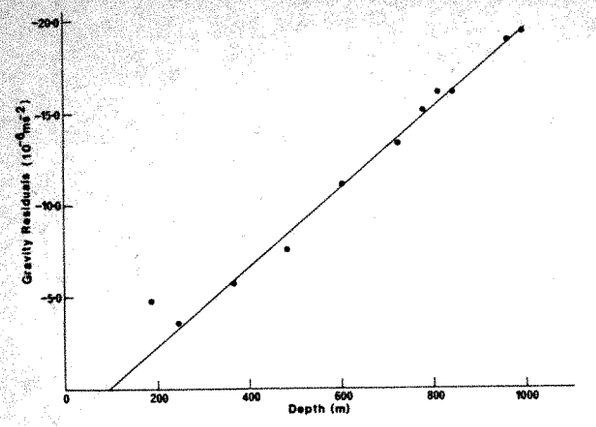


Fig. 2 Gravity residuals, calculated as differences between measured gravity at depth, relative to the surface, and values expected from the laboratory value of G.

laterally extended, but the calculations are similar.) Such a mass would cause a local gravity anomaly of about 30 mgal over a horizontal scale of about 30 km and would give a horizontal gradient greater than the locally observed one. However, the anomaly is comparable with the strongest of the observed gravity anomalies in the region[6] so although it does not provide a convincing explanation for our observations, it introduces an element of doubt that we cannot dismiss with certainty.

Our value of G is the best controlled of the geophysical determinations, all of which are higher than the laboratory value[4], and it falls into the middle of the range. Although we are not prepared, at this stage, to assert that a non-newtonian effect is clear and proven, the circumstantial evidence is very strong indeed. The need for a deep-sea measurement, as originally proposed[2], is now urgent.

This work is supported by the Australian Research Grants Scheme and by Mount Isa Mines Ltd. We appreciate the help of Mount Isa Mines staff, especially, R. Blair, J. Gillam, M. Lee, G. Orthman and J. Rogan, and the opportunity to make full use of company data. F. D. Stacey gave us detailed advice at all stages. This work will be presented elsewhere[7].

Received 28 October; accepted 24 November 1983.

1. Gibbons, G. W. & Whiting, B. F. *Nature* **291**, 636–638 (1981).
2. Stacey, F. D. *Geophys. Res. Lett.* **5**, 377–378 (1978).
3. Stacey, F. D., Tuck, G. J., Holding, S. C., Maher, A. R. & Morris, D. *Phys. Rev.* D**23**, 1683–1692 (1981).
4. Stacey, F. D. & Tuck, G. J. *Nature* **292**, 230–232 (1981).
5. Dahlen, F. A. *Phys. Rev.* D**25**, 1735–1736 (1982).
6. *Gravity Map of Australia*, 1:10,000,000 (Bureau of Mineral Resources, Canberra, Australia, 1979).
7. Holding, S. C. thesis, University of Queensland (1984).

Hyperion: 13-day rotation from Voyager data

P. Thomas*, J. Veverka*, D. Wenkert†, G. E. Danielson† & Merton E. Davies‡

* Center for Radiophysics and Space Research, Cornell University, Ithaca, New York 14853, USA
† California Institute of Technology, Pasadena, California 91125, USA
‡ RAND Corporation, Santa Monica, California 90406, USA

The spin period and orientation of Hyperion have remained uncertain even with high-resolution Voyager images. We have now used low-resolution Voyager images taken over a two-month span to seek a rotational light-curve. The data indicate a spin period of 13 days, with the spin axis nearly parallel to the orbital plane.

Hyperion, an irregularly shaped satellite (maximum dimension about 350 km), orbits Saturn between Titan and Iapetus once every 21.3 days. Because of the satellite's small size and large distance from Saturn, Peale[1] suggested that Hyperion's spin period may not be synchronous with its orbital period. The actual spin or rotation rate of Hyperion is of interest not only for dynamical reasons, but also in the context of understanding the albedo dichotomy on Iapetus[2,3]. One popular explanation of Iapetus holds that dark dust spiralling into Saturn on retrograde orbits preferentially coats the leading hemisphere of Iapetus[3], a scenario that implies that some of this dark dust is also intercepted by Hyperion. If Hyperion, like Iapetus, had a synchronous spin state one might expect to see large albedo differences between its trailing and leading hemispheres. Voyager images reveal no evidence of a hemispherical albedo dichotomy on Hyperion[4].

Initial attempts to derive the rotation period of Hyperion from the highest resolution images obtained by Voyager 2 were not successful[4]. In this study we determine the rotation period by means of a light-curve derived from low-resolution Voyager images. We exclude the high-resolution, high phase-angle images obtained within a period of about one day, because during this period changes in the appearance of Hyperion reflected the rapid motion of the spacecraft rather than the intrinsic rotation of the satellite. Our analysis is restricted to nineteen images obtained through the clear filter ($\lambda_{eff} \sim 0.48\ \mu$m) of the narrow angle cameras: five Voyager 1 images taken over 27 days from 26.5×10^6 km to 4.6×10^6 range, and fourteen Voyager 2 frames taken over 61 days from 59×10^6 to 1.8×10^6 km. The Voyager 1 data were from 14 October–10 November 1980 and Voyager 2 data 24 June–23 August 1981. For each image a disk-integrated brightness was obtained and scaled to a common distance. Corrections for phase effects were made starting from intrinsic phase coefficients of the surface material determined from the high resolution images at phase angles $\alpha = 25°$ to 45°. The resulting average disk-integrated phase coefficients[5] ranged from 0.019 to 0.020 mag per degree, and were used to scale the data to our reference frame (FDS 43323.29) obtained at $\alpha = 6.3°$. The single image below a phase angle of 4.0° ($\alpha = 2.85°$) was scaled using a phase coefficient of 0.03 mag per degree to allow for the possible presence of an opposition surge[6]. The calculated phase coefficients are consistent with those derived for other satellites of Saturn[7,8], and the precise value adopted has little influence on the light-curve analysis.

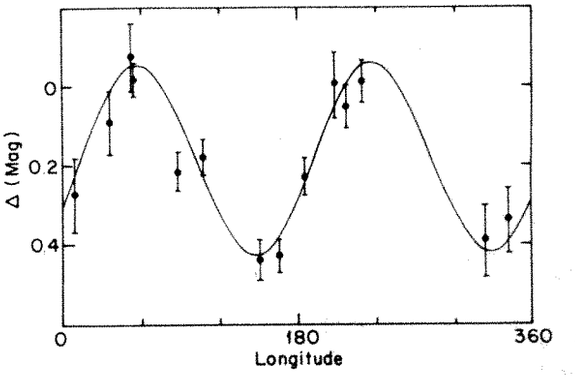


Fig. 1 Hyperion light-curve from Voyager 2 clear filter data, for a period of 13.1 days, rotation axis parallel to Hyperion's orbital plane. The observations are compared to a simple sine curve; error bars are estimated uncertainties due to phase function and photometric noise.

Uncertainties in the disk-integrated values used to construct the light-curve arise from two major sources. First, there is photometric noise given the very small scale of most of the images (1–5 pixels across). Second, for an object as irregular as Hyperion, corrections for differences in solar phase can only be made approximately, and corrections for variations in aspect must be ignored at this level of analysis. The resulting estimated uncertainties in the scaled brightness levels range from 4 to 10%.

0028-0836/84/080716—02$01.00

© 1984 Macmillan

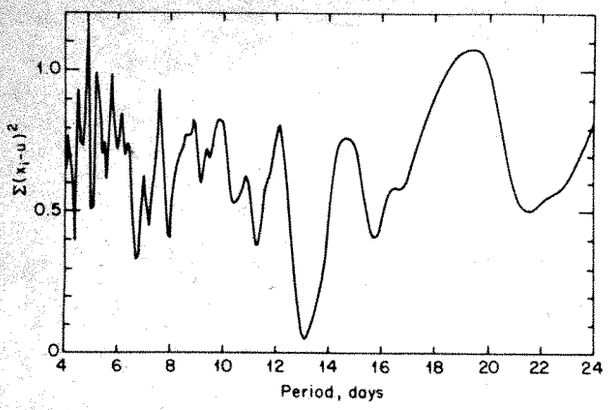


Fig. 2 Residuals of Voyager 2 observations (x_i) compared with a sine curve light-curve (u) as a function of rotation period. Differences in the orientation of the rotation axis produce only small differences in the residuals. The only good fits correspond to periods of 13.1 ± 0.3 days.

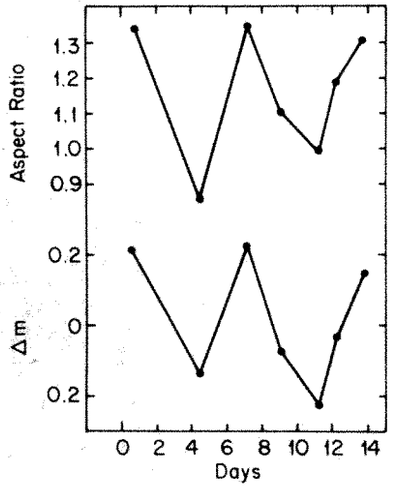


Fig. 3 The aspect of Hyperion and observed light-curve during the final 14 days of Voyager 2 approach data. The aspect ratio is the apparent dimension perpendicular to orbital plane divided by dimension parallel to orbital plane. The similarity of the two curves demonstrates that the light variations are primarily due to variations in the satellite's apparent shape, and that the spin axis is more nearly parallel to the orbital plane than perpendicular to it.

The available data indicate a spin period of 13 days (Figs 1 and 2). The result does not depend strongly on the assumed direction of the spin axis, because during each of the two sequences available the relative angular motions of the spacecraft and Hyperion were small. In Fig. 1 the Voyager 2 data are compared with a simple sine curve of period 13.1 days (the points cover five rotations of the satellite). By themselves, the five Voyager 1 points cannot be used to determine a period, but they are consistent (period and amplitude) with the light-curve shown in Fig. 1. Figure 2 shows the residuals between the Voyager 2 data and model sinusoidal lightcurves as a function of period. The only acceptable fits correspond to periods of 13.1 ± 0.3 days. If we use the estimates of uncertainties (see Fig. 1) a strong minimum occurs at the same period. Because the total uncertainties are not known, a formal χ^2 test is not appropriate.

We now turn to the possibility of determining the orientation of the satellite's spin axis. The last 14 days of Voyager 2 observations (seven images) obtained sufficient resolution to estimate the satellite's apparent shape. The longest dimension was found to be nearly perpendicular to Hyperion's orbital plane. The resulting aspect ratios (north-south dimension divided by east-west dimension) are shown in Fig. 3. It is evident that the light variations observed are due to changes in the apparent shape of the satellite as it rotates around its spin axis and that the rotation axis must be nearly parallel to the orbital plane. The maximum Voyager 1 elongation (at maximum integrated brightness) was also nearly north-south and had an aspect ratio of 1.3.

Recently, Wisdom, *et al.*[9], have suggested that Hyperion's spin state might be chaotic with substantial variations in both the period and the orientation of the spin axis on time scales of several orbital revolutions (period = 21.3 days). Our Voyager 2 data indicate a coherent 13-day spin period over 61 days, or nearly three orbital revolutions. Our data are also consistent with the view that Hyperion's spin period and spin axis orientation were similar 220 days earlier at the time of the Voyager 1 observations.

Our results show that Hyperion is definitely not in synchronous rotation. Consequently it cannot be used to test the hypothesis that dark dust spiralling inward on retrograde orbits causes the pronounced leading side/trailing side albedo asymmetry observed on the surface of Iapetus.

We thank Candy Hansen, Anne Bunker and Andy Collins at the Jet Propulsion Laboratory, Pasadena for their efforts in preserving these satellite sequences in the mission design. This research was supported in part by NASA grant NAGW 193, and Rand Corporation Contract NASW 3602. This is contribution no. 3987 of the Division of Geological and Planetary Sciences at California Institute of Technology.

Received 22 September; accepted 8 December 1983.

1. Peale, S. J. in *Planetary Satellites* (ed. Burns, J.) 87–112 (University of Arizona Press, Tucson, 1977).
2. Cruikshank, D. P. *et al. Icarus* **53**, 90–104 (1983).
3. Squyres, S. & Sagan, C. *Nature* **303**, 782–785 (1983).
4. Smith, B. *et al. Science* **215**, 504–537 (1982).
5. Noland, M. C., thesis, Cornell Univ. (1975).
6. Veverka, J. in *Planetary Satellites* (ed. Burns, J.) 171–209 (University of Arizona Press, Tucson, 1977).
7. Buratti, B. & Veverka, J. *Icarus* (in the press).
8. Thomas, P., Veverka, J., Morrison, D., Davies, M. & Johnson, T. V. *J. geophys. Res.* **88**, 8743–8754 (1983).
9. Wisdom, J., Peale, S. J. & Mignard, F. *Icarus* (submitted).

Spatial structures generated by chemical reactions at interfaces

David Avnir & Michael Kagan

Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Far-from-equilibrium networks of physicochemical processes may exhibit structuring[1], in time (for example, chemical oscillations[2]), in space (stationary patterns[3]) or as a combination of both (chemical waves[4]). Much experimental effort has been invested during the past two decades in temporal and spatio-temporal phenomena[2,4]; and except for periodic precipitation processes[5] no general chemical system was available for a wide experimental study of the evolution of spatial structures. Recently, however, we have revealed a remarkably wide family of processes which produce spatial structures: chemical reactions at liquid interfaces. These include reactions at gas/liquid interfaces[6], and photochemical reactions at liquid/air[7,8] and liquid/liquid[8] interfaces. We now report yet another important class: reactions at membrane surfaces, as shown in Fig. 1. Although hydrodynamic slow currents are apparent at a mature stage of the structure development, the actual mechanism leading to the initial structuring is as yet not clear. We show, however, that these structures are not a visualizing method of patterns caused by evaporative cooling[9,10]: structures are formed also in the absence of such patterning and hydrodynamic currents start only after chemical structures are formed. Some of our observations suggest that chemical reaction is necessary for structure evolution, and that gravity apparently plays no role in this phenomenon.

The recognition that a much wider phenomenon lies behind Möckel's report[11] on structure formation in certain photo-

© 1984 Macmillan Journals Ltd

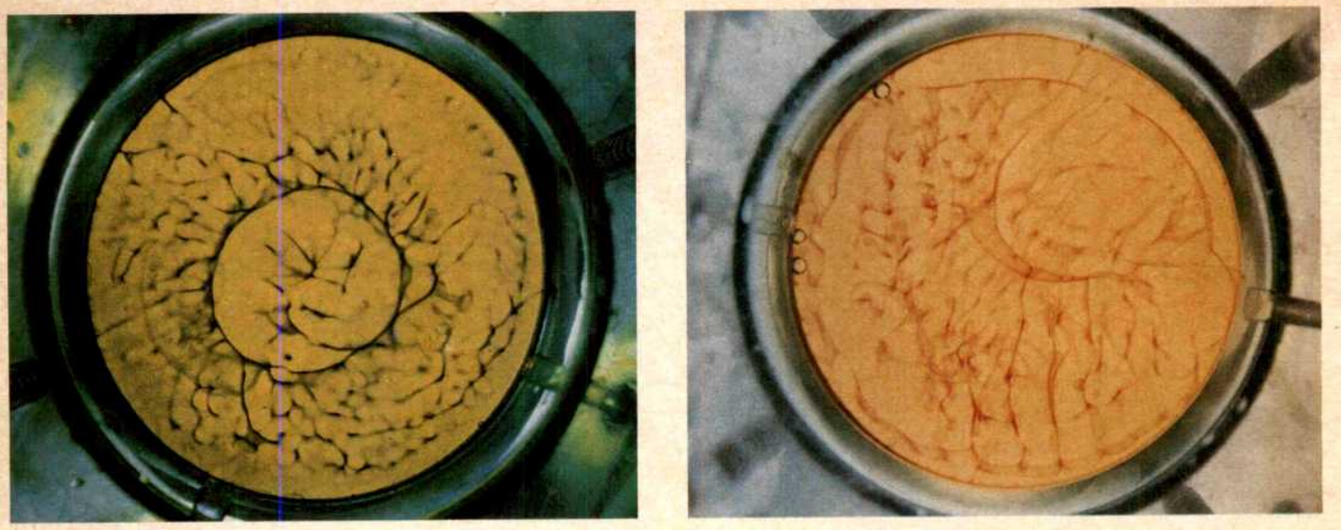


Fig. 1 Chemical structures at membrane surfaces. *a*, Patterns of iodine–starch complex resulting from the diffusion of bromine into KI/starch. *b*, Patterns of $KFe(NCS)_4$ in the reaction of $FeCl_3$ with KSCN.

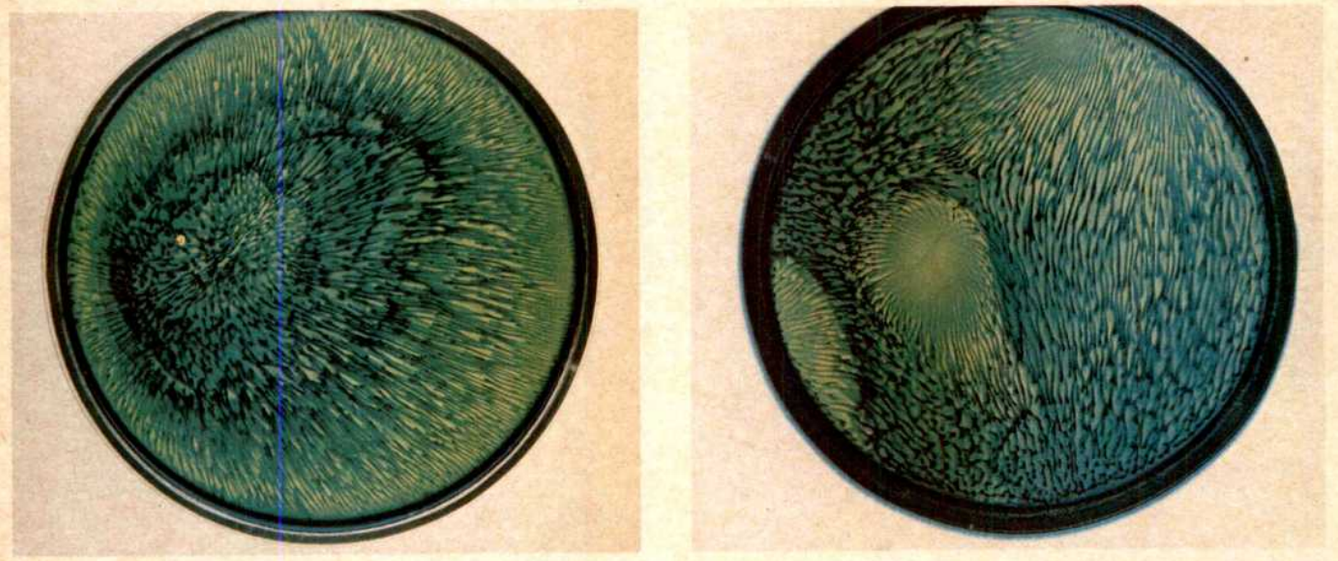


Fig. 2 Structures of Turnbull's blue formed by photoreduction of ferricyanide in a sealed Petri dish. *a*, With an air gap between the liquid surface and the glass seal. *b*, No air gap.

chemical reactions, has been developed through recent exploratory research. First, we reported that almost any photo-halogen cleavage, followed by a thermal halogen reaction produced spectacular far-from-equilibrium structures[7,8]. It was then shown by Micheau *et al.*[9,10], and later by us[12] and by others[13,14], that light-energy can be used quite generally to drive reacting systems away from equilibrium thus forming structures of photo-products. We were able then to substitute energy influx through the liquid interface, by matter influx, and showed that the reaction between a diffusing gas and a reactant in solution proceeds in a nonhomogeneous structured way[6]. An interface which is of special importance because of its role in biology, is that between two reacting solutions separated by a membrane. We report now that dissipative[1] structures are formed near the surface of a dialysis membrane (Spectropore-2, molecular cutoff 12,000) when one species diffuses through the membrane and reacts on the other side; the phenomenon is general from the chemical point of view (Table 1). All types of structure-formation systems described above share in common the sequence of events shown in Fig. 3. The membrane reactions were carried out as follows: A Perspex cell, having two compartments 5 cm in height, separated by the membrane, was filled up with the two solutions, one in each of the compartments. In all reactions (see Table 1 and Fig. 1) a solution of a small species is separated from a dilute solution of a larger substrate, so that diffusion is virtually unidirectional. The coloured product is also a large molecule. The following observations indicated in Table 1 should be especially noted: (1) completely unrelated reactions produce similar patterns. As was found in our previous systems, the nature of the chemical reaction is of secondary importance. In fact, this generality raises the question of how this observation has been overlooked so far. We have referred already to this question when we described the gas/liquid structures[6], and suggested that the observation has been overlooked firstly because of the relative lack of awareness in chemistry to the importance of macroscopic structure-building processes; and secondly, because reactions are usually carried out under stirring: any structure formed would have been destroyed. (2) The

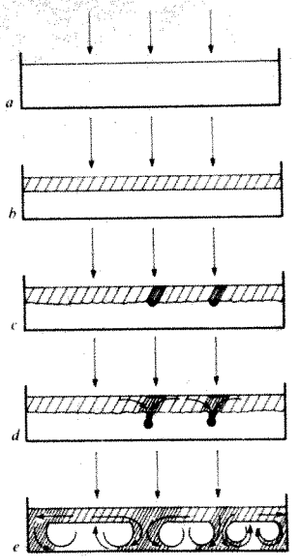


Fig. 3 *a*, Before reaction starts, whether photolytic, gas/liquid or liquid/liquid, the reactants solution is homogeneous and at rest. *b*, The reaction starts over the entire interface. *c*, After about 60 s lines of higher concentration begin to appear. *d*, The product descends along the pattern lines. If the interface is at the bottom the product *ascends*. *e*, If the solution is shallow enough (about 15 mm) the descending product forms hydrodynamic rolls. The solution eventually becomes homogenous again (not shown).



Fig. 4 An iodine–starch pattern formed by photolysis of KI/chloralhydrate/starch at 254 nm.

I_2/starch structures were produced not only through a membrane, but also by photooxidizing an I^-/starch solution[7,11] (Fig. 4, see below), and by I_2 vapours diffusing through starch solution interface[6]. The generality of the nature of the interface in this phenomenon is thus shown. (3) Comparison of reactions 2 and 3 (Table 1) suggests that gravity is not playing a deciding role in the overall mechanism. This is also suggested when photochemical structure-forming systems are irradiated from the bottom through a glass/liquid interface (refs 10, 12 and the Fe(III) photoreduction described below). (4) Note that in the cases where diffusion was not accompanied by a chemical reaction, structures did not form (reactions 10 and 11 in Table 1).

The apparent similarity between the patterns described and bulk hydrodynamic patterns[15] suggests the possibility that the patterns are colour tracing preexisting evaporative hydrodynamic currents (prepatterns)[9,10]. However, we present evidence that chemical patterns evolve in the absence of any evaporative prepatterns.

A number of experiments have been carried out with the photoreduction of Fe(III) to Fe(II) (ref. 16). A Petri dish filled with 5–10 mm of an aqueous solution of 0.2% oxalic acid dihydrate, 0.2% potassium ferricyanide and 0.1% ferric chloride hexahydrate (solution (1)) was irradiated from above at 360 nm (4 × 15 W lamps). The ferrous ions produced in this reaction form $Fe_3[Fe(CN)_6]_2$ (Turnbull's blue). This photochemical reaction, which produces patterns, was found to be experimentally more convenient than our previously reported photochemical reactions[7,8]. Evaporative currents in this system can easily be followed by sprinkling aluminium dust and illuminating from the side with a laser. To avoid the evaporative patterns, the Petri dish was sealed tightly with a glass cover. As expected[15,17], the evaporation patterns disappeared and no other convections could be detected. The liquid/air interface was then irradiated through the seal: beautiful blue structures were formed (Fig. 2*a*). The sequence described in Fig. 3 was very clear: first, the surface was homogeneously charged with the photoproduct (again, evaporation would clearly be seen at this stage); after about a minute, patterns started to grow; and finally bulk movements appeared when the photoproduct started to descend from the line-patterns at the surface, that is, the onset of currents is a later stage, and not a prerequisite, in this phenomenon.

In a second experiment on this photochemical system 25% methanol was added in order to enhance the evaporative patterns. Such binary systems are known to have pronounced patterning[15,18]. The initial experiment was repeated and compared to an open Petri dish irradiation. Although we cannot rule out the possibility that the produced patterns in the open vessel coincide with the evaporative patterns[10], it is our observation that the evaporation in fact interferes with an ordered still growth of the patterns. Furthermore, we found it extremely difficult to get chemical patterns in open vessels containing

Table 1 Structure formation at membrane surfaces

Solution* above membrane	Solution* below membrane	Reaction type	Colour of structure
0.01% Iodine	0.5% Starch	Complexation	Blue
0.1% Bromine	0.5% Starch/1% KI	Oxidation followed by complexation	Blue
0.5% Starch/1% KI	0.1% Bromine		Blue
0.01 M HCl	0.02% Methyl orange	Protonation	Red
0.01 M KOH	0.02% Phenolphthalein	De-protonation	Pink
0.5% Acetaldehyde	20% Morpholine 5% Sodium nitroprusside	Oxidation	Blue
1% $FeCl_3$	0.1% $K_4Fe(CN)_6$	Complexation	Blue
1% $FeCl_3$	0.1% Tannic acid	Complexation	Grey
1% $FeCl_3$	0.1% KSCN	Complexation	Red
0.01% Methylene blue	Water		No structure (blue)
Water	0.02% Methyl orange/0.01 M HCl		No structure (red)

* All solutions in water.

liquids of very high vapour pressure such as methylene dichloride[8].

In a third experiment the Petri dish was filled with solution (1) to the rim, and then covered with a 3 mm sheet of glass, that is, the liquid/air interface was replaced by a liquid/glass interface. Obviously there is no evaporation in this system , yet structures developed in the usual way (Fig. 2*b*). Similarly, structures were obtained when the Petri dish (either closed or open) was irradiated from below. These observations seem to exclude a Marangoni-type effect[14], which requires a free surface[19]. Structures appeared also upon irradiation of a very shallow layer, obtained by 'sandwiching' solution (1) between two glass plates separated by 0.95 mm. Pattern formation upon irradiation of a 1 mm layer was reported by Micheau *et al.* in an open system and attributed to preexisting currents[9,10]. However, a careful observation under a microscope of the sequence of events on irradiating one drop of (1) (~3 mm in diameter, less than 1 mm in height) revealed again the stages of Fig. 3, namely: first pattern formation at the surface and only then slow hydrodynamic currents.

Other evidence shows, as we have reported already[8], that irradiation of the following liquid interfaces, protected from evaporation by another 4 cm liquid layer, produced structures: diphenyl amine/CCl_4 covered by water; diphenylamine sulphonate/KBr/H_2O, covered by cyclohexane; 4-aminophenol·HCl/KBr/H_2O, covered by paraffin oil. The membrane experiments described in this paper again exclude the necessity for evaporative prepatterning for the chemical structures formation: the membrane is covered with a 5 cm layer, and protected at the sides by 2 cm Perspex walls.

Reactions 10, 11 in the Table (dye diffusion only) demonstrate that diffusion by itself is probably insufficient in accounting for the phenomenon, and that coupling with a chemical reaction is necessary. The quantitative evaluation and comparison of one dissipative structure to the other is a complicated, still uncompleted task that has been undertaken by us[12] and by others[17]. However, even at this early stage it seems to us that in many cases (for example, Fig. 4) one can tell qualitatively that the shapes obtained are in fact quite different from usual patterns seen in thermal convections[15].

Models and theories of far-from-equilibrium processes have predicted the feasibility of the formation of interfacial structures[20], yet to the best of our knowledge, the phenomenon we describe is the first wide family of chemical processes forming such structures. Quantitative parametrizations and simulations in the light of such models, and a study of an interesting scaling behaviour of the patterns[12], found during the microscopic experiment described above, are in progress.

Sponsored by the Volkswagen Foundation. Assisted by the F. Haber Research Center for Molecular Dynamics. D.A. is an M. Richter Fellow. Most helpful discussions were carried out with Professors B. Hess, T. Plesser and with Dr S. Müller. We are grateful to the authors of ref. 10 for a preprint.

Received 22 August; accepted 18 November 1983.

1. Nicolis, G. & Prigogine, I. *Self-organization in Nonequilibrium Systems* (Wiley, New York, 1977).
2. Noyes, R. *Ber. Bunsenges. Phys. Chem.* **84,** 295–303 (1980).
3. Orban, M. *J. Am. chem. Soc.* **102,** 4311–4314 (1980).
4. Winfree, A. T. *Sci. Am.* 82–95, June 1974.
5. Kai, S., Müller, S. C. & Ross, J. *J. chem. Phys.* **76,** 1392–1406 (1982).
6. Avnir, D. & Kagan, M. *Naturwissenschaften* **70,** 361–363 (1983).
7. Kagan, M., Levi, A., & Avnir, D. *Naturwissenschaften* **69,** 548–550 (1982).
8. Avnir, D., Kagan, M. & Levi, A. *Naturwissenschaften* **70,** 141–143 (1983).
9. Gimenez, M. & Micheau, J.-C. *Naturwissenschaften* **70,** 90–92 (1983).
10. Micheau, J.-C., Gimenez, M., Brockmans, P. & Dewel, G. *Nature* **305,** 43–45 (1983).
11. Möckel, P. *Naturwissenschaften* **64,** 224 (1977).
12. Kagan, M., Meisels, E., Peleg, S. & Avnir, D. *Lect. Not. Biomath.* (in the press).
13. Zinkovskaya, O. V. *Chem. Abstr.* **98,** 167832h (1983).
14. Wiessenborn, L. & Bausch, R. *Naturwissenschaften* **70,** 307–308 (1983).
15. Berg, J. C., Boudart, M. & Acrivos, A. *J. Fluid Mech.* **24,** 721–735 (1966).
16. Fry, M. S. & Gerwe, E. G. *Ind. Engng Chem.* **20,** 1392 (1928).
17. Müller, S. & Plesser, T. *Lect. Not. in Biomath.* (in the press).
18. Möckel, P. *Naturwissenschaften* **66,** 575–576 (1979).
19. Sterling, C. V. & Scriven, L. E. *A.I.Ch.E. Jl* **5,** 514–523 (1959).
20. Sorenson, T. S., Hennenberg, M., Steinchen-Sanfeld, A. & Sanfeld, A. *Prog. Colloid Polymer Sci.* **61,** 64–70 (1976).

0028–0836/84/080720—02$01.00

A new chemical oscillator containing neither metal nor oxyhalogen ions

Maria Burger* & Richard J. Field

Department of Chemistry, University of Montana, Missoula, Montana 59812, USA

Essentially all known inorganic chemical oscillators[1] conta metal ion[2–4] and/or an oxyhalogen ion, in particular, io ion[3], bromate ion[2,5] or chlorite ion[6]. We report here a chemical oscillator that is based on sulphur chemistry and tains neither a metal ion nor an oxyhalogen ion. The oscil does involve elemental oxygen, and in this way it may be rel to an oscillating reaction recently discovered by Jensen[7]. discovery of new chemical oscillators which are fundamen different from those previously known is of great import as it supplies new testing grounds for general theori dynamic behaviour[8] as well as further information concer the exact mechanistic requirements for chemical oscillato occur.

The chemical oscillator is composed of sulphide ion, sul ion, methylene blue (which serves as a catalyst), and disso oxygen. During the reaction, the methylene blue catalyst cy lates between its oxidized, coloured form (MB^+) and its red leuko form (MBH). Figure 1 shows a typical oscillatory of the absorbance at 668 nm due to MB^+. Sharp puls oxidation are observed in which ~40% of the MBH is conv to MB^+.

The reaction is run in an aqueous medium of *p*H 12–1 in a continuous-flow, stirred tank reactor (CSTR). It is the known example of chemical oscillations in a basic solution three feedstreams contain, respectively, MB^+, Na_2S, Na and O_2 at the appropriate *p*H. The sulphide ion is esser completely tied up as HS^- at *p*H 12–13. Sulphite ion ex the range of feedstream reactant concentrations and re residence times for which oscillations are found. Howeve apparently not a major reactant as oscillations appear absence. Figure 2 shows the region in $[S_2^{2-}]$, $[SO_3^{2-}]$ const space for which oscillations occur at a particular flow oxygen concentration and methylene blue concentration experiment is run in CSTR mode both to maintain the che system far from thermodynamic equilibrium, a necessi sustained chemical oscillations[9], and to keep the reactan centrations, especially that of dissolved oxygen, in the oscil range. The dynamics of the flow does couple with the che dynamics. However, regular but irreproducible oscillation been observed (B. Miewald and R.J.F., unpublished data) the experiment is run in a batch reactor.

The quantitative behaviour of the reaction is rather se to impurities in the Na_2S used, mainly sulphite ion, polysu ion and thiosulphate ion. However, the reaction is quite ducible when care is taken to monitor and control impurities quantitatively.

Oscillations appear only in a rather small range of flow for a particular set of feedstream concentrations, and the c tory region is quite dependent on these concentrations. cally, the steady-state $[MB^+]$ is very close to zero at flow below the oscillatory region while at flow rates higher th oscillatory range it is substantially higher, approximati median $[MB^+]$ during an oscillation. Approach to steady may be very slow both above and below the oscillatory Bistability is a common feature of chemical oscillators CSTR mode[10]. However, we have not yet observed bis in this system. The search continues.

* Permanent address: Institute of Inorganic and Analytical Chemistry, L. Eötvös U Muzeum KRT 4/B, Budapest, Hungary.

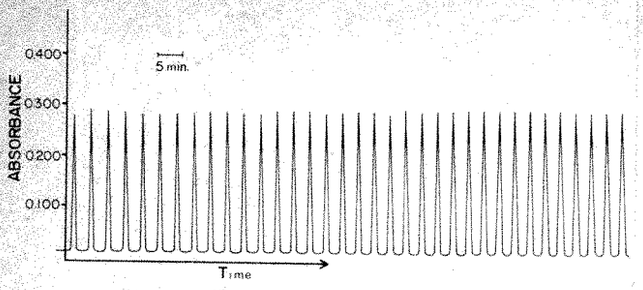


Fig. 1 Oscillations in the concentration of MB^+ as observed by measuring the absorbance at 668 nm. Conditions: $[MB^+]_0 = 3\times10^{-6}$ M: $[SO_3^{2-}]_0 = 1.2\times10^{-3}$ M: $[S^{2-}]_0 = 4.1\times10^{-2}$ M: $[HO^-]_0 = 5\times10^{-2}$ M: $[O_2]_0 = 1.5\times10^{-4}$ M. Temperature, 20 ± 0.1 C. 1/(residence time) = 1.5×10^{-3} s^{-1}.

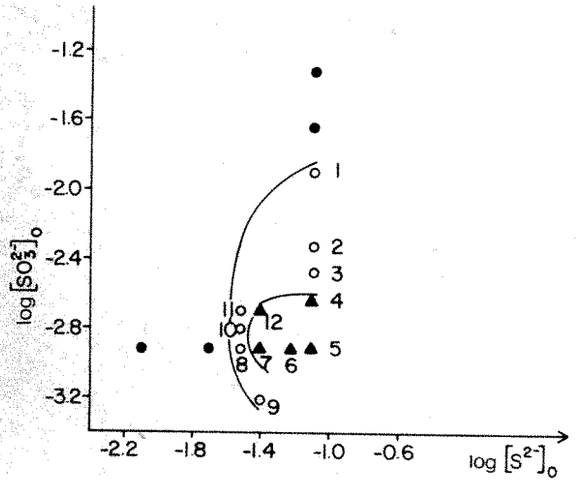


Fig. 2 $[S^{2-}]_0$ versus $[SO_3^{2-}]_0$ constraint-space diagram. Conditions: $[MB^+]_0 = 3.0\times10^{-6}$ M: $[OH^-]_0 = 5\times10^{-2}$ M: $[O_2]_0 = 1.5\times10^{-4}$ M. Temperature, 20 ± 0.1 °C. ○, Transient oscillations as the reciprocal residence times noted below are approached: ●, stable steady state; ▲, sustained oscillations. The reciprocal residence times are: (1) 1.9×10^{-3} S^{-1}; (2) 3.5×10^{-3} S^{-1} and 3.1×10^{-3} S^{-1}; (3) 3.1×10^{-3} S^{-1}; (4) 2.3×10^{-3} S^{-1} and 1.9×10^{-3} S^{-1}; (5) 2.0×10^{-3} S^{-1}; (6) 2.0×10^{-3} S^{-1}; (7) 1.6×10^{-3} S^{-1} and 1.3×10^{-3} S^{-1}; (8) 1.3×10^{-3} S^{-1} and 1.0×10^{-3} S^{-1}; (9) 1.5×10^{-3} S^{-1}; (10) 1.5×10^{-3} S^{-1}; (11) 1.3×10^{-3} S; (12) 1.3×10^{-3} S^{-1}.

The net stoichiometric chemical reaction which drives the oscillations is apparently reaction (1).

$$2SH^- + O_2 \rightarrow 2S + 2HO^- \quad (1)$$

The elemental sulphur may polymerize according to reaction (2),

$$nS + HS^- \rightarrow HS^-_{n+1} \quad (2)$$

or be removed from the system by sulphite ion according to reaction (3).

$$S + SO_3^{2-} \rightarrow S_2O_3^{2-} \quad (3)$$

Substantial amounts of elemental sulphur may precipitate in experiments where $[SO_3^{2-}]$ is low. However, this does not strongly affect the oscillations. Indeed, elemental sulphur may be added to the feedstreams with only minor effect.

This new oscillator was intentionally designed by the expedient of coupling an apparently autocatalytic reaction with a return mechanism. Reaction (4), the reduction of MB^+ by HS^-, is the apparently autocatalytic reaction[11].

$$HS^- + B^+ \rightarrow MBH + S \quad (4)$$

However, we have now found (unpublished data) that the reaction is not genuinely autocatalytic. It is instead inhibited by oxygen, which must be removed before the reaction can begin. A similar inhibition has been observed in the reduction MB^+ by sulphydryl compounds[12] such as cysteine. We have been unable to observe oscillations in the present system when sulphide ion is replaced by cysteine.

The return step is the oxidation of MBH by O_2. There is evidence[13] that this reaction actually is autocatalytic.

An explanation of the mechanism of this oscillator must await further detailed information on the component reactions. This work is underway.

This work was supported by the NSF under grants CHE77-03599 and CHE80-23755. We acknowledge the work of Bruce Miewald who first saw the methylene blue oscillations during the summer of 1978.

Received 16 August; accepted 2 November 1983.

1. Noyes, R. M. & Field, R. J. *Acct. chem. Res.* **10**, 273–280 (1977); in *Oscillations and Traveling Waves in Chemical Systems* (eds Field, R. J. & Burger, M.) (Wiley-Interscience, New York, 1984).
2. Field, R. J., Körös, E. & Noyes, R. M. *J. Am. chem. Soc.* **94**, 8649–8664 (1972).
3. Noyes, R. M. & Furrow, S. D. *J. Am. chem. Soc.* **104**, 45–48 (1982).
4. DeKepper, P. & Epstein, I. R. *J. Am. chem. Soc.* **104**, 49–55 (1982).
5. Noyes, R. M. *J. Am. chem. Soc.* **102**, 4644–4649 (1980).
6. Orbán, M., Dateo, C., DeKepper, P. & Epstein, I. R. *J. Am. chem. Soc.* **104**, 5911–5918 (1982).
7. Jensen, J. H. *J. Am. chem. Soc.* **105**, 2639–2640 (1983).
8. Minorsky, N. *Nonlinear Oscillations* (Krieger, Huntington, 1974).
9. Glansdorff, P. & Prigogine, I. *Thermodynamic Theory of Structure, Stability and Fluctuations* (Wiley-Interscience, New York, 1971).
10. Boissonade, J. & DeKepper, P. *J. phys. Chem.* **84**, 501–506 (1980).
11. West, P. W. & Ramakrishna, T. V. *Analyt. Chem.* **40**, 966–968 (1968).
12. Vishnevskaya, Z. I. & Zhabotinskii, A. M. *Biofizika* **14**, 228–31 (1969).
13. Roelofs, M., Wasserman, E., Jensen, J. & Nader, A. *J. Am. chem. Soc.* **105**, 6329–6330 (1983).

Evidence for stabilization of the Pilbara Block, Australia

T. S. Blake

Department of Geology, University of Western Australia, Nedlands, 6009 Western Australia

The stabilization of continental crust to form cratons represents one of the major features of Earth history[1]. Major structural, geochemical[2,3] and, to a lesser extent, sedimentological changes[4] associated with the development of stable crustal blocks are recorded mainly around 2,500 Myr ago[1]. This change, however, appears to have been globally diachronous and cratonic sequences as old as 3,100 Myr are recorded[5]. I now present the results of a recent regional stratigraphical and sedimentological study on the ~2770 Myr old lower Fortescue Group[6,7] (previously classified lithologically as Proterozoic[8], but following the recent recommendation of a 2,500-Myr Archaean/Proterozoic chronological boundary[9] is referred to as Archaean) in the Pilbara Block[10], Western Australia. A major stabilization of the underlying granitoid–greenstone terrain appears to have pre-dated Fortescue basin formation. This event, which available geochronology suggests was relatively rapid, marked a major changing point in the tectonic evolution of the Pilbara Block.

The lower Fortescue Group (Fig. 1) comprises shallow-dipping, very low-grade[11] flood basalts, clastic sediments and tuffs which rest unconformably on a deformed 3,500–2,850 Myr old granitoid–greenstone terrain[12], the Pilbara Craton[10]. The age of the Fortescue Group is constrained by: (1) 2,847 ± 34 Myr, Pb–Pb whole rock age from a post-tectonic granitoid in the underlying Archaean craton (U.W.A., unpublished data, revises previous Rb–Sr ages[13,14]). (2) 2,768 ± 16 Myr, U–Pb zircon age from a porphyry with the lower Fortescue Group (R. T. Pidgeon, personal communication) that was originally considered intrusive[15] but is now reinterpreted as having an erosive upper contact (upper Unit 2, East Pilbara Basin, Fig. 2). (3) 2,490 ± 20 Myr and 2,470 ± 30 Myr, U–Pb zircon ages from the overlying Hamersley Group[16]. The basal unconformity

0028-0836/84/080721—03$01.00

© 1984 Macmillan Journals Ltd

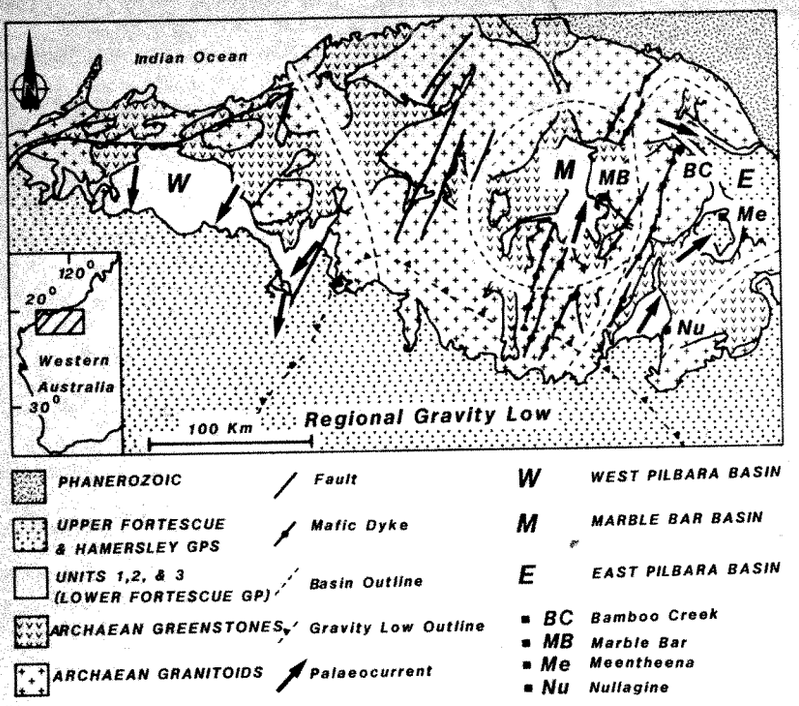


Fig. 1 Location of study area, outline of early Fortescue Group basins and unit 3 palaeocurrents.

(Fig. 2) therefore probably represents a maximum hiatus of ~130 Myr, although the contact between the dated Archaean post-tectonic granitoid and the lower Fortescue Group is not seen.

Three discrete lower Fortescue basins have been recognized, the West Pilbara, Marble Bar and East Pilbara Basins (Fig. 1). These are lithostratigraphically correlated using regional intra-Fortescue unconformities which divide the revised stratigraphy into three major units (Fig. 2). These units sequentially reflect a change in tectonic controls on sedimentation.

Unit 1 comprises a discontinuous basal clastic sedimentary horizon commonly <30 m thick, overlain by up to 2,000 m of subaerial to shallow-water flood basalt. In the Marble Bar Basin, however, up to 500 m of fluvial sandstone and conglomerate exists beneath the basalt. The local derivation of sediment (from Archaean granitoids and greenstones) and converging fluvial palaeocurrents suggest deposition in an enclosed basin centred over greenstones (Fig. 3). Deformation preceded unit 2 sedimentation, resulting in dips of 5–20° towards the centre of the earlier sedimentary basin. Local close to tight folding (dips 50–60°, in places vertical) formed a sinuous central belt of upright, shallow-plunging structures (Fig. 3). This style of deformation is similar to the main upright deformation affecting the underlying greenstone belts which has been assigned to diapiric movements on surrounding granitoids[17].

Unit 2, unconformably overlying Archaean greenstones and unit 1, exists only in the East Pilbara and Marble Bar Basins (Fig. 2). In the East Pilbara Basin it comprises a poorly exposed fluvial and lacustrine sequence overlain by an intrusive and extrusive acid porphyry complex. In the Marble Bar Basin, unit 2 mainly comprises subaerial to shallow-water basaltic lavas with complex interdigitations of sediments near the base. Most of the sediments, which are of granitoid provenance, are preserved as three, up to 80 m thick, upward-coarsening sequences (shale/silt to sandstone/pebble conglomerate) deposited axially in an elongate basin ~15 km wide and at least 40 km long. Initial fluvial sedimentary input was from the north with a later influx from the south-east. The coarsening upward sequences probably resulted from fluvial progradation into a lacustrine environment. The cyclicity of sedimentation suggests an active fault control, although the exact positions of these faults are unclear. Most observed fractures were generated by major post-unit 2 movements. The style of sedimentation, in possibly fault-controlled belts with laterally persistent stratigraphy and a more

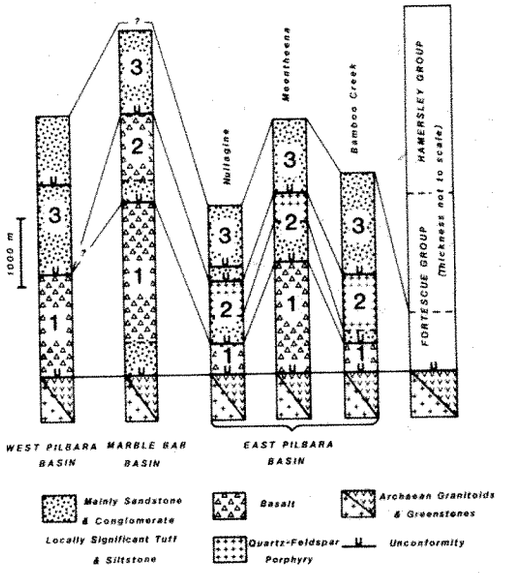


Fig. 2 Stratigraphical position of units 1, 2 and 3 and revised stratigraphy (cumulative maximum thicknesses).

uniform provenance, is in marked contrast to the sedimentation in unit 1, although in both units sediment accumulated over greenstone belts.

Unit 3 is dominated by granitoid derived sandstones with pebble conglomerates and minor coarser conglomerate, except in the West Pilbara Basin where it is mainly composed of epiclastic volcanic sediments. Fluvial palaeocurrents (Fig. 1) indicate axial sediment dispersal in linear troughs centred over greenstone belts. These basins were ~20 km wide and at least 100 km long (satellite imagery suggests the main north–north-east trending East Pilbara Basin structure may have been >250 km long). Fault-controlled local marginal alluvial fan systems (up to 1 km thick) suggest deposition in part in elongated graben structures. Active basin margin faulting apparently became less important towards the top of unit 3, sedimentation patterns remained linear and parallel to the underlying troughs, although basins became significantly wider and marginal sedimentation overlapped earlier growth faults.

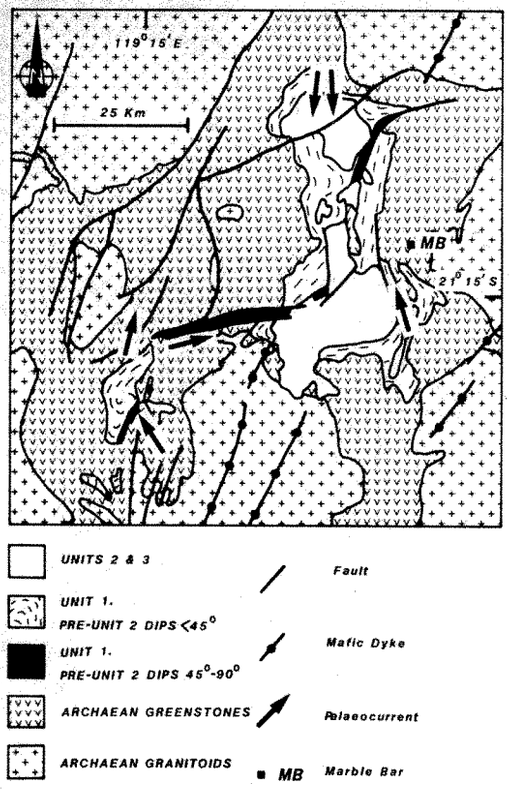


Fig. 3 Marble Bar Basin, unit 1 palaeocurrents and deformation belt.

Unit 3 palaeoccurent data (Fig. 1) indicate a northward palaeoslope in the Marble Bar and East Pilbara Basins and a southward palaeoslope in the West Pilbara Basin. Unit 3 was not deposited between these two regions where units higher in the Fortescue Group overlap the Archaean basement. Bouguer anomalies[18] show a regional gravity low in this area, probably reflecting a preponderance of low density granitoids in the underlying Archaean craton. Such an area would have been prone to gravitational uplift and appears to have been a topographic high during deposition of the lower Fortescue Group[19].

In the East Pilbara large (up to 100 km long and 150 m wide) north–north-east trending undeformed mafic dykes cross-cut Archaean granitoids and greenstones. The relationship between these and the lower Fortescue Group is generally inconclusive, although the latest phases clearly post-date unit 2 and probably unit 3.

The lower Fortescue Group sedimentary stratigraphy reflects an increasing fracture control on sedimentation culminating in >250 km long, mostly north–north-east trending, fractures during unit 3. Initially, sedimentary basin geometry and deformation may have been locally controlled by minor intra-cratonic diapiric movements on Archaean granitoids. The effusion of extensive flood basalts during unit 1 (now preferentially preserved over greenstone belts), however, suggests that basins were initiated under a tensional regime. Fracturing became less important during the upper part of unit 3 and a more regional subsidence is apparent, resulting in the probable unification of all three initial basins during upper Fortescue and Hamersley times. The north–north-east trending mafic dykes (possibly feeders to some of the basalts) cross-cut the Archaean craton irrespective of lithology or structural trends, exploiting the same fracture system that controlled sedimentary basins. This disregard for Archaean crustal trends and the inferred tapping of deep magma sources suggest failure of the lithosphere rather than the crust.

The initiation of Fortescue Group accumulation appears to have been caused by the superimposition of a regional tensional regime on a stabilized Archaean granitoid–greenstone terrain, although inferred minor diapiric movements suggest that some internal mobility remained. A significant lithospheric thickening and associated stabilization during the 130 Myr between the latest Archaean plutonism and initial Fortescue deposition would account for a change from crustal to lithospheric dominated tectonics. Although the causes of such an event remain speculative, a localized rapid decrease in heat flow[20,21] would have such an effect. In this context, it is noteworthy that the proposed period of cratonization ($\leq$130 Myr) is of the order of the present thermal time constant[22]. A rapid local decline in heat flow and subsequent lithospheric thickening and stabilization, as opposed to a secular global decrease, accords with the worldwide diachronous record (3,100–2,500 Myr) of continental cratonization.

I acknowledge financial support from Carpentaria Exploration Co. Pty Ltd and the University of Western Australia. M. J. Bickle, R. Buick, B. Krapez, P. Morant, J. Thornett and R. K. Wright criticised the manuscript.

Received 5 May; accepted 7 December 1983.

1. Cloud, P. *Geol. Soc. S. Afr. Mem. Lect. Ser.* **14,** (1976).
2. McLennan, S. M. & Taylor, S. R. *Geol. Soc. Aust. Abstr.* **9,** 172–173 (1983).
3. Veizer, J. & Compson, W. *Geochim. cosmochim. Acta* **40,** 905–914 (1976).
4. Houston, R. S. & Karlstrom, K. E. *Uranium-Bearing Quartz-Pebble Conglomerates* (University of Wymoming Press, Laramie 1979).
5. Hunter, D. R. *Precambrian of the Southern Hemisphere* (Elsevier, Amsterdam, 1981).
6. Macleod, W. N. *et al. West. Aust. Geol. Surv. a. Rep.* **1962,** 44–54 (1963).
7. Trendall, A. F. *West. Aust. Geol. Surv. a. Rep.* **1978,** 63–71 (1979).
8. Dunn, P. R. *et al. Aust. Bur. Miner. Res. Rec. 1966/72* 172 (1966).
9. Sims, P. K. *Precamb. Res.* **13,** 379–380 (1980).
10. Drummond, B. J. *Bur. Miner. Res. J. Aust. Geol. Geophys.* **8,** 35–51 (1983).
11. Smith, R. E. *et al. J. Petrol.* **23,** 75–102 (1982).
12. Jahn, B. M. *et al. Geochim. cosmochim. Acta* **45,** 1633–1652 (1981)
13. de Laeter, J. R. & Blockley, J. G. *J. geol. Soc. Aust.* **19,** 363–370 (1972).
14. de Laeter, J. R. *et al. West Aust. Geol. Survey a. Rep.* 1974, 73–79 (1975).
15. Hickman, A. H. *Explanatory Notes for Nullagine* 250,000 *sheet* (Geol. Surv. West. Aust., 1978).
16. Compston, W. *et al. Geol. Soc. Aust. Abstr.* **3,** 40 (1981).
17. Hickman, A. H. *Geol. Surv. West. Aust. Bull.* **127,** (1983).
18. *Bur. Min. Res. Geol. Geophys. 1:500,000, Bouguer Anom. Maps.* F50/B2-11, F50/B2-12, F51/B2-5, F51/B2-9 (1972).
19. Horwitz, R. C. & Smith, R. E. *Precamb. Res.* **6,** 293–322 (1978).
20. Tarling, D. H. *J. geol. Soc. Lond.* **137,** 459–467 (1980).
21. Hunter, D. R. *Precamb. Res.* **1,** 295–326 (1974).
22. Parsons, B. & Schlater, J. G. *J. geophys. Res.* **82,** 803 (1977).

Cambrian and Ordovician conodonts from the Robertson Bay Group, Antarctica and their tectonic significance

Clive F. Burrett & Robert H. Findlay

Geology Department, University of Tasmania, GPO Box 252C, Hobart, Tasmania, Australia 7001

The Robertson Bay Group is a thick (>3,000 m) sequence of turbidites deposited in one or more submarine-fan complexes[1–3]. It is exposed throughout the eastern half of North Victoria Land and has been assigned variously to the Upper Precambrian[4] (on the basis of acritarchs and lack of body fossils), or to the Lower Cambrian[5] or Cambrian[2] (partly on the basis of trace fossils). We report here Upper Cambrian and Lower Ordovician conodonts in a limestone block within rocks mapped[3,6] as Robertson Bay Group in the southeastern part of North Victoria Land. This discovery necessitates a reappraisal of tectonic reconstructions of the pre-Mesozoic units of southeast Australia, New Zealand and Antarctica[7–17]. A reconstruction is proposed which includes correlation of the Lower Ordovician Greenland Group, New Zealand and the Upper Cambrian–Lower Ordovician Wierah Formation of southern Tasmania.

During the third German North Victoria Land Expedition (GANOVEX III) in the southern summer 1982–83, 4 kg

© 1984 Macmillan Journals Ltd

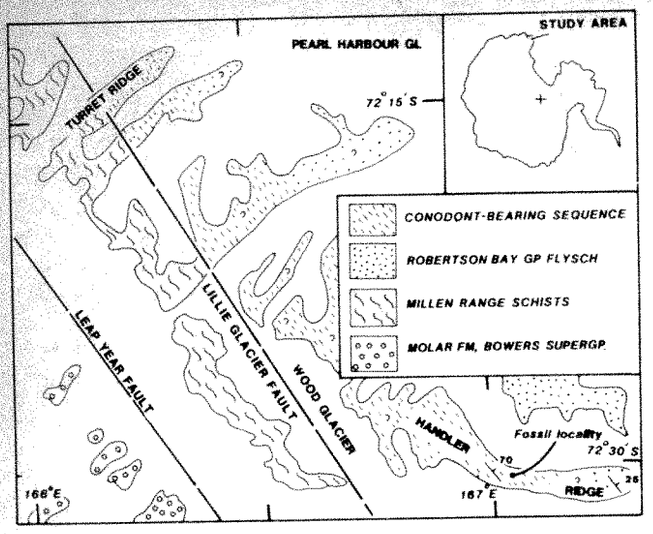


Fig. 1 Geological map of southeast part of North Victoria Land, Antarctica. East–west distance across map is 46 km.

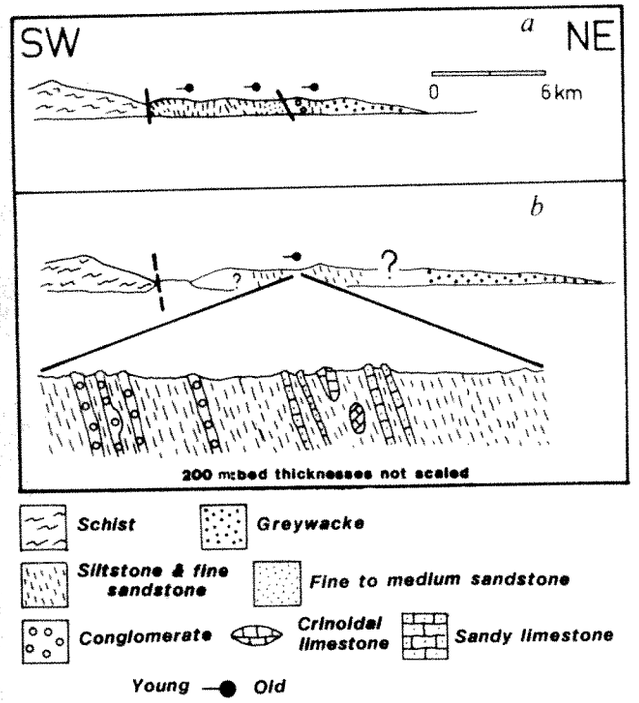


Fig. 2 Diagrammatic cross sections across *a*, Turret Ridge, and *b*, Handler Ridge.

of crinoidal intrabiosparite were obtained at Handler Ridge (Fig. 1) from a 1×0.5 m carbonate block within a sequence dominated by strongly cleaved, fine-grained, siliciclastics interbedded with subordinate, thin, matrix-supported siliceous conglomerate with pebbles of vein-quartz, limestone, chert and fine-grained sandstone and mudstone (Fig. 2). One fist-sized carbonate clast adjacent to the block has yielded a trilobite fauna currently being studied by T. Wright and colleagues in the United States. A similar sequence is exposed on Turret Ridge, some 30 km northwest (Fig. 1). Unfortunately, logistic problems prevented detailed stratigraphic logging of the section but the sequence appears to grade northeast into typical Robertson Bay Group turbidites.

Folds at the fossil locality plunge steeply (60–90°) in contrast to the generally shallowly plunging (0–25°) structures found in the Robertson Bay Group at the east end of Handler Ridge

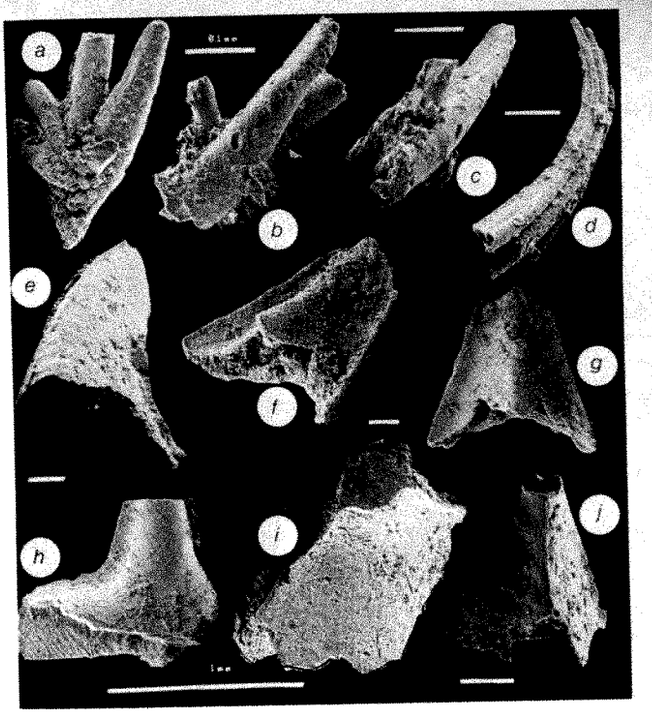


Fig. 3 Conodonts from limestone block within Robertson Bay Group, Handler Ridge. *a–c*, Fused assemblage of four elements of *Proconodontus posterocostatus* Miller 1980; *d*, *'Prooneotodus' tenuis* (Müller 1959); *e*, *Proconodontus* aff. *muelleri* Miller 1969; *f–g*, *Prosagittondontus* aff. *dunderbergiae* Müller 1959; *h–i*, *Iapetognathus* sp.; *j*, *Westergaardodina* aff. *bicuspidata* Müller 1959. Scale bar, 0.1 mm except for *h–i*, 1 mm.

and predominant[18,19] elsewhere in the Robertson Bay Group. However, this contrast is not diagnostic of a tectonic distinction between the rocks at the fossil locality and the more typical Robertson Bay Group rocks to the east, as the folds throughout Handler Ridge possess the same northwest-trending axial plane cleavage. This cleavage occurs throughout the Robertson Bay Group, and is axial plane to primary non-cylindrical folds in Robertson Bay Group rocks on the northeast side of Pearl Harbour Glacier[19] and elsewhere[18].

On acetic acid dissolution, the limestone samples yielded a fauna of paraconodonts, conodontophorids, inarticulate brachiopods and phosphatocopine ostracods. Also present are poorly preserved, indeterminate, asaphid trilobites.

The conodont fauna includes *'Prooneotodus' tenuis* (Müller 1959), *Prosagittodontus* aff. *dunderbergiae* (Müller 1959), *Furnishina* spp., *Problematoconites* sp., *Westergaardodina* aff. *bicuspidata* Müller 1959, *Proconodontus* aff. *muelleri* Miller 1969 and the Lower Trempealeauan zonal index[20], *Proconodontus posterocostatus* Miller 1980. Also present is a compound conodontophorid with an erect, laterally compressed cusp and wide basal cavity which is referable to the Tremadoc genus *Iapetognathus* Landing 1982 (Fig. 3) known from North America and Sweden. On present evidence[20,21] *Proc. posterocostatus* is restricted to the Lower Trempealeau (Upper Cambrian) and *Iapetognathus* to the Tremadoc (Lower Ordovician). The remaining conodonts are mainly Upper Cambrian species though some range into the Ordovician. It is probable that the abundant carbonate intraclasts within the limestone block contain the Upper Cambrian conodonts; judging from its unusually abraded and pitted nature, the *Iapetognathus* may have been reworked also. Therefore the conodont fauna provides only a lower age limit of Lower Tremadoc on the limestone block. An upper age limit of Upper Ordovician is provided by the asaphids[22].

Whole rock K–Ar radiometric dating[23] on slates in the Robertson Bay Group yield ages ranging between 450 and

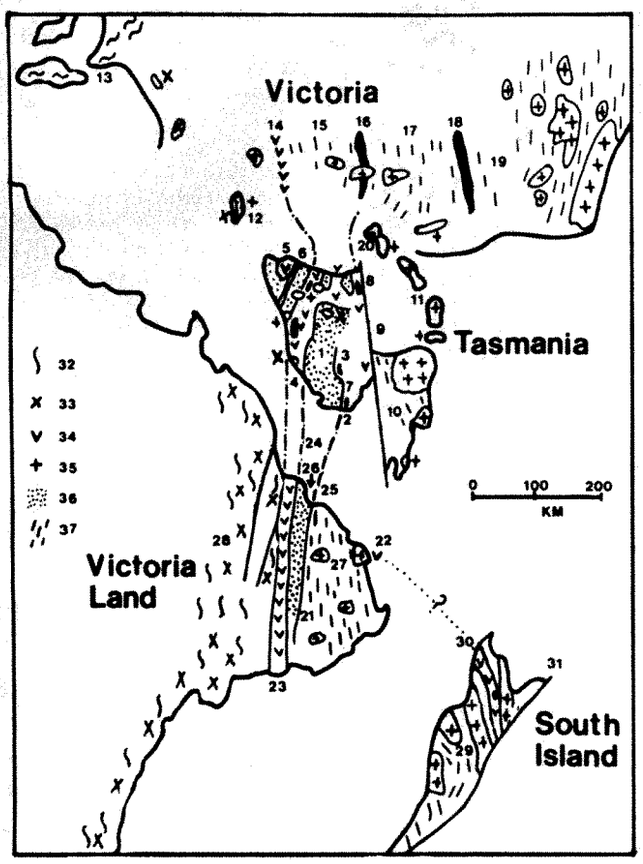


Fig. 4 Possible reconstruction of major blocks prior to Cretaceous break-up of Gondwanaland. 1, Tyennan Block; 2, South Coast Tasmania; 3, Adamsfield Trough with ultramafics; 4, Dundas Trough; 5, Smithton Basin; 6, Arthur Lineament; 7, Lake Edgar–New River Fault; 8, Beaconsfield ultramafic and Cambrian sediments and volcanics; 9, Tamar Fracture; 10, Mathinna Beds; 11, Bassian Rise granites; 12, King Island; 13, Kanmantoo Fold Belt; 14, Stavely Greenstone Belt; 15, Ballarat Trough; 16, Heathcote Greenstone Belt; 17, Melbourne Trough; 18, Mt Wellington Greenstone Belt; 19, East Victorian Metamorphic Belt; 20, Cape Liptrap-Waratah Bay; 21, Handler Ridge; 22, Unger Island volcanics; 23, Bowers Trough; 24, Leap Year Fault, 25, Lillie Glacier Fault; 26, Millen Range schist; 27, Robertson Bay Group; 28, high grade metamorphics, migmatites of the Wilson Group and correlates; 29, Greenland Group; 30, Central Belt of Cambrian volcanics and sediments (N. W. Nelson); 31, Alpine Fault; 32, relatively high grade metamorphics of the Wilson Group in Victoria Land and the Kanmantoo Fold Belt in South Australia; 33, late Precambrian-Ordovician granitoids; 34, Cambrian volcanic and siliciclastic sequences; 35, Devonian–Lower Carboniferous granites; 36, metamorphics of the Tyennan Block and the Millen Range; 37, relatively low grade turbiditic Palaeozoic fold belts of Victoria, Tasmania and New Zealand.

510 Myr. These ages are thought[23] to be metamorphic ages related to the Cambro-Ordovician Ross Orogeny, and therefore the sequence at Handler Ridge can be no younger than 450 Myr (that is, no younger than Llandeilo[24]).

The Robertson Bay Group, a simply-deformed sequence of Lower Ordovician and ?Cambrian turbidites intruded by Late Devonian granites, invites comparison with the Lower Ordovician–Lower Devonian turbiditic Mathinna Beds[25] of eastern Tasmania which are also intruded by Late Devonian granites. However, the Mathinna Beds contain neither conglomerates nor limestones, and a closer similarity appears to be with the recently described Wierah Formation on the south coast of Tasmania[26,27]. This formation overlies disconformably a sequence of turbidites belonging to the Point Vivian Formation. Thin limestone beds from the turbiditic lower part of the Wierah Formation contain an uppermost Cambrian conodont/phosphatocopine ostracod/brachiopod/trilobite fauna.

There is a considerable stratigraphic gap (~500 m) above the Wierah Formation but it is succeeded at Surprise Bay by a deep-water turbiditic carbonate sequence of Caradoc age (Upper Middle Ordovician)[28,29] in contrast to the peritidal carbonates that covered the western half of Tasmania in the Middle-Upper Ordovician.

Sedimentological evidence from the Upper Cambrian and Ordovician sediments on the Tasmanian south coast indicates palaeoslopes towards the south and southeast (refs 28 and 29 and K. Bischoff, personal communication). Derivation of Australian sediments, during the Ordovician, from the Robertson Bay Group, as suggested recently[30], is therefore unlikely. Instead, an Upper Cambrian to Ordovician basin may have existed to the (present day) south of Tasmania, parts of which are now present in southern Tasmania, North Victoria Land, and could be represented also by the Lower Ordovician turbiditic[31] Greenland Group of South Island, New Zealand (Fig. 4).

If the Robertson Bay Group and the Wierah Formation are aligned as in Fig. 4 then the Bowers Trough (no. 23 in Fig. 4), consisting of a Cambrian siliciclastic and volcanic sequence is aligned with the geologically similar Dundas Trough (no. 4 in Fig. 4) in western Tasmania. This alignment has been suggested several times before[8,14,16]. The recently proposed Lillie Glacier Fault[19] in North Victoria Land (no. 25 on Fig. 4) is aligned with the Lake Edgar–New River Fault (no. 7 on Fig. 4) and the newly described[19] schists in the Millen Range (no. 26 on Fig. 4) are aligned with the Precambrian metamorphics of the Tyennan Block (no. 1 on Fig. 4). Eastern Tasmania, along with the Bassian Rise granites (no. 11 on Fig. 4) and the Lower Palaeozoic sequence of Waratah Bay–Digger Island[32] in Victoria (no. 20 on Fig. 4) are displaced along the Tamar Fracture[25] (no. 9 on Fig. 4).

Future work in the Robertson Bay Group will assist delineation of the extent of the Lower Ordovician sediments and their relationships to surrounding areas.

This work results from the opportunity to participate in GANOVEX III, and we thank the expedition leader, F. Tessensohn for permission to publish. We thank T. O. Wright, K. Bischoff, B. Stait, and D. H. Green for their help, and the ARGS for financial support.

Received 17 August; accepted 17 October 1983.

1. Wright, T. *Geol. Jb.* **B41,** 127–138 (1981).
2. Field, B. & Findlay, R. in *Antarctic Earth Science* (ed. Oliver, R.) 123–126 (Australian Academy of Science, Canberra, 1983).
3. Tessensohn, F., Duphorn, K., Jordan, H., Kleinschmidt, G., Skinner, D., Vetter, U., Wright, T. & Wyborn, D. *Geol. Jb.* **B41,** 31–88 (1981).
4. Laird, M. & Bradshaw, J. *N.Z. Antarct. Rec.* **4**(2), 3–10 (1982).
5. Cooper, R., Jago, J., McKinnon, D., Shergold, J. & Vidal, G. in *Antarctic Geoscience* (ed. Craddock, C.) 629–633 (University of Wisconsin Press, Madison, 1982).
6. Le Couteur, P. & Leitch, E. in *Antarctic Geology* (ed. Adie, R.) 229–236 (North-Holland, Amsterdam, 1964).
7. Cooper, R. *N.Z. Jl Geol. Geophys.* **18,** 1–20 (1975).
8. Griffiths, J. *Nature* **234,** 203–207 (1971); **249,** 336–338 (1974); *Bull. geol. Soc. Am.* **88,** 1203–1210 (1977).
9. Laird, M., Cooper, R. & Jago, J. *Nature* **265,** 107–110 (1977).
10. Cooper, R. *J. R. Soc. New Zealand* **9,** 29–84 (1979).
11. Harrington, H. *Nature* **245,** 109–112 (1973).
12. Solomon, M. & Griffiths, J. in *The Tasman Geosyncline,* 19–46 (University of Queensland Press, Brisbane, 1974).
13. Grindley, G. & Davey, F. in *Antarctic Geoscience* (ed. Craddock, C.) 15–29 (University of Wisconsin Press, Madison, 1982).
14. Cooper, R. & Grindley, G. (eds) *Late Proterozoic–Devonian sequences of S.E. Australia, Antarctica and New Zealand and their correlation* (Geological Society of Australia, Sydney, 1982).
15. Jago, J. in *Gondwana Five* (eds Creswell, M. & Vella, P.) 199–204 (Balkema, Rotterdam, 1981).
16. Laird, M. *J. R. Soc. N.Z.* **11,** 425–438 (1982).
17. Quilty, P. in *Antarctic Symposium* 18–40 (Anzaas, Hobart, 1981).
18. Kleinschmidt, G. & Skinner, D. *Geol. Jb.* **B41,** 155–199.
19. Findlay, R. & Field, B. in *Antarctic Earth Science* (ed. Oliver, R.) 107–112 (Australian Academy Science, Canberra, 1983).
20. Miller, J. *Paleontol. Contr. Univ. Kans.* **99,** 1–44 (1980).
21. Fortey, R., Landing, E. & Skevington, D. in *Cambrian-Ordovician boundary* (eds Bassett, M. & Dean, W.) 95–129 (National Museum of Wales, Cardiff, 1982).
22. Moore, R. *Treatise on Invertebrate Paleontology Part O Arthropoda 1.* (University of Kansas Press, Lawrence, 1959).
23. Adams, C., Gabites, J., Wodzicki, A., Laird, M., Bradshaw, J. in *Antarctic Geoscience* (ed. Craddock, C.) 543–554 (University of Wisconsin Press, Madison, 1982).
24. Ross, R., Naeser, C. & Lambert, R. *Am. Ass. petrol. geol. Stud.* **6,** 347–354 (1978).

25. Williams, E. *Tectonophysics* **48**, 159–205 (1978).
26. Spry, A. & Banks, M. (eds) *Geology of Tasmania* (Geological Society Australia, Adelaide, 1962).
27. Berry, R. & Harley, S. *Pap. Proc. R. Soc. Tasmania* **117**, 59–76 (1983).
28. Burrett, C., Stait, B. & Laurie, J. *Mem. Ass. Australas. Palaeont.* **1**, 177–193 (1983). *Geol. Soc. Aust. Abstr.* **9**, 198–199 (1983).
29. Burrett, C., Stait, B., Sharples, C. & Laurie, J. in *Handbook of the Ordovician System* (eds Bruton, D. & Williams, S.) (Universitetsforlaget, Oslo, in the press).
30. Wyborn, D. *Bur. Min. Resour. Geol. Geophs. Yb.* **81**, 12–16 (1982).
31. Laird, M. *N.Z. Jl Geol. Geophys.* **15**, 372–393 (1972).
32. Douglas, J. & Ferguson, J. *Geology of Victoria* (Geological Society of Australia, Melbourne, 1976).

Biomass conversion to liquid fuels: potential of some Arizona chaparral brush and tree species

Edwin A. Davis*, James L. Kuester† & Marvin O. Bagby‡

* Rocky Mountain Forest and Range Experimental Station, Forestry Sciences Laboratory, and † Department of Chemical Engineering, Arizona State University, Tempe, Arizona 85287, USA
‡ Agricultural Research Service, USDA, Northern Regional Research Center, Northern Agricultural Energy Center, Peoria, Illinois 61604, USA

Arizona chaparral brush and tree species can be converted into high-grade liquid hydrocarbon fuels by indirect liquefaction in which pyrolysis is followed by catalytic liquefaction. Here we have examined various Arizona chaparral species and other woody plants for oil and hydrocarbon content and as biomass feedstocks for pyrolytic conversion to high-grade liquid fuels. Although no tested species contained enough oils or hydrocarbons to be considered useful sources, gasification data show that they are all promising biomass feedstocks for pyrolytic conversion to liquid fuels. Olefin yield from biomass gasification, which is an indicator of liquid hydrocarbon yield, may be influenced by feedstock composition. If some particularly outstanding biomass feedstocks were discovered, then manipulation of the vegetation to favour those species could be a management alternative to prescribed burning or chemical control. Because most of the chaparral species tested produced pyrolysis gas of comparable olefin content, however, harvesting of chaparral without regard to species composition is indicated.

Brushlands in the southwestern United States have been viewed as a low-value, relatively unproductive resource at best[1–5], or an extreme fire hazard at worst[6–9]. Attempts have been made to improve the productivity of chaparral and other brushlands using brush control treatments for the purpose of increasing streamflow duration and amount[10,11], augmenting forage for grazing[10,12,13], improving deer habitat[14,15], and reducing the spread of wildfires[16,17]. Under present practices these benefits must justify expenditures for treatment costs. Of the various benefits, increased grazing capacity generates the only return on capital investment. In the process of controlling brush, either through the use of prescribed burning or chemicals, a potentially valuable energy resource is sacrificed. An alternative to this process would be to harvest the brush on a rotation basis as a renewable energy resource for conversion into liquid fuels, densified fuelwood, or energy-rich chemicals.

In the present study, plant material for testing consisted of branches, stems and leaves of mature shrubs. The samples were reduced in a series of grinding steps (chipper, hammer mill and Wiley mill) to pass through a 6-mm screen. Analytical samples were ground still further to pass through a 1-mm screen. Analytical data are reported on an oven-dry basis. The solvent extraction scheme used to characterize the biomass feedstocks in terms of oil, hydrocarbon and polyphenol fractions is one used extensively by the US Department of Agriculture to discover energy-rich plants[18,19].

Test species were evaluated with respect to their oil and hydrocarbon contents in comparison with those of selected reference species (Table 1). None of the brush or tree species ranked with guayule in hydrocarbon content or with gopher plant in oil content. The polyphenol fraction is of interest as an indicator of tannin content and of reactive substances potentially valuable for making adhesives, phenolic resins, antioxidants and other products[20]. Of the species tested, sugar sumac gave the highest polyphenol value (16.3%) which compares favourably with the reference plant, smooth sumac (20.2%), once considered a commercial source of tannin[21]. Black greasewood contained considerably more crude protein than the other species; it compares well with gopher plant, which had the highest value, and with terminal twigs of several deer-browse shrubs[22] even though the greasewood sample contained much more woody material relative to terminal leafy twigs. None of the test species rated well as potential hydrocarbon- or botanochemical-producing species.

Biomass conversion options include biological processes to produce methane or alcohols, and thermochemical processes to produce heat, low- or medium-quality gas, oxygenated pyrolytic oils by direct liquefaction, or high-quality hydrocarbon liquid fuels by indirect liquefaction[23,24]. Thermochemical conversion via indirect liquefaction offers promise for converting biomass to high-grade transportation fuel equivalent to that derived from petroleum; the product would be compatible with existing engine design and the fuel distribution system. Three indirect liquefaction routes for biomass have been reported: (1) Naval Weapons Center (NWC) system[25]; (2) Mobil's system[26,27]; and (3) Arizona State University's (ASU) system[23]. All three systems use a synthesis gas. The NWC recommended an externally heated tubular reactor; Mobil technology did not address the gasification step; and the ASU system uses a fluidized bed with a circulating solid heat transfer system which converts unfractionated synthesis gas to a diesel-type fuel in a catalytic second stage.

A wide variety of feedstocks can be processed in the ASU system to a gas with a heating value of ~500 Btu per scf (standard cubic feet). Because biomass contains very little sulphur, the hydrogen sulphide content of the gas beyond the wet scrubber is negligible. Potential products include medium-quality gas, 1-propanol, paraffinic fuels (diesel, kerosene, jet), and high-octane gasoline. A key step is to tailor the pyrolysis gas composition with respect to carbon monoxide, hydrogen and olefins for use as a synthesis gas for the liquid fuels system.

The capability of the ASU system has been demonstrated with several feedstocks, for example almond prunings (from California orchards) and guayule bagasse (from bushes in Mexico). Composition of the pyrolysis gas is of high fuel value (about 500 Btu scf^{-1}) for all feedstocks, with minor variations in gas composition[28]. The water–alcohol phase for both feedstocks from the liquefaction reactor typically contains ~90% water by weight, 9% 1-propanol, and less than 1% other alcohols. Gas chromatograms of the liquid hydrocarbon products from various feedstocks are very similar in the C_7–C_{17} range and are similar to that of no. 2 diesel fuel; the major difference is the presence of C_7–C_9 compounds in the biomass liquid fuel products[28]. The biomass product should be directly usable as a transportation fuel without distillation. Properties of the undistilled biomass liquid fuel match those of JP-4 jet fuel more closely than those of no. 2 diesel fuel[28].

Factor studies in the laboratory have shown that pyrolysis gas composition is an adequate predictor of the capacity of biomass feedstocks to yield liquid fuel. Indicators of a high-quality pyrolysis gas are high olefin content and a H_2/CO ratio of 1.0–1.5. Most dry biomass feedstocks without water addition yield a H_2/CO ratio of 0.5–0.9. This ratio can be increased to the 1.0-1.5 range (and higher) by adding steam to the pyrolysis reactor fluidization gas to produce hydrogen and to deplete carbon monoxide via the water gas shift reaction ($CO + H_2O \rightarrow H_2 + CO_2$), with the reaction possibly catalysed by ash from the feedstock[28]. The optimal pyrolysis reactor fluidization gas appears to be a combination of steam and liquefaction reactor off-gas to supply higher molecular weight paraffinic hydrocar-

TABLE 1 Biomass feedstock analysis and gasification results

Biomass feedstocks		Feedstock analysis						Operating conditions			Pyrolysis reactor performance					
Species	Common name	Ash (%)	Crude protein (%)	Polyphenol (%)	Oil (%)	Hydrocarbon (%)	Heating value (Btu lb^{-1})	Fluidizing gas (lb h^{-1})	Temp. (°C)	Residence time (sec)	Gas composition (mol%) H_2	CO	CO_2	Paraffins	Olefins	H_2/CO ratio
Range and woodland feedstocks																
Quercus turbinella	Shrub live oak	3.1	4.6	7.4	1.1	0.1	8,524	22	760	5.2	28.0	41.3	4.5	18.0	8.1	0.67
Cercocarpus betuloides	Mountain mahogany	3.1	4.8	8.1	1.2	0.1	9,076	22	749	4.2	27.6	37.8	4.8	16.9	12.8	0.72
Arctostaphylos pungens	Pointleaf manzanita	1.7	2.0	10.4	0.9	0.3	8,444	22	749	5.2	24.5	35.5	10.6	16.3	12.8	0.68
A. pringlei	Pringle manzanita	2.2	2.1	11.4	0.6	0.3	8,775	22	760	4.6	25.0	40.7	6.8	16.4	11.1	0.61
Garrya wrightii	Wright silktassel	3.2	1.8	4.6	2.6	0.2	9,265	22	716	4.7	25.6	38.7	5.4	18.3	11.9	0.66
Rhus ovata	Sugar sumac	5.3	3.7	16.3	2.8	nil	—	22	649	4.1	28.9	31.9	10.6	18.2	10.4	0.90
Larrea tridentata	Creosote bush	3.8	6.4	5.7	0.5	0.1	8,822	22	749	4.5	26.0	39.4	7.7	16.6	10.2	0.65
Prosopis velutina	Velvet mesquite	4.4	6.5	4.8	1.0	0.1	8,507	21	921	3.5	33.0	44.4	5.1	18.5	5.0	0.74
Cupressus arizonica	Arizona cypress	4.5	1.6	9.5	2.0	nil	7,955	22	732	4.6	26.6	38.4	7.0	17.5	10.3	0.69
Juniperus osteosperma	Utah juniper	4.2	3.4	6.7	1.3	0.2	8,589	22	704	4.5	28.8	39.5	6.4	17.9	7.3	0.72
Pinus edulis	Pinyon pine	3.3	2.6	5.0	3.0	0.3	9,060	22	771	5.3	25.8	41.8	4.4	17.6	10.0	0.61
Sarcobatus vermiculatus	Black greasewood	5.0	10.6	2.5	0.6	0.1	—	—	—	—	—	—	—	—	—	—
Reference feedstocks																
Pseudotsuga menziesii	Douglas-fir (bark)	5.1	—	17.2	5.0	0.2	10,076	23	760	4.0	16.6	53.4	3.0	19.7	7.1	0.31
Prunus amygdalus	Almond (prunings)	2.8	—	7.3	0.6	0.3	8,235	22	727	5.2	23.0	44.9	6.6	16.4	9.2	0.51
Parthenium argentatum	Guayule (raw)	5.2	4.3	8.9	2.3	10.4	10,820	20	710	4.6	17.3	35.0	8.5	28.6	10.6	0.49
P. argentatum	Guayule (bagasse)	3.9	3.7	5.9	2.0	4.5	7,838	21	593	6.5	19.7	38.7	7.1	24.1	10.2	0.51
P. argentatum	Guayule (cork)	2.1	6.2	6.6	6.0	2.3	11,376	22	704	7.5	20.6	22.1	3.8	31.1	22.3	0.92
Euphorbia lathyrus	Gopher plant	3.1	12.7	7.6	9.9	0.4	8,725	31	743	6.4	33.0	26.3	7.6	18.7	14.1	1.26
Rhus glabra	Smooth sumac	7.7	7.1	20.2	5.9	0.2	—	—	—	—	—	—	—	—	—	—

The analytical values are based on a moisture-free whole plant basis. Ash and crude protein (calculated as Kjeldahl nitrogen times 6.25) were determined for separate subsamples. The pyrolysis fluidized medium was sand, and the fluidizing gas was recycled pyrolysis gas.

bons for cracking to olefins and hydrogen. Liquid fuel yields of ~40 gallons per ton of biomass can be expected when the pyrolysis gas has an olefin content of ~10 mol%.

A variety of rangeland brush and tree species representative of chaparral, desert shrub and woodlands in Arizona were tested through the gasification step. All biomass feedstocks were tested using recycled pyrolysis gas at 21–22 lb h^{-1} without steam addition. Although these conditions are not considered optimal for maximizing liquid fuel production, a relative assessment of the feedstocks can be made. Reference feedstocks were tested in similar conditions. The olefin content of the pyrolysis gas from 11 range and woodland species varied from 5 to 13 mol% (Table 1). Pointleaf manzanita, birchleaf mountain mahogany, Wright silktassel and Pringle manzanita yielded the greatest amounts of olefins. Velvet mesquite and Utah juniper yielded the smallest amounts of olefins and compare closely with the reference feedstocks, fir bark and almond prunings. Outstanding olefin yield was obtained from the reference feedstock guayule cork, which thus far is superior to all other biomass feedstocks tested, including raw guayule and guayule bagasse. This finding was the basis of the effort to identify high olefin-yielding biomass feedstocks and to correlate chemical composition with olefin yield. Rank correlation analysis did not indicate significant correlation between olefin yield and the other variables. A detailed chemical characterization of guayule cork may prove helpful in identifying plant constituents that correlate with high olefin yields in the pyrolysis gas. This in turn could provide a key to discovering high olefin-yielding plants.

We conclude that relatively unproductive chaparral and woodlands are a valuable renewable energy resource from which high-grade liquid fuels can be produced. Side benefits from harvesting these vegetation types would be similar to the main benefits obtained from prescribed burning: range improvement for cattle and wildlife, temporary increases in water yield, and fire hazard reduction. An important difference, however, is that harvesting the biomass for fuel production recovers the stored solar energy, whereas prescribed burning or chemical control methods waste it. Of course, many situations would occur where, because of slope steepness or terrain, harvesting would be impracticable and burning or chemical control would be the method of choice. Conversions from brush to grass in which the brush is killed by means of chemicals offer many years of increased water yield[10,11], but an associated side effect is an increase in the nitrate concentration of the stream water[29]. Harvesting of resprouting brush should not result in the loss of nitrate from the watershed soil to the stream water because the nitrogen cycle would not be broken as it is when the brush is killed.

The problems associated with a programme of harvesting brush to make fuel are being addressed and prospects for their solution are encouraging[30–34]. The design of an operational mobile biomass conversion system that is self-sustaining with respect to energy is essential for range biomass harvesting to be economically feasible. The California mobile pyrolysis unit is an example of such a unit being built to produce low-Btu gas and pyrolytic oil[35]. However, the primary product of this system is a highly oxygenated oil that would require considerable refining to be useful as a transportation fuel. Research on harvesting rotations for maximum sustained yields and fertilization requirements to replace nutrients lost through harvesting will eventually be needed. Of immediate importance is the demonstration of the operational validity of the concept. The research reported here is just a beginning in that it demonstrates that rangeland brush and trees can be converted into high-grade liquid fuels.

This research was partially supported by the US Department of Energy (contract DE-AC02-76CS40202) and the US Department of Agriculture (grants 59-2043-0-2-094-0 and 59-2043-1-6-062-0).

Received 21 September; accepted 11 November 1983.

1. Minnich, R. A. in *Chaparral for Energy Information Exchange*, 124–130 (U.S. Dep. Agric., 1976).

2. *An Assessment of the Forest and Range Land Situation in the United States*, For. Resource Rep. no. 22 (U.S. Dep. Agric., 1981).
3. Vogl, R. in *Proc. Symp. Dynamics and Management of Mediterranean-type Ecosystems*, San Diego, 561 (Gen. Tech. Rep. PSW-58, U.S. For. Serv., 1982).
4. Leisz, R. R. in *Proc. Symp. Dynamics and Management of Mediterranean-type Ecosystems*, San Diego, 3–5 (Gen. Tech. Rep. PSW-58, U.S. For. Serv., 1982).
5. Johnson, H. in *Proc. Symp. Dynamics and Management of Mediterranean-type Ecosystems*, San Diego, 6–7 (Gen. Tech. Rep. PSW-58, U.S. For. Serv., 1982).
6. Biswell, H. J. in *Proc. Symp. Dynamics and Management of Mediterranean-type Ecosystems*, San Diego, 564 (Gen. Tech. Rep. PSW-58, U.S. For. Serv., 1982).
7. Klinger, K. K. & Wilson, C. C. *Fire Control Notes* **29**, 3–6 (1968).
8. Rothermel, R. C. & Philpot, C. W. *J. For.* **71**, 640–643 (1973).
9. Leisz, D. R. & Wilson, C. C. *J. For.* **78**, 94–95 (1980).
10. Hibbert, A. R., Davis, E. A. & Scholl, D. G. *Rocky Mount. For. Range exp. Stn Res. Pap.* RM-126 (1974).
11. Hibbert, A. R., Davis, E. A. & Knipe, O. D. in *Proc. Symp. Dynamics and Management of Mediterranean-type Ecosystems*, San Diego, 382–389 (Gen. Tech. Rep. PSW-58, U.S. For. Serv., 1982).
12. Cable, D. R. *Rocky Mount. For. Range exp. Stn Res. Pap.* RM-155 (1975).
13. Jordan, G. L. *Range Seeding and Brush Management on Arizona Rangelands*, Tech. Bull. T81121 (Ariz. Agric. exp. Stn, 1981).
14. Reynolds, H. G. in *16th a. Ariz. Watershed Symp.*, Phoenix, Arizona, 10–17 (Ariz. Water Comm. Rep. no. 2, 1972).
15. Holecheck, J. L. *J. Soil Water Conserv.* **36**, 265–269 (1981).
16. Brown, T. C. & Boster, R. S. *Rocky Mount. For. Range exp. Stn Res. Pap.* RM-119 (1974).
17. Green, L. R. in *Proc. Symp. Dynamics and Management of Mediterranean-type Ecosystems*, San Diego, 464–471 (Gen. Tech. Rep. PSW-58, U.S. For. Serv., 1982).
18. Buchanan, R. A., Cull, I. M., Otey, F. H. & Russell, C. R. *Econ. Bot.* **32**, 131–145 (1978).
19. Buchanan, R. A., Otey, F. H., Russell, C. R. & Cull, I. M. *J. Am. Oil Chem. Soc.* **55**, 657–662 (1978).
20. Buchanan, R. A., Otey, F. H. & Bagby, M. O. in *Recent Advances in Phytochemistry* Vol. 14 (eds Swain, T. & Kleiman, R.) 1–22 (Plenum, New York, 1980).
21. Clarke, I. D., Rogers, J. S., Sievers, A. F. & Hopp, H. *U.S. Dep. Agric. Tech. Bull.* no. 986 (1949).
22. McCulloch, C. Y. & Urness, P. J. *Spec. Rep.* no. 3 (Arizona Game and Fish Dep., 1973).
23. Kuester, J. L. in *Biomass as a Nonfossil Fuel Source* Symp. Ser. 144 (ed. Klass, D. L.) 163–184 (Am. Chem. Soc., Washington, D.C., 1981).
24. Kam, A. Y. in *Energy from Biomass and Wastes*, IV, 589–615 (Institute of Gas Technology, Chicago, 1980).
25. Diebold, J. P., Benham, C. P. & Smith, G. D. *Conversion of Organic Wastes to Unleaded High Octane Gasoline* (Final Rep. EPA-IAG-D6-0781, 1979).
26. Meisel, S. L., McCullough, J. P., Lechthaler, C. H. & Weisz, P. B. *Chemtech* **6**, 86–89 (1976).
27. Weisz, P. B., Haag, W. O. & Rodewald, P. G. *Science* **206**, 57–58 (1979).
28. Kuester, J. L. *Conversion of Cellulosic Wastes to Liquid Fuels* Rep. DOE/CS/40202-T5 (U.S. Dep. Energy, 1981).
29. Davis, E. A. in *Proc. Symp. Dynamics and Management of Mediterranean-type Ecosystems*, San Diego, 333–338 (Gen. Tech. Rep. PSW-58, U.S. For. Serv., 1982).
30. Koch, P. & McKenzie, D. W. *J. For.* **74**, 809–812 (1976).
31. Abelson, P. H. *Science* **208**, 1325 (1980).
32. Riggan, P. R. & Dunn, P. H. in *Proc. Symp. Dynamics and Management of Mediterranean-type Ecosystems*, San Diego, 149–157 (Gen. Tech. Rep. PSW-58, U.S. For. Serv., 1982).
33. Toland, J. R. S. in *Proc. Symp. Dynamics and Management of Mediterranean-type Ecosystems*, San Diego, 186–192 (Gen. Tech. Rep. PSW-58, U.S. For. Serv., 1982).
34. *International Bio-Energy Directory* (ed. Bente, P. F. Jr) (Bio-Energy Council, Washington, D.C., 1981).
35. Kosstrin, H. M. in *Proc. Energy Generation and Cogeneration from Wood*, 64–66 (Forest Products Research Society, Madison, Wisconsin, 1980).

Shallow-water sclerosponges on Jamaican reefs and a criterion for recognition of hurricane deposits

Terence P. Scoffin

Department of Geology, University of Edinburgh, Edinburgh EH9 3JW, UK

Malcolm D. Hendry

Department of Geology, University of West Indies, Mona, Kingston, Jamaica, West Indies

We report here that excavation into the *Acropora palmata* rubble on the reef crest at 4 m water depth on Discovery Bay reefs, Jamaica, revealed the presence of numerous sclerosponges (*Ceratoporella nicholsoni*) living attached directly to the coral branches 1–2 m below the rubble surface. The coral branches excavated were deposited during a storm event (in August 1980) and consequently lack the zoned crusts showing a succession from photophilic (light-loving) to sciaphilic (shade-loving) secondary frame-builders which normally develop during slow steady incremental coral branch accumulation. Thus the nature and sequence of encrustations on coral rubble can indicate the speed of its accumulation.

Before 1980 the shallow reef front (0 to 7 m water depth) of the Discovery Bay west fore-reef consisted predominantly of erect, thick branches of living *A. palmata*[1,2]. In August 1980 large waves generated by hurricane Allen struck these reef and the bulk of the *Acropora* corals were broken and m subsequently died. In July 1983, although there had been sc recovery of *Acropora* corals, there was mainly a loose (estimated at 0.5–2 m thick) of dead *A. palmata* rubble occu ing the shallow reef front and extending leeward into ramp exposed up to 1.5 m above sea level. We excavated a hole a water depth of 4 m, into the loose rubble of the reef cres reveal the internal structure of the accumulation and to n the type and nature of encrusting organisms.

The hole excavated was 3 m in surface diameter and 2 m de and free of sediment less than cobble size. The dead *A. palm* plates extracted ranged from 30 to 150 cm long, 15 to 40 wide and 1 to 10 cm thick. They were stacked at all angles most lay sub-horizontally. A depth zonation of encrusting org isms was clearly evident as the hole was deepened to a de representing a vertical stack of 10–15 *A. palmata* branc Table 1 lists the principal calcareous encrusting organi encountered; most were living at the time of examination. organisms found in each 20 cm depth zone are listed in decr ing abundance and recorded for the upper and lower surf of the *A. palmata* plates. A gradual change from photopl to sciaphilic organisms occurs with depth. The overlapping *palmata* plates shield areas beneath from direct light, altho small gaps in the lattice of branches allowed patches of ph philic organisms to survive in the shafts of light penetra down to a maximum depth of ~80 cm below the rubble surf The crusts of calcareous red algae reached thicknesses of 8 at the surface, but below about 30 cm they were less than 2 thick. Of special interest was the occurrence of numer sclerosponges (*Ceratoporella nicholsoni*), maximum diam 6 mm, average diameter 3 mm, in abundances up to 50 100 cm^2 on the undersurfaces of *A. palmata* branches betw 1 and 2 m below the rubble surface. Almost all the organi listed in Table 1 (except some serpulids and cheilost ectoprocts and the thicker crusts of algae near the surface) v growing directly on the dead *A. palmata* plates; they were overlapping other groups of organisms to form composite cr

Although reef-encrusting organisms have differing rate colonization of new substrates[4,5], 3 years is probably enough for the succession to be complete[5]. Thus, the ver sequence of encrusting organisms observed down the excava is probably a stable community which, if undisturbed, w eventually form crusts of constant composition on the c fragments.

During his study of the calcareous encrusting organism the Recent and Pleistocene reefs of Barbados, Martindale[4] n that *A. palmata* fragments were normally coated by a crust to 10 cm thick) consisting of thick photophilic algal layers to the coral substrate, gradually giving way to thinner lam of sciaphilic algae and foraminiferans, bryozoans and serpu Martindale interpreted this common composite successio being a result of the progressive accumulation of *A. pal* branches, causing a gradual reduction of light, and also a re tion in water circulation and a probable increase in turbi The absence of such a sequence of encrustation on the ru of the Discovery Bay reef crest relates to the instantan deposition of the thick pile of fresh coral branches.

Studies of sclerosponge ecology on Jamaican reefs[6–8] shown that these calcareous sponges can tolerate conditio exceedingly low light intensity and also low levels of nut supply. On Jamaican reefs, *C. nicholsoni* grows out in the down to at least 200 m depth, and is the principal frame-bu between 70 m and 105 m depth[8]. Above 60 m depth it grad becomes more cryptic as the twilight zone recedes further the reef, and above 40 m depth it is almost exclusively ca nicolous. The occurrence of many *C. nicholsoni* skel encrusting the fragments of shallow-water corals indicate existence at some previous time of a deep pile of fine sedim free rubble, through which light could scarcely penetrate water could circulate. The juxtaposition of the sclerospong relatively fresh shallow-water coral fragments indicates tha

0028-0836/84/080728—02$01.00

Table 1 Principal calcareous encrusting organisms

Excavation depth	Substrate upper surface	Substrate under surface
0–20 cm	Calcareous red algae (up to 8 mm-thick crusts) *Porolithon* sp., *Lithophyllum* sp., *Neogoniolithon* sp.	Calcareous red algae (crusts <2 mm) *Mesophyllum* sp., *Neogoniolithon* sp. Encrusting foraminiferans *G. plana*, *H. rubrum*
20–40 cm	Platy corals (~10 cm diameter) (*Agaricia agaricites*, *Porites astreoides*), calcareous red algae (crusts <2 mm), *Porolithon* sp., *Lithophyllum* sp., *Neogoniolithon* sp., *Mesophyllum* sp. Delicate branching *Stylaster* sp. corals occur in concave downward-facing bends or joints of *A. palmata* branches	*Neogoniolithon* sp., *Mesophyllum* sp., *G. plana*, *H. rubrum*, *Carpentaria utricularis*, cheilostome ectoprocts, serpulids (?*Hydroides* sp. ?*Filagrana* sp.), thecidioid brachiopods
40–60 cm	*Lithophyllum* sp. *Mesophyllum* sp. *Neogoniolithon* sp. *Gypsina plana* *Homotrema rubrum* Cheilostome ectoprocts *Agaricia agaricites*	*Mesophyllum* sp. *Neogoniolithon* sp. *H. rubrum* Cheilostome ectoprocts *G. plana* Serpulids *C. utricularis*
60–80 cm	*Mesophyllum* sp. Serpulids *H. rubrum* Nestling sponges ?*Orina calcinea*	Cheilostome ectoprocts Serpulids Thecidioid brachiopods *C. nicholsoni* (7 per 100 cm^2)
80–100 cm	*H. rubrum* Cheilostome ectoprocts Serpulids *C. nicholsoni* (2 per 100 cm^2) Solitary coral (?*Astrangia* sp.) Nestling sponges ?*Orina calcinea* ?Rhizamminid foraminifera	Cheilostome ectoprocts Serpulids *C. nicholsoni* (20 per 100 cm^2) *H. rubrum* Thecidioid brachiopods
100–120 cm	Cheilostome ectoprocts *H. rubrum* Serpulids *C. nicholsoni* (4 per 100 cm^2) Solitary corals (?*Astrangia* sp.) ?Rhizamminid foraminifera	Cheilostome ectoprocts Serpulids *C. nicholsoni* (30 per 100 cm^2) *H. rubrum* Thecidioid brachiopods
120–140 cm	Serpulids Cheilostome ectoprocts *C. nicholsoni* (20 per 100 cm^2) Thecidioid brachiopods	*C. nicholsoni* (50 per 100 cm^2) Thecidioid brachiopods Solitary corals (?*Astrangia* sp.) *H. rubrum* Serpulids Cheilostome ectoprocts
140–160 cm	Serpulids *C. nicholsoni* (20 per 100 cm^2) Cheilostome ectoprocts	*C. nicholsoni* (50 per 100 m^2) Cheilostome ectoprocts
160–180 cm	*C. nicholsoni* (5 per 100 cm^2) Serpulids	*C. nicholsoni* (20 per 100 cm^2) Serpulids
180–200 cm	Thin cover of sand-sized sediment Encrustations of platy corals, *A. agaricites* (dead)	*C. nicholsoni* (10 per 100 cm^2) Serpulids

pile of rubble accumulated at one depositional event, otherwise a progressive sequence from photophilic to sciaphilic encrusters, would be expected on each coral fragment.

Presently, in sheltered hollows at the surface of the rubble, 10-cm diameter platy corals (*Agaricia agaricites* and *Porites astreoides*) are attached to, and to an extent binding, the *A. palmata* fragments. Unless broken by another major storm, these platy corals, with time, should be preserved as a crudely horizontal platy encrustation on the top of the storm rubble but beneath further 'calm weather' deposits of *A. palmata*, which, no doubt, will soon cover the reef crest. The occurrence of dead encrustations of platy *A. agaricites* at the base of the excavation suggests that we had reached the top of the previous increment of rubble.

Clearly, the depositional histories of cliff or core sections of coral rubble cannot be completely unravelled by studies of the fragments' texture and disposition alone; the nature and sequence of assemblages of encrusting organisms, however, can help to elucidate the rate of supply of coral branches.

We thank Bruce Greenidge for diving assistance and Henry Reiswig for help with animal identifications.

Received 26 September; accepted 28 November 1983.

1. Kinzie, R. A. III *Bull. mar. Sci. Gulf Carib.* **23**, 93–155 (1973).
2. Woodley, J. D. *Nature* **287**, 387 (1980).
3. Woodley, J. D. *et al. Science* **214**, 749–755 (1981).
4. Martindale, W. thesis, Univ. Edinburgh (1976).
5. Choi, D. R. & Ginsburg, R. N. *Proc. 4th int. Coral Reef Symp., Manila 1981* **2**, 781 (1982).
6. Hartman, W. D. & Goreau, T. F. in *The Biology of the Porifera* Vol. 25 (ed. Fry, W. G.) 205–243 (Academic, London, 1970).
7. Jackson, J. B. C., Goreau, T. F. & Hartman, W. D. *Science* **173**, 623–625 (1971).
8. Lang, J. C., Hartman, W. D. & Land, L. S. *J. mar. Res.* **33**, 223–231 (1975).

Real and apparent movement nulled

R. L. Gregory & J. P. Harris

Brain and Perception Laboratory, Department of Anatomy, Medical School, University of Bristol, Bristol BS8 1TD, UK

It is a controversial issue whether 'real' movement, seen from the successive stimulation of retinal receptors by a moving image, and 'apparent' (phi) movement, which is produced by stimuli separated in space and time, are transmitted by the same or different neural channels. With this question in mind, we have devised ways to oppose real against opposite-direction apparent movement, to see whether they cancel. Cancelling, especially in a variety of conditions, would be expected for a single but not for separate neural movement channels. As we report here, we find that opposed-direction real and apparent movements will cancel, allowing null measurements to be made.

The simplest method is to rotate a sector disk at constant speed, in, say, a clockwise direction, and illuminate it with two sources—flashes of light at a constant rate, and a continuous light. An electronic stroboscope provides short (1 μs) flashes which are set at a repetition rate to make the sector disk rotate apparently backwards (anticlockwise). The continuous light signals the disk's true (clockwise) rotation as for normal viewing. As the stroboscope flashes are short, the retinal images of the

© 1984 Macmillan Journals Ltd

sectors are essentially stationary for each flash, and so only apparent movement is signalled (anticlockwise) while the continuous light signals clockwise real movement. The question is: is there an intensity ratio of the intermittent and continuous lights at which the opposed real and apparent movements cancel? We find that there is a critical balance of intensities at which systematic movement ceases. At this cancelling null point, all that remains is rapid flicker, or a random jiggle. This is phenomenally similar to what happens when two oppositely moving gratings are viewed superimposed, to cancel two real movements. The finding that opposed real and apparent movements can cancel suggests that they are carried by the same neural channel.

At a lower relative intensity of the continuous light, the apparent movement from the stroboscope dominates and the disk rotates anticlockwise; at higher intensities, it rotates clockwise by real movement. The mean luminance, which is not critical, was in the lower photopic range.

As the disk can have any number of equally spaced sectors, and as the apparent movement occurs at several flash rates, there are many independently controllable parameters, which is exceedingly useful. The real edge velocity of the sectors, given by the rotational velocity of the disk, may be varied independently of the apparent movement, as for any rotational velocity this is given by the number of sectors and the flash rate which are independently variable. For some uses, a band of bars is better than a sector disk, which may have its centre blanked off to restrict the range of velocities along the radii of the sectors. The disks were rotated between 10 and 100 r.p.m., as selected with a stepped ratio gear box driven by a synchronous motor.

It is interesting that with large jumps of the flashed sectors, although apparent movement is maintained cancelling does not occur. The appearance is of two oppositely rotating disks passing through each other, both movements being seen, rather than cancelling. Thus, it seems that only 'short-range' movement[1] will cancel for nulling, and that 'long-range' movement[2], which does not cancel, is signalled by different neural channels. This occurs, for example, in the following conditions: six equally spaced white and six black sectors on an 18-cm disk, rotating at 50 r.p.m., viewed from 2 m. Illumination is: flashes of 1 μs at 8 flashes per s, with a peak luminance of about 10 Cd m^{-2}. This flash rate gives the highest attainable apparent velocity in this situation. The displacements of the signalled sector positions exceed the short-range limit for most of their radii. The added constant light is adjusted for of 2 Cd m^{-2}. These rather critical conditions give simultaneous counter-rotations when the apparent and real movements are balanced instead of nulling. When this is viewed with a fourfold reduction of size at the eye, to bring the flash image jumps within short-range movement, it is seen as nulled rather than as opposed movements.

In the more generally holding conditions for nulling, when colour filters are added, to make, say, the stroboscope flashes blue and the continuous light red, nulling still occurs in spite of colour contrast between the opposed real and apparent movements. Following prolonged fixation of this double-colour nulled movement, no significant after-effect appears, either for white light or when the stationary test pattern is viewed with blue or with red light. These observations provide further evidence that the real and apparent movements are being transmitted by the same neural channel, and that the movement channel is independent of the colour systems. This is confirmed by the observation that, following several minutes' fixation of the rotating disk set up with nulled movement, there is no significant movement after-effect. As would be expected, after-effects are seen in the opposite directions to the adapting real or apparent movements following stimulation either side of the null point.

If the nulled movement is viewed through a defocusing lens to blurr the edges of the moving sectors, the null point remains essentially unchanged. However, when viewed through a neutral density filter (or a pin hole), placed before the eyes so that both illuminations are reduced equally, the null point changes. The real movement from the continuous source is now less effective than the apparent movement from the stroboscope—as now it rotates anticlockwise. This occurs dramatically with a filter of less than half a log unit, and the shift of the null point is greater with higher filter factors. The null can be restored by resetting the luminance ratio, so this effect can readily be measured. It is evidently associated with the eye's adaptation level, and so may indicate its integration time, which increases with dark adaptation; for when a bright light is shone into the eyes, producing light adaptation, the null point shifts in the opposite direction to the shift with the dark filter. This effect is greater for low illuminations of the sector disk, while the dark filter is more effective at higher illuminations, which is to be expected for an adaptation effect. The impairment of real movement may be explained in these terms, for increased integration time must impair temporal resolution of the receptors, which will affect the sequential signalling of real movement as the image runs continuously along the receptors, but can hardly affect the signalling of flashes separated beyond the eye's longest integration time, of a few milliseconds.

Nulling can be achieved without a continuous light—by lengthening the stroboscope flashes. This is done with an electromechanical shutter driven by variable-frequency and duration pulses, and arranged to interrupt a projector illuminating the disk. The real and apparent movements can now be cancelled and nulled with no continuous light, by setting the flash duration so that the real movement which is signalled during each flash equals the opposed apparent movement, given by the repetition rate. Nulling is obtained in this way by adjusting the flash duration around 5–10 ms. The dark filter changes this null point, less than for the short flash plus continuous light. In both cases increase in the eye's integration time with dark adaption impairs continuous movement without impairing the apparent movement, at least for these low flash rates of under 10 Hz.

Repeated texture patterns, in place of the sectors, should allow pattern-signalled movement to be compared with edge signalled movement. It has been shown that apparent movement is lost with isoluminant colour contrast for alternating texture patterns, though not for clearly separate features or objects[3]. The movement nulling technique will be used with isoluminant colour contrast displays, both with repeated texture patterns and with clearly separated sectors or dots, to explore these effects of luminance and colour contrast for textures and individual features, both with the short-range movement, which allows cancelling and nulling, and with long-range movement which, as it does not cancel, seems to work by different neural mechanisms from those of short-range apparent and real movement.

Received 15 September; accepted 5 December 1983.

1. Braddick, O. *Vision Res.* **14**, 519–528 (1974).
2. Anstis, S. M. *Phil. Trans. R. Soc.* B**290**, 153–160 (1980).
3. Ramachandran, V. S. & Gregory, R. L. *Nature* **275**, 55–56 (1978).

Induction of the ipsilateral retinothalamic projection in *Xenopus laevis* by thyroxine

Sally G. Hoskins* & Paul Grobstein

Departments of Biology and of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois 60637, USA

Hormones are important in the development of behaviour and there is now abundant evidence that they also affect the morphological development of the nervous system[1–6]. In principle, hormones could act by inducing new patterns of connectivity between widely separated structures in the nervous system or by influencing local connectivity. Most available work documents effects of the latter sort. We present here evidence for

* Present address and address for reprint requests: Department of Biological Sciences, Columbia University, 901 Fairchild Center, New York, New York 10027, USA.

0028–0836/84/080730—04$01.00

the former. Our results indicate that thyroxine, the hormone which causes metamorphosis in the frog *Xenopus laevis*, can induce precociously in a pre-metamorphic tadpole the ipsilateral retinothalamic projection, a retinofugal pathway which normally develops during metamorphosis. Our evidence suggests that the hormone's presence in one eye alone is sufficient to cause axons of some ganglion cells in that eye to grow to targets in the ipsilateral thalamus and to form terminal fields of appropriate morphology. Since the axons of the induced pathway project ipsilaterally, unlike axons in the normal pre-metamorphic tadpole, virtually all of which project contralaterally, our results are also relevant to questions concerning the control of axonal trajectory in the optic chiasm.

The ipsilateral retinothalamic projection in *X. laevis* first appears during metamorphosis[7–9]. In adult frogs, the projection arises primarily from a temporo-ventral subregion of the retina[8,10] and terminates in several nuclei of the rostral and caudal thalamus[11]. We have studied the development of the projection using anterograde transport of horseradish peroxidase (HRP). A complete description of this material is in preparation. Our observations, like those of investigators using ^{3}H-proline[8] and degeneration[7] techniques, show the ipsilateral retinothalamic projection to be a late-developing pathway. Reaction product was either absent or extremely sparse in ipsilateral thalamic terminal zones of tadpoles at stage 54[12], approximately 3.5 weeks old (Fig. 1*b*). In frogs analysed 1 week after the completion of metamorphosis, about 7 weeks old, there was substantial labelling in the ipsilateral terminal fields (Fig. 1*a*).

To determine whether the development of the ipsilateral retinothalamic projection, like other metamorphic changes, depends on thyroxine, we reared tadpoles in 10% Holtfreter's solution and 0.01% propylthiouracil (PTU), beginning at stages 48–50. PTU prevents endogenous production of thyroxine in the thyroid gland by blocking the iodination of tyrosine[13]. PTU-reared tadpoles cease morphological development at stage 54, but remain healthy and continue to increase in size for extended periods[14]. The eyes of PTU-reared animals, like those of stage 54 tadpoles, continue to grow as new cells are added symmetrically to the dorsal and ventral peripheries (Fig. 2). Despite the continuing retinal growth, the ipsilateral projections of PTU-reared stage 54 tadpoles (Fig. 1*c*) remain like those of normal tadpoles at stage 54. When released from PTU, the giant tadpoles complete metamorphosis normally and develop typical ipsilateral retinothalamic projections.

To confirm the thyroxine requirement for development of an ipsilateral projection, we pressure-injected small amounts (3–17 ng) of L-thyroxine in 50 nl Wesson oil into one eye of PTU-reared tadpoles using a calibrated glass micropipette, and continued to house the animals in PTU. Other PTU-reared tadpoles were injected intraocularly with oil alone. Between 3 and 12 weeks after the injection, the optic projection from either the treated or untreated eye was examined by application of HRP to the optic nerve. Some of the tadpoles, particularly those which had received higher doses of thyroxine, advanced slightly in developmental stage during the experimental period, progressing to stages 54–57 (22 cases); other animals, which had received lower doses of thyroxine, or oil alone, remained by all external criteria at the developmental stage characteristic of PTU-reared animals (7 cases).

In 14 of the animals which had advanced slightly in stage, the ipsilateral projections made by the hormone-treated eye were assessed. In 10 of these cases, the optic projection to the ipsilateral thalamus (evaluated by intensity and distribution of label in the thalamic terminal zones as described below) was clearly better developed than that of normal tadpoles at comparable stages (stages 55–57). In several instances, these ipsilateral



Fig. 1 The retinothalamic projection in *X. laevis* 1 week after the conclusion of metamorphosis (*a*), at tadpole stage 54 (*b*) and in a stage 54 tadpole which had been reared in PTU for 25 weeks (*c*). We applied crystals of horseradish peroxidase (HRP) to the central stumps of severed optic nerves. 24–48 h later, the animals were killed and the brains prepared for sectioning. Frozen sections (40 μm) were cut on a sliding microtome, and processed using 0.5% cobalt chloride and 1% diaminobenzidine (DAB) solutions for histochemical demonstration of the HRP reaction product[22]. Photomicrographs are of transverse sections through the rostral thalamus at the level of the nucleus of Bellonci, the largest thalamic terminal field, in animals in which one optic nerve was exposed to HRP. In all three cases, reaction product is present in the optic tract (ot) and in the nucleus of Bellonci (nb; solid arrows) on the contralateral (contra) side of the brain. In the post-metamorphic case, but not in the two tadpoles, reaction product is also evident in the ipsilateral (ipsi) nucleus of Bellonci (nb; open arrows). Scale bars: 100 μm.

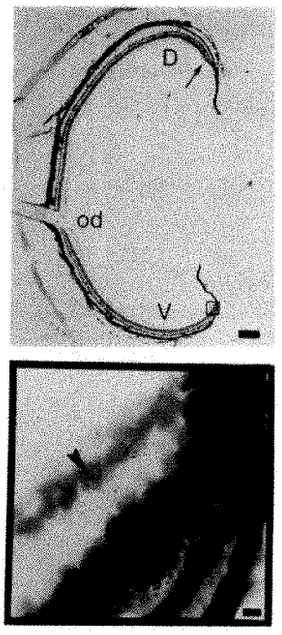


Fig. 2 Retinal growth during PTU-rearing. This PTU-reared tadpole, whose ipsilateral retinothalamic projection is illustrated in Fig. 1*c*, received an intra-abdominal injection of ^{3}H-thymidine (5 μl, specific activity 81.6 Ci mmol^{-1}, NEN), and was subsequently returned to PTU. Eleven weeks later, HRP was applied to one optic nerve of the animal, and its retinal projection was analysed. At this time, the eyes were fixed separately, sectioned at 10 μm and processed for autoradiography. The upper part of the figure is a parasagittal section through one retina, taken at the level of the optic disk (od). Dorsal (D) and ventral (V) are indicated. The lower part of the figure is a higher magnification view of the boxed region of the ventral periphery, showing ^{3}H-thymidine-labelled retinal ganglion cells (arrowhead) peripherally and unlabelled cells centrally. The location of a similar border in the dorsal retina is indicated by the arrow in the upper part of the figure. Scale bars: 100 μm (upper), 10 μm (lower).

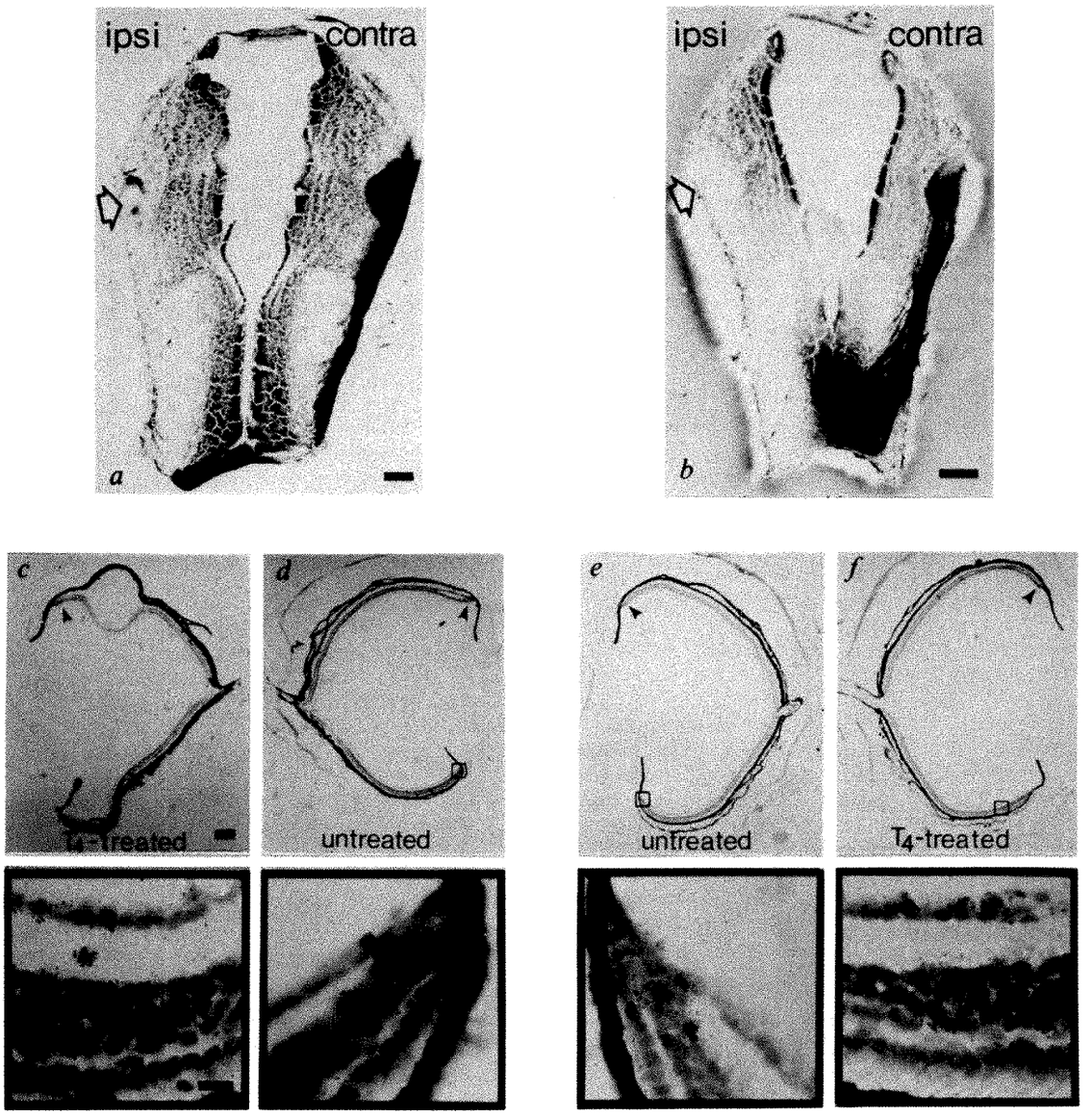


Fig. 3 Effects of injecting thyroxine into one eye of PTU-reared tadpoles. Each animal received an intraocular injection of thyroxine within 24 h of a previous intra-abdominal injection of ^{3}H-thymidine, like that described in the legend to Fig. 2. Photomicrographs are of brain and retinal sections oriented like those of Figs 1 and 2. The sections on the left are from a stage 54 PTU-reared tadpole in which the optic projection from the thyroxine-treated (T_4-treated) left eye was examined following 3 weeks of further rearing in PTU. Sections on the right are from a similar case in which the right eye was thyroxine-treated and the projection from the untreated left eye was examined following 3.5 weeks of further rearing in PTU. The thyroxine-treated left eye developed an ipsilateral projection (open arrow in *a*), while the untreated left eye did not (open arrow in *b*). In both cases, the thyroxine-treated eye added more cells ventrally than dorsally (*c*, *f*), while the untreated eye grew symmetrically (*d*, *e*). As in Fig. 2, arrows and boxes in the low-power retinal sections indicate the locations of ^{3}H-thymidine labelling borders as well as the regions which are shown in the higher magnification views. Scale bars: *a*–*f*, 100 μm; insets, 10 μm.

projections resembled those of postmetamorphic frogs. Four of the tadpoles in this group had ipsilateral projections which were not enhanced, but instead had developed to the extent typical of the animals' morphological stages.

Optic nerve projections from the other, untreated eye were assessed in the remaining eight thyroxine-treated tadpoles which had progressed to stages 55–57. In seven such cases, the ipsilateral projections from the other, untreated eye were those typical of normal untreated tadpoles at stages 55–57. A single stage 56 animal of this group had an enhanced ipsilateral projection. The three tadpoles injected with oil alone showed no change in developmental stage during the experimental period, and the ipsilateral projections from the oil-injected eyes were those characteristic of stage 54 tadpoles. Thus, injections of thyroxine and oil, but not of oil alone, can cause the development of advanced retinal projections to the ipsilateral thalamus. Eyes treated directly with thyroxine were much more likely to produce enhanced projections (10 of 14 cases) than were the untreated eyes of similar tadpoles (1 of 8 cases). These results clearly indicate that exogenous thyroxine can cause development of an ipsilateral projection in tadpoles in which the endogenous production of thyroxine has been prevented[9]. They also suggest that thyroxine acts directly on the eye, since the development of the ipsilateral projection was more advanced in thyroxine-treated than in non-treated eyes, and raise the possibility that a local action of thyroxine may be sufficient to cause development of the projection.

The thyroxine-treated animals which remained at morphological stage 54 throughout the experimental period also produced enhanced projections to the thalamus ipsilateral to the thyroxine-treated eye, providing more direct evidence that local hormone action may be sufficient to induce the projection. We analysed the projection from the thyroxine-treated eye in each of three tadpoles which remained at stage 54 for 3–4 weeks

following injection of 3.75 ng of thyroxine into one eye. Figure 3*a* is a transverse section through the brain of one of these animals, taken at the level of the nucleus of Bellonci (NB), the largest and most heavily innervated terminal field in the ipsilateral thalamus of adult frogs. The distribution of reaction product within this nucleus in the thyroxine-treated case illustrated is similar to that found in the terminal fields of young post-metamorphic animals (Fig. 1*a*), being most dense dorsally and medially with a less densely labelled core. Such a differential distribution of reaction product is not seen in ipsilateral thalamic terminal zones of normal developing tadpoles until stage 60, at which time the overall density of reaction product is still quite low[9]. Similar dense, differentially distributed deposits of reaction product were seen in the ipsilateral NBs of the other two tadpoles in this group. Reaction product was also found in the other usual ipsilateral terminal zones, including the uncinate field, which is not normally innervated until stage 64 (ref. 9).

In a fourth, similarly treated animal which remained at stage 54 following intraocular injection of thyroxine, we analysed the projection from the untreated eye. The projection was typical of that seen in normal tadpoles at stage 54 (Fig. 3*b*). Label was absent in all ipsilateral terminal zones except NB where there was extremely sparse labelling similar to that sometimes seen in normal tadpoles at stage 54 as well as in PTU-reared animals, both untreated and oil-injected.

Beginning at stage 55, the eyes of normally developing *X. laevis* tadpoles undergo a shift in the pattern of cell proliferation, resulting in greatly increased addition of cells to the ventral as compared with the dorsal retinal periphery. This shift can be elicited precociously if thyroxine is injected into the eye of younger tadpoles[15]. The presence or absence of an altered pattern of retinal histogenesis thus provided an additional means of testing whether the action of thyroxine was localized.

The retinas of the thyroxine-treated eyes underwent the expected shift in pattern of histogenesis during the 3.5-week period of the experiment, while those of the untreated eyes did not (Fig. 3*c–f*). Dense concentrations of silver grains mark the cells which underwent their final mitotic division in the presence of the ^{3}H-thymidine label and hence formed the border of the retina at the beginning of the experimental period. In the ventral retina of thyroxine-treated eyes (Fig 3*c*, *f*, and insets), this grain border was displaced centrally as new cells were added during the experimental period. In the dorsal retina, few new cells were added, and the grains remained near the periphery. In the untreated eyes (Fig 3*d*, *e*) of the same tadpoles, in contrast, both dorsal and ventral grain borders remained symmetrically and peripherally located, indicating that the histogenetic pattern of these untreated eyes did not change during the experimental period. The distribution of silver grains in the retinas of such untreated eyes was like that seen in both eyes of PTU-reared, untreated tadpoles.

The production of ipsilateral terminal fields like those of post-metamorphic frogs in the brains of pre-metamorphic tadpoles is a striking example of the hormonal induction of a neuronal pathway. That hormonal induction of the ipsilateral projection was not observed in an earlier, unpublished study (cited in ref. 8) may have been due to the use of triiodothyronine pellets, rather than thyroxine injections. Our evidence that an ipsilateral projection from one eye can be induced in the absence of many if not all of the metamorphic changes normally occurring when the projection develops, also has interesting implications for understanding what controls the patterns of projection made by axons at the optic chiasm.

The induction of an ipsilateral projection from one eye in the absence of a similar projection from the other clearly indicates that interactions between corresponding fibres from the two eyes[16] are not essential for the development of an ipsilateral projection (see also refs 8, 10). The finding also makes it unlikely that the appearance of an ipsilateral projection can be accounted for simply in terms of a critical change at the chiasm since if this were the case enhanced projections should have been seen from both eyes of hormone-treated tadpoles. In general, our findings suggest that the primary change responsible for the development of the ipsilateral projection is a change in the cells of the eye itself. By three criteria (external morphological stage, patterns of retinal histogenesis, and projections of the untreated eye) the action of thyroxine in the animals which remained at stage 54 was restricted to the treated eye.

Both normally and in the experimental situation described here, the development of an ipsilateral projection is correlated with an asymmetric pattern of proliferation of ganglion cells, during which growth occurs primarily in ventral retina[17–19]. Retrograde transport and birthdating studies in normal *X. laevis* indicate that the vast majority of the ipsilaterally-projecting retinal ganglion cells of post-metamorphic frogs lie in retinal regions produced late in development, after the retina has begun to proliferate asymmetrically[20]. The region of the retina which will exhibit enhanced growth is, however, apparently determined much earlier, during embryonic stages. If eye rudiments are rotated or grafted at stages well before stage 54, both the ipsilateral projection[8] and the ventral growth burst[21] are later found to originate from ganglion cells descended from the region previously situated temporoventrally. The ipsilateral projection of normal frogs, then, is formed during metamorphosis by neurones whose eventual fate was determined during embryogenesis. This suggests that the role of thyroxine in the development of the ipsilateral retinothalamic projection is twofold: to produce a new population of ganglion cells, and to permit the expression of a previous determination, one which causes the axons of some of these cells to project ipsilaterally at the optic chiasm. Whether the two events are causally related is a question warranting further investigation.

We thank Drs Héctor Cornejo, R. W. Guillery and Margaret Hollyday for reading and commenting on the manuscript, J. Heberle for help with figures, and Richard Critchlow, Lisa Won and Lee Zwanziger for animal care. This work was supported by NSF BNS 791422.

Received 15 September; accepted 28 November 1983.

1. Jacobson, M. *Developmental Neurobiology* 219–249 (Plenum, New York, 1978).
2. Goy, R. W. & McEwen, B. S. *Sexual Differentiation of the Brain* 1–211 (MIT, Cambridge, 1980).
3. Kollros, J. J. in *Metamorphosis, A Problem in Developmental Biology* 2nd edn (eds Gilbert, L. I. & Frieden, E.) Ch. 13 (Plenum, New York, 1981).
4. Morgan, B. L. G. in *Brain and Behavioral Development* (eds Dickerson, J. W. T. & McGurk, H.) Ch. 4 (Surrey, London, 1982).
5. Konishi, M. & Gurney, M. E. *Trends Neurosci.* **5**, 20–23 (1982).
6. Truman, J. W. & Levine, R. B. *Current Methods in Cellular Neurobiology* (eds Baker, J. & McElvy, J.) (Wiley, New York, in the press).
7. Khalil, S. H. & Szekely, G. *Acta biol. sci. hung.* **27**, 253–260 (1976).
8. Kennard, C. *J. Embryol. exp. Morph.* **65**, 199–217 (1981).
9. Hoskins, S. G. & Grobstein, P. *Soc. Neurosci. Abstr.* **7**, 289 (1981).
10. Hoskins, S. G. & Grobstein, P. *Soc. Neurosci. Abstr.* **6**, 647 (1980).
11. Levine, R. L. *J. comp. Neurol.* **189**, 1–29 (1980).
12. Nieuwkoop, P. D. & Faber, J. *Normal Table of* Xenopus laevis (*Daudin*), 163–188 (North-Holland, Amsterdam, 1967).
13. Green, W. L. in *The Thyroid* (eds Werner, S. C. & Ingbar, S. H.) Ch. 4 (Harper & Row, New York, 1971).
14. MacLean, N. & Turner, S. *J. Embryol. exp. Morph.* **35**, 261–266 (1976).
15. Beach, D. H. & Jacobson, M. *J. comp. Neurol.* **183**, 615–624 (1979).
16. Lund, R. L., Cunningham, T. J. & Lund, J. S. *Brain Behav. Evol.* **8**, 51–72 (1973).
17. Hollyfield, J. G. *Devl Biol.* **24**, 264–286 (1971).
18. Jacobson, M. *Brain Res.* **103**, 541–545 (1976).
19. Beach, D. H. & Jacobson, M. *J. comp. Neurol.* **183**, 603–614 (1979).
20. Hoskins, S. G. & Grobstein, P. *Soc. Neurosci. Abstr.* **8**, 513 (1982).
21. Beach, D. H. & Jacobson, M. *J. comp. Neurol.* **183**, 625–632 (1979).
22. Adams, J. C. *J. Neurosci.* **2**, 141–145 (1977).

Evolutionary relationships of ratites and carinates: evidence from ontogeny of the tarsus

C. McGowan

Department of Vertebrate Palaeontology, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada M5S 2C6

de Beer[1] proposed that ostriches and their allies, the ratites, are degenerate birds that evolved, neotenously, from carinates (flying birds). Others[2–5] have argued for their primitiveness, but the view that ratites are derived within birds has prevailed[6–8]. The problem is soluble only when avian phylogeny

0028-0836/84/080733-03$01.00

© 1984 Macmillan Journals Ltd

is understood, and Ostrom[9] makes a strong case for the evolution of birds from theropod dinosaurs. Included in his evidence was the ascending process of the astragalus, a feature first shown by Huxley[2] to be unique to theropods and birds (Fig. 1). Ostrom's critics[10], however, argue that the avian process is not associated with the astragalus, being instead an independent ossification, the pretibial bone[11]. The present ontogenetic study reveals both processes in birds: the ascending process exemplified by ratites and the pretibial bone by carinates. This implies that, since carinates possess the unique pretibial bone, they could not have given rise to the ratites, which are primitive for this feature.

Three series of embryos were obtained (at daily intervals), for the common quail (*Coturnix coturnix*) and for the chicken (*Gallus domesticus*), and these were double stained with Alizarin red and Alcian blue[12]. In addition, developing chickens, emus (*Dromaius novaehollandiae*) and ostriches (*Struthio camelus*) were monitored radiographically. Data were also obtained from X rays, embryos and immature skeletons for the ostrich, emu, kiwi (*Apteryx australis*), cassowary (*Casuarius casuarius*), red-winged tinamou (*Rhynchotis refescens*), brush-land tinamou (*Nothoprocta cinerascens*) and various carinates, specified below. Ontogeny in carinates will be considered first, followed by that of ratites.

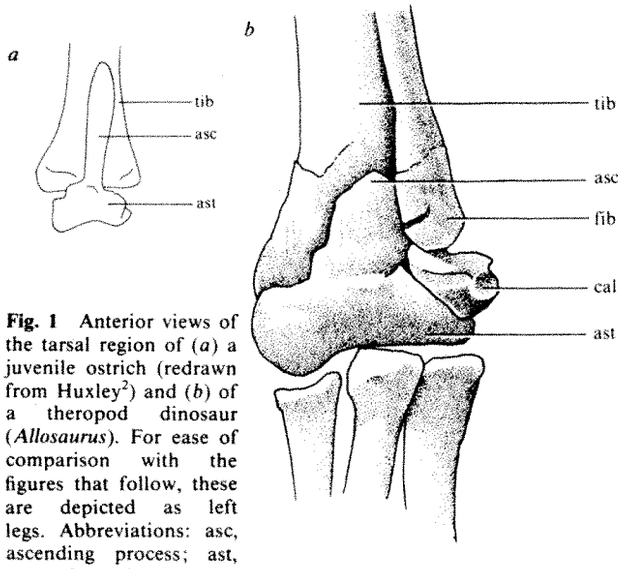


Fig. 1 Anterior views of the tarsal region of (*a*) a juvenile ostrich (redrawn from Huxley[2]) and (*b*) of a theropod dinosaur (*Allosaurus*). For ease of comparison with the figures that follow, these are depicted as left legs. Abbreviations: asc, ascending process; ast, astragalus; cal, calcaneum; fib, fibula; tib, tibia. Note that although the ascending process arises from the astragalus, it lies predominantly on the lateral aspect of the tibia.

Before ossification of the tarsus, which began on day 12 in one of the quails, and day 15 in the chickens, the distal end of the tibia is entirely cartilaginous. There are prominent lateral (calcaneal) and medial (astragalar) condyles, joined proximally, with a cartilaginous spur projecting proximally from the former (Fig. 2*a*; s). This spur, which is the precursor of the pretibial bone, begins ossifying from its tip (Fig. 2*b–d*; pre). Several days later the astragalus and calcaneum begin to ossify. These ossifications appear as small nodules embedded in their respective condyles (Fig. 2*e*; ast, cal). The astragalus is initially about twice as large as the calcaneum in both species. The pretibial bone eventually fuses with the (ossified) calcaneum; in the chicken this occurs some 40 days after hatching (Fig. 2*f*).

Similar relationships between the pretibial bone and calcaneum have been seen in stained embryos of the ring-billed gull (*Larus delawarensis*) and in radiographs of the Adelie penguin (*Pygoscelis adeliae*), common shag (*Phalacrocorax aristotelis*), common starling (*Sturnus vulgaris*), Canada goose (*Branta canadensis*), common eider (*Somateria mollissima*), mallard (*Anas platyrhynchos*), red-headed duck (*Aythya americana*), snow goose (*Chen caerulescens*) and the Mikado pheasant (*Syrmaticus mikado*). Caution is required in drawing conclusions from radiographs alone, but it seems that tarsal development in carinates is uniform.

Huxley[2] was sure that the ascending process of the ostrich was part of the astragalus, but this can be established only if the fate of the calcaneum is known. The youngest ostrich examined, a near-hatching embryo, has a prominent and well-ossified ascending process, expanded distally into a disk of bone occupying about half the width of the distal end of the tibia (Fig. 3*a*; asc, dsc). The disk is separate from, but in contact with, the medial condyle, the latter being largely ossified. The lateral condyle, with which the medial condyle is continuous, is still almost entirely cartilaginous, and is drawn up into a process which appears to be homologous with the precursor of the carinate pretibial bone (Fig. 3*a*; s). However, in marked contrast to carinate ontogeny this process does not ossify later. At this stage the ascending process cannot be said to be part of either astragalus or calcaneum. Radiographs of two 5-week-old juveniles show that the ascending process becomes contiguous with the astragalus, the identity of the astragalus being confirmed by the appearance of a small calcaneum lying lateral to it (Fig. 3*b*; cal). By the 11th week the calcaneum has fused with the astragalus (Fig. 3*c*). Three emu chicks, X-rayed weekly from 4 to 12 weeks after hatching, each showed a putative astragalus,

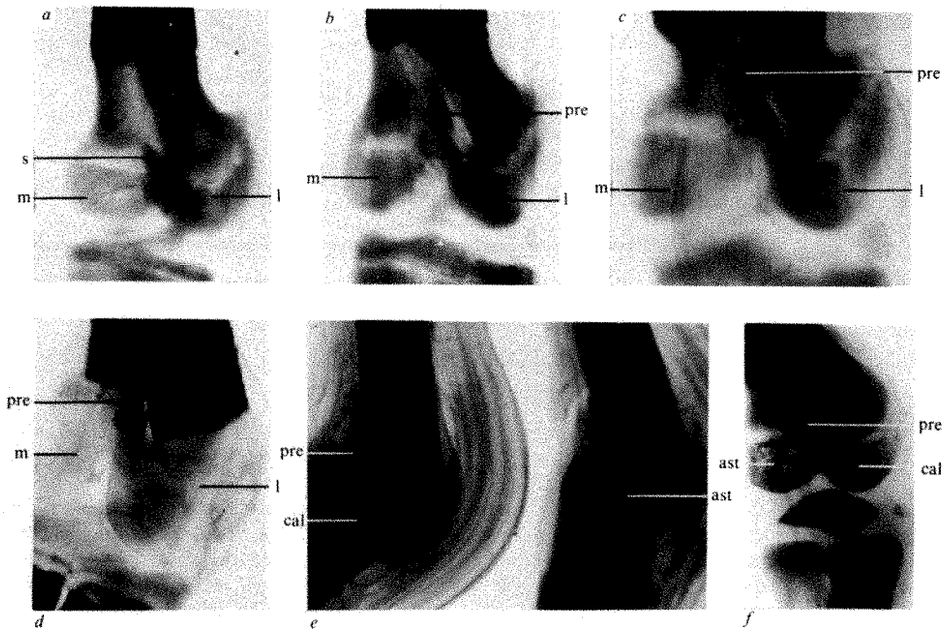


Fig. 2 Development of the carinate tarsus. Left leg depicted, unless otherwise stated. *a*, *b*, 12-day-old quail embryos, anterolateral views; *c*, 14-day-old quail embryo, anterolateral view; *d*, 19-day-old chicken, anterolateral view; *e*, 16-day-old quail, lateral view of left leg and medial view of right leg; *f*, sub-adult chicken, 40 days after hatching, anterolateral view. Abbreviations as in Fig. 1 with additions: l, lateral condyle; m, medial condyle; pre, pretibial bones; s, cartilaginous spur.

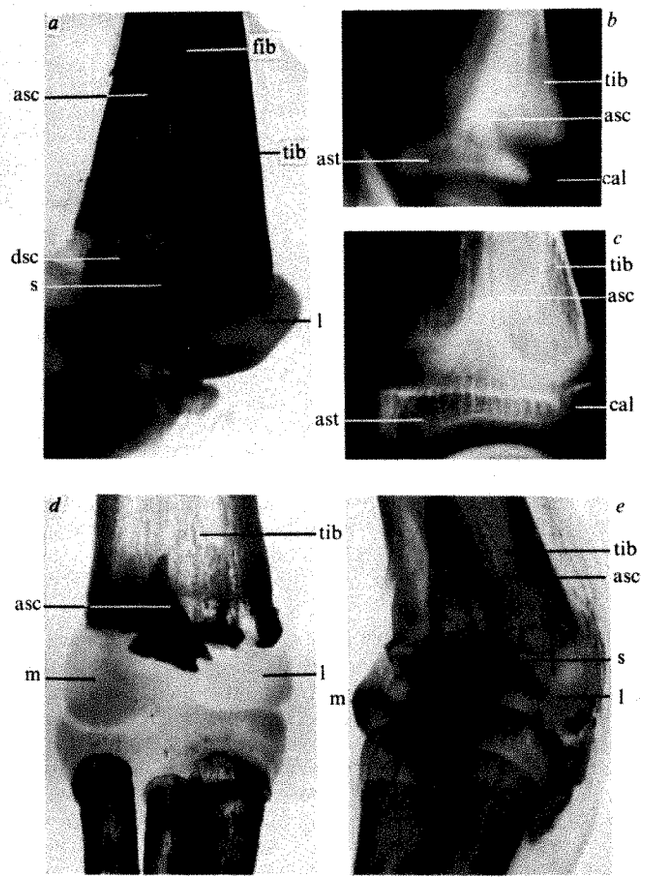


Fig. 3 Development of the ratite and tinamou tarsus. Left legs depicted. *a–c*, Ostrich; *d*, *e*, red-winged tinamou. *a*, Ostrich embryo shortly before hatching, anterolateral view; *b*, *c*, X rays of 5-week-old and 11-week-old juvenile ostriches, anterior views; *d*, late tinamou embryo, shortly before hatching, anterior view; *e*, hatchling tinamou, approximately 1 week old, anterolateral view. Abbreviations as in Fig. 2, with addition: dsc, distal disk of ascending process.

with an ascending process, but no evidence of an ossified calcaneum. However, in one of four stained, near-hatching embryos, there is a small ossified calcaneum, confirming the identity of the astragalus. Radiographs of six embryonic and five subadult kiwis show a prominent ascending process but, as in the emu X rays, no evidence of an ossifed calcaneum. Parker[13], however, showed that the diminutive calcaneum had become incorporated into the astragalus, with its associated ascending process, prior to ossification. Evidence for the cassowary and rhea is presently incomplete, but radiographs show a putative astragalus with a prominent ascending process, as in the other ratites. Holmgren's[14] avian studies focusing on the earlier (precartilaginous) stages of development, are of limited interest here; however, he drew the erroneous conclusion that ankle development in the ostrich scarcely differed from that in the chicken, gull and duck.

Tinamous possess a mosaic of ratite and carinate features and it is therefore important to consider their tarsus. Examination of late embryos of the red-winged tinamou reveals a prominent bony ascending process lying midway between the (conjoined) lateral and medial condyles (Fig. 3*d*; asc). There are no other ossifications at this stage. In a week-old hatchling a prominent spur can be seen arising from the lateral condyle, similar to that seen in the ostrich (Fig. 3*e*; s). There is no evidence of a calcaneal ossification, but there is a small astragalus. The ascending process of the red-winged tinamou is, therefore, not an ossification of the spur from the lateral condyle, and so is not homologous with the pretibial bone of carinates but corresponds instead to the ratite condition.

There are therefore two different tarsal conditions in birds. Carinates have a pretibial bone, an ossification of a cartilaginous spur which fuses with the calcaneum. This is a synapomorphy for carinates, implying that they did not give rise to the ratites, contrary to de Beer's[1] hypothesis. Ratites (including tinamous) have an ascending process, an ossification which fuses with the astragalus, as in theropods[15]. The ratite tarsus is, therefore, in the primitive condition. Indeed, most of the features that distinguish ratites from carinates are now considered to be primitive rather than derived[3–5,16]. They include the palaeognathous palate[17,18], a feature that has been used as evidence for neoteny[19], albeit on slender evidence[6]. Ratites should therefore be viewed as primitive rather than as derived birds. This does not imply that ratites descended directly from a stage in bird ancestry before the power of flight had been acquired, only that their ancestral stock was more primitive than carinates, a point made elsewhere[16,20].

I thank the Metropolitan Toronto Zoo, especially K. Mehren and E. Smith; for supplying specimens, C. Edwards for alizarin preparations, C. Gilmore for histological preparations, J. Mulock for Fig. 1, P. Purves for typing, A. J. Baker, R. Johnson and S. L. Olson for valuable discussions and criticisms of the manuscript, J. H. Ostrom and A. J. Charig for criticisms of the manuscript. Research was supported by Natural Sciences and Engineering Research Council of Canada grant A 9550.

Received 29 September; accepted 24 November 1983.

1. de Beer, G. *Bull. Br. Mus. nat. Hist. Zool.* **4,** 59–70 (1956).
2. Huxley, T. H. *Q. Jl Geol. Soc.* **26,** 12–31 (1870).
3. Lowe, P. R. *Proc. zool. Soc. Lond.* **1928,** 185–247 (1928).
4. Lowe, P. R. *Ibis* **5,** 398–432 (1935).
5. Friant, M. *Acta anat.* **39,** 300–328 (1959).
6. Bock, W. J. *Proc. Int. orn. Congr.* **13,** 39–54 (1963).
7. Cracraft, J. *Ibis* **116,** 494–520 (1974).
8. de Beer, G. in *A New Dictionary of Birds* (ed. Thomson, A. L.) 681–685 (Nelson, London, 1964).
9. Ostrom, J. H. *Nature* **242,** 136 (1973); Ostrom, J. H. *Q. Rev. Biol.* **49,** 27–47 (1974); Ostrom, J. H. *A. Rev. Earth planet. Sci.* **3,** 55–57 (1975); Ostrom, J. H. *Proc. Cent. natn. Rech. Sci.* **218,** 519–532 (1975); Ostrom, J. H. *Biol. J. Linn. Soc.* **8,** 91–182 (1976).
10. Martin, L. D., Stewart, J. D. & Whetstone, K. N. *Auk* **97,** 86–93 (1980).
11. Morse, E. S. *Ann. Lyceum Nat. Hist.* **10,** 141–158 (1874).
12. Watson, A. G. *Anat. Rec.* **187,** 743 (1977).
13. Parker, T. J. *Phil. Trans. R. Soc.* **182,** 25–134 (1892).
14. Holmgren, N. *Acta zool. Stockh.* **14,** 185–295 (1933); **36,** 243–328 (1955).
15. Welles, S. P. *J. Paleont.* **59,** 401 (1983).
16. McGowan, C. *J. zool. Res.* **197,** 173–219 (1982).
17. Gingerich, P. D. *Nature* **243,** 70–73 (1973).
18. Houde, P. & Olson, S. L. *Science* **214,** 1236–1237 (1981).
19. McDowell, S. *Auk* **65,** 520–549 (1948).
20. Tucker, B. W. *Int. orn. Congr.* **8,** 222–224 (1938).

Molecular genetic evidence for early evolutionary origin of budding peptidoglycan-less eubacteria

Erko Stackebrandt*, Wolfgang Ludwig*, Wolfgang Schubert†, Friedrich Klink‡, Heinz Schlesner§, Telse Roggentin§ & Peter Hirsch§

* Lehrstuhl für Mikrobiologie, Technische Universität München, Arcisstrasse 16, D-8000 München 2, FRG
† Lehrstuhl für Organische Chemie I, Technische Universität München, Lichtenbergstrasse 4, D-8046 Garching, FRG
‡ Biochemisches Institut, Medizinische Fakultät der Universität Kiel, Olshausenstrasse 40-60, D-2300 Kiel, FRG
§ Institut für Allgemeine Mikrobiologie der Universität Kiel, Olshausenstrasse 40-60, D-2300 Kiel, FRG

Recent studies on the cell wall composition of budding, non-prosthecate bacteria have shown that representatives of the genera *Planctomyces* and *Pasteuria* (*sensu* Staley, 1973) lack the peptidoglycan moiety[1], which is the characteristic feature of eubacterial cell walls[2,3], but possess an as yet unidentified protein sheet instead; in this respect *Planctomyces* and *Pasteuria* strains resemble certain archaebacteria more than eubacteria[3,4]. To determine their phylogenetic positions, the 16S ribosomal RNAs of three budding, peptidoglycan-less strains with and without stalks were subjected to partial

0028-0836/84/080735—03$01.00

© 1984 Macmillan Journals Ltd

sequence analyses. As we report here, the comparison of data with those obtained from about 320 eubacterial and 40 archaebacterial strains revealed that the strains investigated are not members of the archaebacterial kingdom but represent an ancient line of descent of the eubacterial kingdom. Furthermore the diphtheria toxin reaction[5] of *Planctomyces* and *Pasteuria* shows that they behave like eubacteria.

The three strains of budding, non-prosthecate bacteria investigated share the main characteristics of members of the *Planctomyces–Blastocaulis* group of bacteria[6]. They were isolated from brackish surface water of the Kiel Fjord, Baltic Sea[1]. Strain IFAM 1317 (IFAM = Institut für Allgemeine Mikrobiologie, University of Kiel) and strain IFAM 1310 resemble *Planctomyces maris*[7] and *Pasteuria ramosa* (*sensu* Staley, 1973), respectively, while strain IFAM 1319 shows no distinct similarity to any of the described species of this group. Cultivation of these organisms followed described methods[1]. Isolation of 16S rRNA[8–10] and sequence analysis[10–13] were done as described. Similarity coefficients, S_{AB} values[14], were calculated using a CDC computer.

Table 1 shows the similarity coefficients, calculated from the 16S rRNA oligonucleotide catalogues of the three strains investigated (not shown) and more than 350 bacterial reference strains. The S_{AB} values show strains IFAM 1310 and IFAM 1319 to be rather closely related (0.70), while they show less rRNA sequence homology with the *Planctomyces maris*-like strain IFAM 1317 (0.30 and 0.32, respectively). The degree of relatedness found between these three organisms and representatives of the various lines of descent of the eubacterial kingdom indicates that they are significantly more remote (with S_{AB} values ranging between 0.06 and 0.21, with an average value of 0.15), while almost no relationship can be detected to members of the archaebacterial kingdom (0.04–0.12, average 0.10). The range of the latter S_{AB} values is of the same order of magnitude as that found between representatives of the eubacterial and archaebacterial kingdoms (0.06–0.14)[15]. The lack of relationship between archaebacteria and budding, non-prosthecate and peptidoglycan-less bacteria agrees with results of the diphtheria-toxin reaction, recently used to distinguish archaebacteria and eubacteria[5,16] (Table 2). Cell supernatants of strains IFAM 1310 and IFAM 1317 do not contain proteins that served as substrate for the diphtheria toxin; in this negative reaction they resemble eubacteria[5,16]. Archaebacteria and eukaryotes, on the other hand, serving as a control, give significant diphtheria toxin-dependent protein labelling.

While it is clear that *Planctomyces* and relatives are not related to archaebacteria, the rRNA analyses do not indicate their status as a third line of descent of prokaryotes independent of those of archaebacteria and eubacteria. Our data indicate that the peptidoglycan-less bacteria have to be considered as eubacteria, with average S_{AB} values between *Planctomyces* and relatives and the eubacteria, being lower (0.15) than those found in the various lines of descent of eubacteria (0.22)[15,17]. The exact phylogenetic position, however, cannot yet be determined, since it is not known whether the 16S rRNA cistrons of *Planctomyces*- and *Pasteuria*-like strains have evolved at the same rate as those of eubacteria, which, with certain exceptions[18,19], appear isochronic. If one assumes that the evolutionary rate of the strains investigated differs from that of the majority of eubacteria, the lack of peptidoglycan and of certain universal oligonucleotides present in the 16S rRNA of isochronically evolving eubacteria[19,20] (that is, CACAAG, CUAACG, AAUACG, UUCCCG, AUACCCUG, AUUCCUACG) can be taken as positive evidence for this theory. In this case, the phylogenetic position of *Planctomyces* and relatives is within the main radiation of eubacterial species, either representing an individual line of descent or specifically related to members of one of its lines (as, for example, the fast-evolving mycoplasmas and relatives were found to be related to certain strains of *Clostridium*[18]).

However, a degenerate evolution of the budding, non-prosthecate and peptidoglycan-less eubacteria is not proven. The possibility therefore remains that these organisms represent the descendants of an ancient group of eubacteria that branched off immediately after the divergence of the 'ur-eubacteria' from

Table 2 Diphtheria toxin reaction with cell supernatants from budding, peptidoglycan-less eubacteria (I), peptidoglycan-containing eubacteria (II), archaebacteria (III) and eukaryotes (IV)

		TCA-precipitable radioactivity (pmol NAD per mg supernatant protein)	
		With toxin	Without toxin
I	Strain IFAM 1310	0.23	0.22
	Strain IFAM 1317	0.16	0.11
II	*Escherichia coli*	0.20	0.15
	Hyphomicrobium sp.	0.22	0.16
III	*Methanococcus vannielii*	32.95	0.25
	Thermoplasma acidophilum	15.60	0.34
IV	Wheat germ	31.84	0.52
	Calf liver	18.81	0.36

The reaction, containing 10 μl toxin solution (1.3 μg protein nitrogen), 10 μl NAD (adenine ^{14}C, 277 Ci mol^{-1}, 75 pmol) and 50 μl 150,000*g* supernatant containing 1–4 mg protein, was carried out at 37 °C for 30 min, and trichloroacetic acid (TCA)-precipitable radioactivity measured.

Table 1 Binary comparison among the 16S rRNA catalogues (S_{AB} values)

		IFAM 1310	IFAM 1319	IFAM 1317
Strain resembling *Pasteuria ramosa sensu* Staley[22]	IFAM 1310	—	0.70	0.30
	IFAM 1319	0.70	—	0.32
Strain resembling *Planctomyces maris*	IFAM 1317	0.30	0.32	—
Eubacteria				
Purple bacteria and their non-phototrophic relatives*		0.06–0.11	0.06–0.15	0.06–0.15
Cyanobacteria*		0.09–0.15	0.10–0.16	0.09–0.16
Spirochaeta†		0.08–0.14	0.07–0.11	0.11–0.16
Chloroflexus/Herpetosiphon group†		0.07–0.08	0.07–0.10	0.11–0.16
Cytophaga/Flavobacterium group†		0.08–0.13	0.09–0.12	0.13–0.18
Mycoplasmas and relatives*		0.07–0.10	0.07–0.09	0.09–0.16
*Deinococcus**		0.09–0.12	0.09–0.13	0.13–0.17
Clostridia and relatives*		0.08–0.17	0.09–0.15	0.11–0.18
Actinomycetes and relatives*		0.08–0.15	0.08–0.15	0.10–0.21
Archaebacteria				
Methanogenic bacteria*		0.06–0.12	0.04–0.10	0.06–0.12
*Thermoplasma acidophilum**		0.08	0.07	0.07
Sulfolobus and related taxa†		0.06–0.08	0.06–0.08	0.05–0.08

* The oligonucleotide catalogues of the reference strains have been published (see ref. 20).
† Unpublished data from the groups of C. R. Woese (University of Illinois, Urbana) and E.S.

the universal common ancestor of extant life, the 'progenote'[21], and before the main radiation of eubacterial lines of descent[17,19]. According to this theory the lack of certain universal oligonucleotides in the 16S rRNA catalogues implies not a loss due to high mutation rate, but rather that these universal eubacterial oligonucleotides evolved later in the evolution of eubacteria, after the ancestor of *Planctomyces* and relatives had already branched off. The eubacterial universal oligonucleotides missing in the catalogues of *Planctomyces* and relatives are also missing in archaebacteria which diverged even earlier than did the budding, peptidoglycan-less eubacteria. Likewise, the lack of the peptidoglycan moiety reflects a primitive state of eubacterial cell walls, with the 'invention' of peptidoglycan following at a later stage in the evolution of eubacteria.

This work was supported by a research grant from the Deutsche Forschungsgemeinschaft. We thank C. R. Woese for providing us with unpublished data.

Received 22 September; accepted 6 December 1983.

1. König, H., Schlesner, H. & Hirsch, P. *Arch. Microbiol.* (in the press).
2. Woese, C. R., Magrum, L. J. & Fox, G. E. *J. molec. Evol.* **11**, 245–252 (1978).
3. Kandler, O. *Zbl. bakt. Hyg., I. Abt. Orig. C* **3**, 149–160 (1982).
4. Kandler, O. & König, H. *Arch. Microbiol.* **118**, 141–152 (1978).
5. Kessel, M. & Klink, F. *Nature* **287**, 250–251 (1980).
6. Schmidt, J. M. & Starr, M. P. *Curr. Microbiol.* **1**, 325–330 (1978).
7. Bauld, J. & Staley, J. T. *J. gen. Microbiol.* **97**, 45–55 (1976).
8. Johnson, J. L. in *Manual of Methods for General Bacteriology* (eds Gerhardt, P. *et al.*) 450–475 (American Society for Microbiology, Washington, 1981).
9. Kirby, K. S. *Meth. Enzym.* **12B**, 87–99 (1968).
10. Stackebrandt, E., Ludwig, W., Schleifer, K. H. & Gross, H. J. *J. molec. Evol.* **17**, 227–236 (1981).
11. Stackebrandt, E., Seewaldt, E., Ludwig, W., Schleifer, K. H. & Huser, B. A. *Zbl. bakt. Hyg., I. Abt. Orig. C* **3**, 90–100 (1982).
12. Jay, E., Bambara, R., Padmanaghan, R. & Wu, R. *Nucleic Acids Res.* **1**, 331–353 (1974).
13. Domdey, H., Jank, P., Sänger, H. L. & Gross, H. J. *Nucleic Acids Res.* **5**, 1221–1236 (1978).
14. Fox, G. E., Pechman, K. R. & Woese, C. R. *Int. J. syst. Bact.* **27**, 44–57 (1977).
15. Woese, C. R. & Fox, G. E. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5088–5090 (1977).
16. Kessel, M. & Klink, F. *Zbl. bakt. Hyg., I. Abt. Orig. C* **3**, 140–148 (1982).
17. Fox, G. E. *et al. Science* **209**, 457–463 (1980).
18. Woese, C. R., Maniloff, J. & Zablen, L. B. *Proc. natn. Acad. Sci. U.S.A.* **77**, 494–498 (1980).
19. Stackebrandt, E. & Woese, C. R. in *Molecular and Cellular Aspects of Microbial Evolution* (eds Carlile, M. J., Collins, J. F. & Moseley, B. E. B.) 1–31 (Cambridge University Press, 1981).
20. Woese, C. R. *et al. Nature* **254**, 83–86 (1975).
21. Woese, C. R. & Fox, G. E. *J. molec. Evol.* **10**, 1–6 (1977).
22. Staley, J. T. *Can. J. Microbiol.* **19**, 609–614 (1973).

Possible artefactual basis for apparent bacterial growth at 250 °C

Jonathan D. Trent, Roger A. Chastain & A. Aristides Yayanos

Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA

Baross and Deming[1] reported that thermophilic marine bacteria isolated from the vicinity of a submarine hot-spring grow at temperatures up to at least 250 °C. They did not, however, conduct the appropriate control experiments to eliminate the possibilities of chemical artefacts or contamination. Here, in experiments using the same growth medium, the same temperature and pressure apparatus[2] and the same sampling and analytical procedures, we report results nearly identical to theirs. We conclude that their evidence indicating bacterial growth at 250 °C may be due to artefacts produced in the medium and to contaminants introduced during sample processing.

The 'black smoker' hydrothermal vent, discovered at a depth of 2,650 m at 21° N on the East Pacific Rise, releases 350 °C water into the ocean[3]. Water remains liquid at this temperature due to hydrostatic pressure. It has been suggested that the absence of liquid water, rather than high temperature *per se*, limits bacterial growth[1]. This idea has been logically extended to predict that extreme thermophiles could inhabit submarine hydrothermal vents[4].

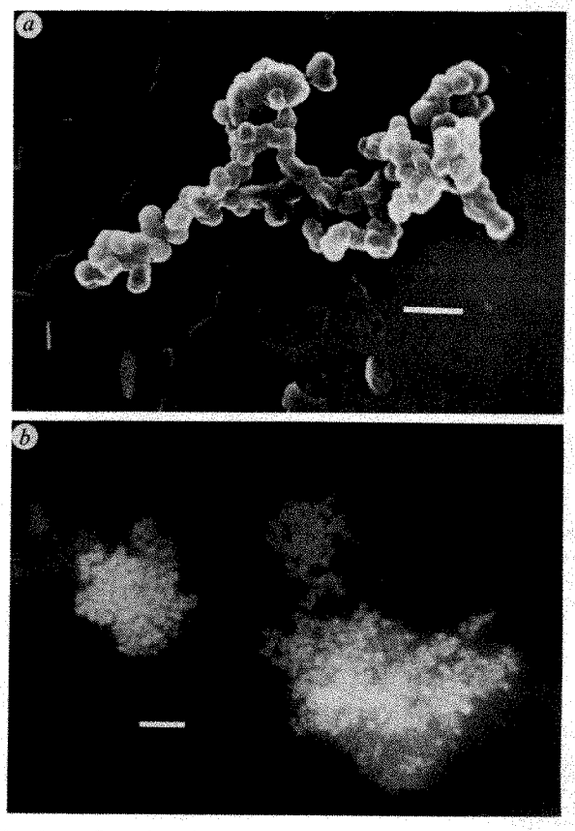


Fig. 1 *a*, Scanning electron micrograph of particles resembling bacteria from uninoculated medium after 4 h at 250 °C and 265 atm pressure. Scale bar, 1 μm. *b*, These particles are not DAPI-stained but autofluoresce, giving them some resemblance to DAPI-stained bacteria. Scale bar, 3 μm.
Methods: Experiments were conducted in a high-temperature and high-pressure apparatus[2]. The temperature of this system at pressure is monitored by a thermocouple and a gauge thermometer inserted into the wall of the pressure chamber. The temperature inside the chamber was 238 ± 2 °C when the wall temperature read 249 ± 2 °C. Samples were taken before the medium was heated, when it reached maximum temperature, and then at approximately 1h intervals for 5–6 h. Samples were fixed with 2% E.M.-grade glutaraldehyde and stored at 4 °C until analysed. For epifluorescent microscopy, glutaraldehyde-fixed samples were transferred in duplicate to sterile tubes. One subsample was untreated and used to measure autofluorescence and the other was stained with DAPI at a final concentration of 0.02 μg ml^{-1} for 1–3 h. Between 0.01 and 1.0 ml of sample was transferred into 5 ml of diluent (synthetic seawater with 0.25% formalin and 0.05% azide) and filtered onto 0.2-μm Nucleopore filters stained with Irgalan black. Over 200 objects were counted for each sample.

Baross and Deming's experiments were conducted using apparatus in this laboratory and their samples were analysed at Oregon State University. Their strongest evidence for the existence of 'black smoker' bacteria was based on growth curves, protein concentrations and transmission electron micrographs. Experiments were done at 265 atm pressure and 150, 200, 250 and 300° Celsius. An uninoculated control was done at 200 °C but no data are given. Controls were not done at any other temperature. Only at 250 °C is there a reasonable correlation between increases in cell concentrations and protein, and transmission electron micrographs of cells were from that temperature.

We requested a culture from Baross to verify their results independently. Although we did not receive the culture, we conducted experiments with uninoculated media and with media inoculated with a non-thermophilic marine bacterium, *Vibrio harveyi*. These experiments were designed to look for artefacts arising in the medium itself or in medium supplemented with cellular material.

0028–0836/84/080737—04$01.00

© 1984 Macmillan Journals Ltd

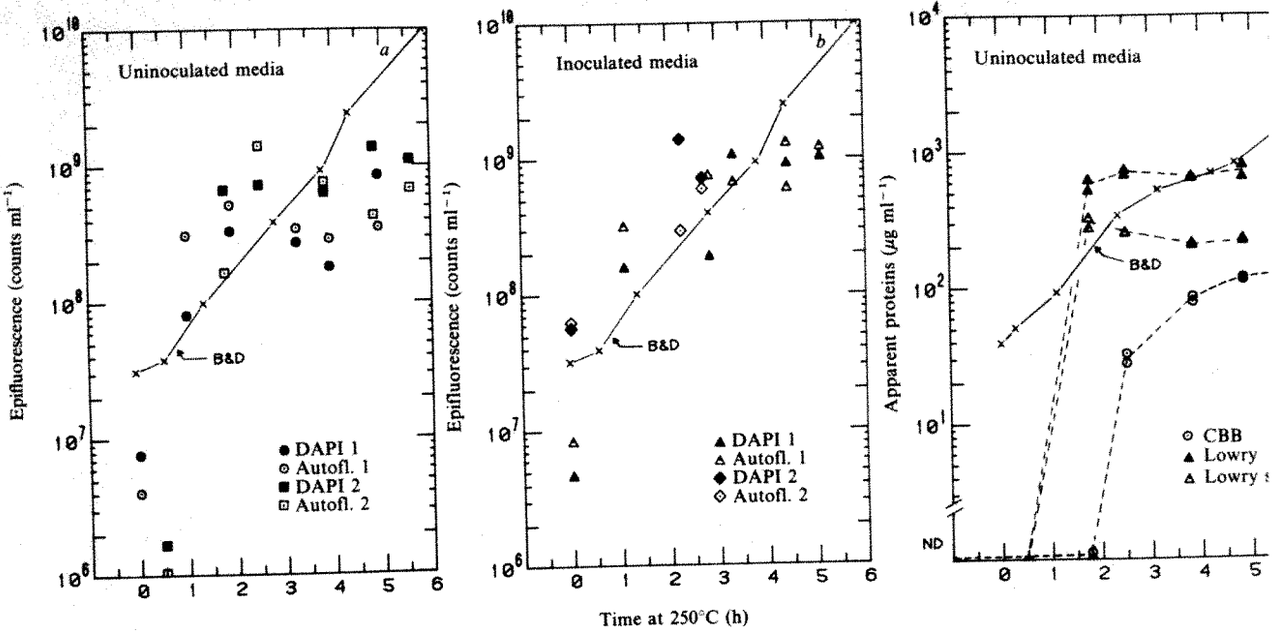


Fig. 2 *a*, In both experiments 1 and 2, the addition of DAPI did not increase counts above autofluorescence, indicating that DAPI stai did not occur. The solid curve labelled B & D shows the data reported by Baross and Deming. *b*, Experiments in which bacteria were ad to media gave the same results as uninoculated media. The solid curve labelled B & D shows Baross and Deming's data. *c*, Before hea there is no measurable protein in the media using either the Coomassie brilliant blue (CBB) or the Folin–Lowry (Lowry) techniques. A heating to 250 °C for 1 or 2 h, colour develops, suggesting the presence of proteins. Part of the absorption in the Lowry technique is du the presence of a precipitate; centrifuging samples to remove turbidity decreases absorption (Lowry sp.). The solid curve labelled B & from the original paper by Baross and Deming[1]. 'ND' signifies not detected.

Methods: The medium is described as 'minimal salts' (MS) supplemented with: *a*, 0.03% $(NH_4)_2SO_4$; *b*, 0.001% $Ca(NO_3)_2$; *c*, 0.0(Bacto-yeast extract (Difco); *d*, 0.3% HEPES buffer (Sigma); *e*, 0.2% $Na_2S_2O_3 \cdot 5H_2O$; *f*, 0.02% $MnSO_4 \cdot H_2O$; *g*, 0.001% $FeSO_4 \cdot 7H_2C$ 0.05%Na_2HPO_4; *i*, 0.5% $NaHCO_3$; *j*, trace element mix[12]. Stock media consisted of seven solutions: minimal salts plus *a*–*d*, made as solution at 2 times concentration; *e*–*i*, made as five separate solutions at 10 times concentrations; and *j*, made at the concentration at w it is used. All stock media were made with Nanopure water, stored at room temperature and except for *g*, which was filter sterilized, all sterilized by autoclaving. For inoculated controls, 1 ml of stationary phase *V. harveyi* ($\sim 5 \times 10^8$ cells) was centrifuged, rinsed with 1 n medium, re-centrifuged and suspended in the experimental medium. Protein concentrations in glutaraldehyde-fixed samples were meas using the methods reported by Baross and Deming[1]. Aliquots of 0.5 ml were centrifuged at 13,000*g* for 1.5 min at 25 °C. The supern was aspirated and the pellet was precipitated by addition of 0.5 ml of cold 5% TCA for 1 h on ice. The sample was repelleted by centrifug at 13,000*g*, 1 min at 4 °C; the precipitate was washed with cold 5% TCA as above, and pelleted a third time. The final pellet was solubi with 200 μl of 1 M NaOH, and the *p*H adjusted to ~7 with 200 μl of HCl. The data reported in *c* are from the above method but varia on this method included direct protein measurements on unprocessed samples, TCA precipitation before initial centrifugation and no addition of HCl to adjust *p*H. All procedures gave some colour development with heated media, suggesting that the Folin–Lowry an Coomassie techniques should be used with caution, or that other protein assays should be used to evaluate these samples.

Our results showed no significant differences between uninoculated media and *V. harveyi* inoculated media at high temperature and pressure. A flocculent, white precipitate due to the mixing of manganese sulphate, sodium phosphate and sodium bicarbonate was present in the unheated media. This precipitate contained no epifluorescent particles above background and no measurable proteins. The amount of precipitate in the media decreased during heating and changed consistency from an amorphous floc to distinct micrometre-sized spheres (Fig. 1*a*). These spheres resembled bacteria in size and shape and were seen alone or in clumps (Fig. 1*a*, *b*).

For their growth curves, Baross and Deming measured changes in particles stainable with 4′,6′-diamidino-2-phenylindole (DAPI), a DNA-specific stain routinely used to count bacteria[5]. The spheres we observed in heated media were autofluorescent and were not DAPI stained (Fig. 2*a*, *b*). Baross and Deming report observations of autofluorescence in their samples but they attribute it to methanogenic bacteria.

In our experiments the concentration of spheres increased for the first 2 or 3 h then stabilized at $\sim 10^9$ per ml. In Baross and Deming's experiment at 250 °C, particles reached a concentration of 10^{10} per ml (Fig. 2*a*, *b*, curve B & D). We think this difference between our 'controls' and their experiment is not significant given the variability we observed due to mixing, sampling and counting.

Samples were processed for protein using the procedures described by Baross and Deming. Protein concentrations were measured using the Folin–Lowry and the Coomassie b blue techniques[6]. We found that heated media change time and gave positive readings in both protein assays (F A number of non-protein substances have been found t fere with the Folin–Lowry[7] and Coomassie brilliant blue[8] We do not know the reason for the positive protein reac our heated media.

Apparent protein concentration, measured with the technique, reached a plateau shortly after the media r maximum temperature. Subtracting our apparent protei trol' values from Baross and Deming's 250 °C 'bacteria' (Fig. 2*c*, curve B & D), leaves only one data point background at the end of their experiment. As in the epifluorescent microscopy, the difference between our d theirs may be attributed to differences in sampling pro and processing. For example, if precipitates are not r by centrifugation or otherwise corrected for, apparent concentrations are significantly higher (compare Lov Lowry sp., Fig. 2*c*).

Baross and Deming present transmission electron graphs of bacterial cells kept at 250 °C for 7 h. We u techniques to prepare samples for transmission electror scopy. Both methods resulted in satisfactory fixation of *veyi* in the unheated medium (Fig. 3*a*). In heated me first method revealed the formation of micrometre-sized that with few exceptions did not resemble cells (Fig. 3*b* the second method (A. Soeldner, personal commun

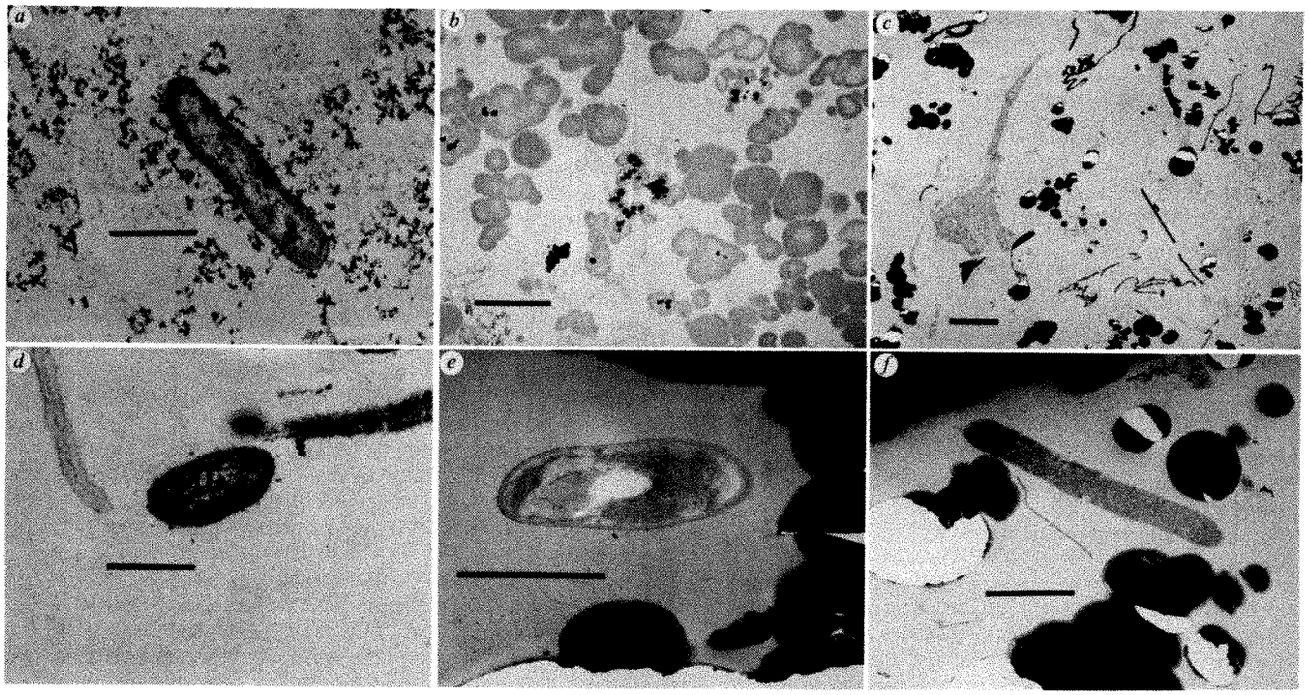


Fig. 3 *a*, A transmission electron micrograph of unheated medium inoculated with the non-thermophilic bacterium *V. harveyi*. The cell is surrounded by the precipitate present in the starting medium. Scale bar, 1 μm. *b*, After heating to 250 °C at 265 atm pressure, all *V. harveyi* cells disappeared. Using an embedding technique with filtered agar, only micrometre-sized spheres were found in a sample after 4 h at temperature and pressure. Scale bar, 3 μm. *c*, The same sample shown in *b* processed using the embedding procedure used by Baross and Deming, shows the same spheres more darkly stained and a variety of other objects including cells. Scale bar, 3 μm. *d–f*, Examples of cells and cell-like objects found in thin sections from uninoculated media heated to 250 °C for 4 h and processed using the same transmission electron microscopic procedure used by Baross and Deming. Scale bars: *d*, 0.5 μm; *e*, *f*, 1 μm.

Methods: Immediately after sampling, specimens were fixed for electron microscopy by adding glutaraldehyde to a final concentration of 2%. Fixed material was washed with 0.2 M cacodylate buffer (*p*H 7.8) and postfixed with 1% OsO_4 in buffer. In one procedure fixed samples were embedded in 0.22 μm filtered 2% Nobel agar (Difco) in buffer and dehydrated in ethanol. In the other procedure samples were embedded in unfiltered Ionagar (Oxoid no. 2) in buffer, dehydrated in acetone and stained with uranyl acetate before final dehydration (70% step). All specimens were infiltrated with Spurr resin. Silver-grey thin sections were post-stained with 4% uranyl acetate and 0.1% lead citrate.

which was used by Baross and Deming, we found many objects that resembled cells (Fig. 3*c–f*). The critical difference between the two methods was the use of 0.2-μm filtered embedding agar in the first method and the use of unfiltered agar in the other method. It thus seems likely that the cells seen by Baross and Deming in samples incubated at 250 °C and 265 atm did not survive exposure to these conditions but were contaminants added to the samples after they were cooled and decompressed.

The production of methane, hydrogen and carbon monoxide and the presence of protein amino acids are also presented as evidence for black smoker bacteria. Gas production in Baross and Deming's experiments was measured after 3 h at 300 °C. No gas data are reported from 250 °C. Their uninoculated media control was done at 200 °C rather than 300 °C and reportedly did not produce gases. We did not analyse our 'control' samples for gas production.

We determined protein amino acids on three of our samples using Baross and Deming's procedures. One sample was from uninoculated and unheated medium, a second sample was from medium uninoculated and heated for 4 h at 250 °C, and a third sample was from medium inoculated with *V. harveyi* and heated for 4.5 h at 250 °C. These samples were analysed by the Amino Acid Dating Laboratory at Scripps Institution of Oceanography. A total of 200 nmol ml^{-1} (predominantly serine, glutamic acid, glycine and aspartic acid) was measured in the trichloroacetic acid (TCA)-precipitable fraction of our unheated, uninoculated sample. Other amino acids were present at the detection limits of the analytical facility (~1 nm). In samples of both the uninoculated and inoculated media that were heated to 250 °C, total amino acids in the TCA-precipitable fraction were 120 and 80 nmol ml^{-1}, respectively, with the same four amino acids found in unheated samples predominating. We think the levels of amino acids measured in our control samples were due to contamination in the pressure apparatus or from reagents used in sample processing.

Amino acid data reported in Baross and Deming's Table 1 are fractions (addition of their data totals 1.0002). Their caption states that concentrations of amino acids can be derived from their total colorimetrically determined protein data minus 25% for unknown peaks. Since we have shown that their colorimetric protein determinations are suspect, it is not clear what concentrations of amino acids are actually present in their samples, or if they are greater than the levels of contamination we found in uninoculated media. They report a number of amino acids, such as serine and threonine, which are known to be unstable at elevated temperatures[9]. Contamination is a significant problem when measuring trace amounts of amino acids[10,11]. Since Baross and Deming's amino acid data are based on a single determination, without controls, we do not consider them good evidence for the existence of black smoker bacteria.

In Baross and Deming's experiments only their results from 250 °C were suggestive of bacterial growth. There were no controls done at this temperature and thus no way to eliminate artefacts. The experiments reported here indicate that artefacts are produced in media heated to 250 °C at 265 atm pressure in the experimental system used[2]. These artefacts cast some serious doubts on their reported growth curves and protein data. Contamination and abiotic micrometre-sized spheres could account for the micrographs of 'cells' Baross and Deming reported from their 250 °C samples. Data on gas production and amino acids are not presented in sufficient detail to be seriously considered supporting evidence for the existence of black smoker bacteria.

This work was supported by NSF grant OCE-8208419 and a contract from Sandia National Laboratories. We thank R. Van Boxtel, A. S. Dietz, P. A. Farnsworth, J. A. Baross and A. Soeldner for technical assistance, J. L. Bada and D. Darling

for amino acid analysis, and S. L. Miller, H. Craig and K. H. Nealson for reviewing our manuscript.

Received 19 September; accepted 7 December 1983.

1. Baross, J. A. & Deming, J. W. *Nature* **303**, 423–426 (1983).
2. Yayanos, A. A., Van Boxtel, R. & Dietz, A. S. *Appl. envir. Microbiol.* **46**, 1357–1363 (1983).
3. East Pacific Rise Study Group *Science* **213**, 31–40 (1981).
4. Baross, J. A., Lilley, M. D. & Gordon, L. I. *Nature* **298**, 366–368 (1982).
5. Porter, K. G. & Feig, Y. S. *Limnol. Oceanogr.* **25**, 943–948 (1980).
6. Hanson, R. S., Phillips, J. A. in *Manual of Methods for General Bacteriology* (ed. Philipp, G.) 328–364 (Am. Soc. Microbiol, Washington DC, 1981).
7. Bonitati, J., Elliot, W. B. & Miles, P. G. *Analyt. Biochem.* **31**, 399–404 (1969).
8. Rosenthal, K. S. & Koussale, F. *Analyt. Chem.* **55**, 1115–1117 (1983).
9. Vallentyne, J. R. *Geochim. cosmochim. Acta* **28**, 157–188 (1964).
10. Wolman, Y. & Miller, S. L. *Nature* **234**, 548–549 (1971).
11. Bada, J. L. *et al. Nature* **301**, 494–497 (1983).
12. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Y. *J. gen. Microbiol.* **111**, 1–61 (1979).

Reply from John A. Baross* and Jody W. Deming†

* School of Oceanography, Oregon State University, Corvallis, Oregon 97331, USA
† Chesapeake Bay Institute and Department of Biology, The Johns Hopkins University, Shady Side, Maryland 20764, USA

The conclusion of Trent *et al.* that no bacterial growth occurred in our experiments[1] is not tenable because of crucial differences between the results of our work and theirs, and because of additional data not addressed by these authors. If the scores of morphologically diverse bacteria we observed in thin sections of a 250 °C sample were contaminants, they would have appeared in the 300 °C sample also reported[1], since both samples were processed simultaneously using the same reagents. Such was not the case. Recent amino acid analyses[2] from our original experiments[1] indicate that at 250 °C the total amino acid content of particulate protein increased from 1.6 to 145 $\mu g\ ml^{-1}$ in 5.4 h, a doubling time of approximately 1 h. The same doubling time was calculated for concentrations of each of the 15 amino acids (including serine and threonine) detected by the standard methods we used[1,2]. We continue to work with the original culture of black smoker bacteria, as well as the viable culture recovered from the titanium syringe upon termination of the 250 °C experiment, and one of us will dive with the French in March 1984 to search for similar bacteria in unexplored smokers at 13° N.

1. Baross, J. A. & Deming, J. W. *Nature* **303**, 423–426 (1983).
2. Baross, J. A., Deming, J. W. & Becker, R. R. *Proc. 3rd int. Symp. Microbial Ecology: Current Perspectives in Microbial Ecology* (American Society for Microbiology, Washington, DC, in the press).

Role of an upstream regulatory element in leucine repression of the *Saccharomyces cerevisiae leu2* gene

Alfonso Martinez-Arias*‡, H. Joseph Yost† & Malcolm J. Casadaban*†

* Department of Biophysics and Theoretical Biology, and
† Committee on Genetics, University of Chicago, Chicago, Illinois 60637, USA

The expression of a number of eukaryotic genes has been shown to involve at least two sequences located upstream of the actual transcription unit[1–7]: one of these sequences, centred on a widely conserved TATAAT sequence, is thought to be involved in determining the precise site of initiation of transcription[1,8]; the other has a gene-specific sequence, can function at a variable distance upstream of the initiation site[3,9], and is involved in the regulation of transcription[3,4,7,10]. By constructing β-galactosidase gene fusions, to facilitate measuring gene expression *in vivo*, we have now defined a *cis*-acting regulatory element of the *Saccharomyces cerevisiae leu2* gene. This element is located within a 280 base pair (bp) fragment which occurs 125 bp upstream of the *leu2* translation initiation codon and which contains a short G+C-rich palindromic sequence. A fragment of the *Escherichia coli* transposable element Tn*9* which contains a similar palindromic sequence can functionally replace the natural *leu2* regulatory element. Our results are contrary to previous speculations that the *leu2* gene is regulated by an attenuation mechanism[11,12].

Biochemical studies of the *leu2* locus of *S. cerevisiae*, which encodes β-isopropylmalate dehydrogenase, an enzyme of the leucine biosynthetic pathway, have indicated that enzymatic activity is repressed when cells are grown in the presence of leucine and threonine[13]. DNA sequence analysis of the region upstream of *leu2*[12] has revealed the complex arrangement (Fig. 1) of a Ty*1* transposable element, a leucyl tRNA gene and an open reading frame with the potential for encoding a 24 amino acid polypeptide containing 6 leucine residues. This led to speculation about the regulatory mechanisms that may be operating at this locus[11,12]. To investigate further the nature of this regulation an *Xho*I–*Bst*EII fragment, which extends from the Ty*1* δ element to the 13th *leu2* codon, was fused *in vitro* to the *E. coli* β-galactosidase gene on the vector pMC1790[14,15], as described in Fig. 1. Comparable fusions to the β-galactosidase gene have been used to study the regulated expression of several other genes in yeast[16–18]. The resulting fusion plasmid pMC1876 expresses a level of β-galactosidase in transformed[19] yeast cells that is repressed fivefold when the cells are grown in the presence of 1 mM leucine and 1 mM threonine (Table 1).

The *leu2* control region in the fusion plasmid was dissected as illustrated in Fig. 2 and assayed as summarized in Tables 1 and 2. A deletion from the upstream side extending to the middle of the $tRNA_3^{leu}$ gene sequence (Δ1) results in a small increase in β-galactosidase expression but no qualitative changes in the pattern of regulation. Total removal of $tRNA_3^{leu}$ gene sequences in this region (Δ8) leads to a further increase in β-galactosidase expression. This suggests that the tRNA gene is not directly involved in the regulation of this locus, although its presence does exert a small effect on *leu2* expression, perhaps due to steric interactions between factors bound to these two closely linked genes[20]. Removal of sequences up to the *Hin*cII site located 125 bp upstream of the *leu2* translation initiation codon (Δ2) results in a 100-fold decrease in β-galactosidase activity and the loss of leucine-specific regulation. This indicates that regions important for regulation exist between the tRNA gene and the *Hin*cII site. Moreover, as the Δ2 deletion does not remove the major transcription initiation site (A. Andreadis and P. Schimmel, personal communication) the regulation of this locus presumably takes place primarily at the level of transcription.

To explore this further, we exchanged the DNA fragment between the *Hin*cII site and the *Bst*EII site in Δ1 with a 250 bp piece of DNA containing the transcription and translation initiation regions from the *cyc1* gene of *S. cerevisiae* fused to the β-galactosidase gene[17,21]. When this fusion, which has a hybrid promoter (HYΔ1), was assayed in yeast, β-galactosidase activity was regulated by leucine and threonine (Table 2). This fusion does not respond to other amino acids in the culture medium nor to catabolite repression, which is the normal regulatory pattern of the *cyc1* gene. This indicates that the *leu2* regulatory element is located upstream of the *Hin*cII site and well before the *leu2* coding region. It is interesting, however, that the levels of β-galactosidase expressed by HYΔ1 are lower than those of the wild-type *leu2* promoter, and that the ratio of the levels of expression in repressing and non-repressing conditions is larger. This might reflect differences in translation or stability of the hybrid proteins from the fusions, or it might reflect a requirement for a precise interaction between the upstream regulator and the initiation site-proximal sequences, which is altered in the hybrid promoter.

In the DNA sequence[12] between the Δ8 and the Δ2 end points, we noted the presence of a short palindromic sequence

‡ Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

0028–0836/84/080740—03$01.00

© 1984 Macmillan Journals Ltd

Table 1 Repression of *leu2* expression

Plasmid*	leu2 activity†			
	No amino acids	Leu+Thr	His	No amino acids: Leu+Thr
pMC1876	640	140	720	4.6
Δ1	940	320	1,000	3.0
Δ8	1,400	390	1,400	3.6
Δ2	12	13	13	0.9
Δ26	19	20	ND	0.9
InΔ1	930	410	ND	2.3

* Yeast strain M12B (*α,trp*1-289, *ura*3-52, obtained from K. Struhl and R. Davis) cells were transformed[19] with the indicated plasmids. The cells were grown in SD minimal media[26] supplemented with uracyl or tryptophan (40 μg ml^{-1}) and the indicated amino acids (1 mM each).

† The leu2 assays were performed in triplicate on exponentially growing cells at a concentration of 10^7 cells per ml as described previously[15]. Units of activity are nmoles *o*-nitro-phenyl-β-D-galactoside (ONPG) cleaved per min per mg of total protein in the extract. The different levels are not due to changes in the segregation properties of the plasmids (data not shown). ND, not determined.

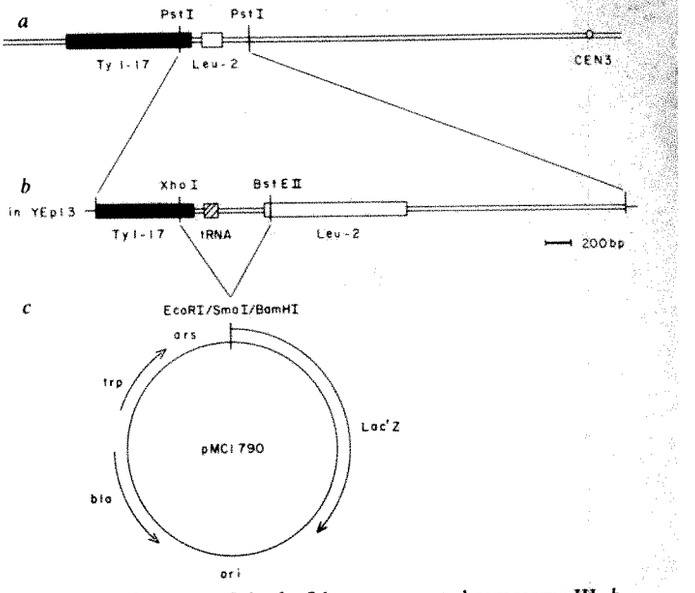


Fig. 1 *a*, Location of the *leu2* locus on yeast chromosome III. *b*, The *Pst*I fragment of the original *leu2* gene cloned in YEp13[27]. The *leu2* gene is transcribed from left to right[15]. There is a Ty*1* element, a $tRNA_3^{Leu}$ gene and an open reading frame (ORF) upstream from the *leu2* coding region[12]. *c*, The fusion plasmid pMC1876 was formed by inserting a blunt-ended *Xho*I–*Bst*EII fragment from YEp13 into the *Sma*I site of vector pMC1790[13], as described previously[14]. This construction has the first 13 *leu2* codons fused in phase to the eighth codon of *lacZ*, so that a hybrid protein with β-galactosidase activity is encoded. *E. coli* strain MC1066[14] carrying plasmid pMC1876 is ampicillin resistant, Trp^+ and expresses β-galactosidase activity detectable on M63 Xgal medium[28]. Note that inserts into the *Sma*I site of pMC1790 are flanked on the left by an *Eco*RI site and on the right by a *Bam*HI site.

(5′ TGAGAGGCCGGAACCGGCTTTTCA 3′) containing two *Hpa*II sites (CCGG) and centred 192 bp from the *leu2* translation initiation codon. Short repeats similar to this have been implicated in the transcriptional regulation of several eukaryotic loci[3,7,22]. To test the involvement of this sequence in the regulation of *leu2* expression, the 6 bp of DNA between the two *Hpa*II sites were deleted (Δ26, Fig. 2) from the fusion (Δ8) lacking the $tRNA_3^{Leu}$ gene. When placed into yeast, this deletion–fusion displayed a very low constitutive β-galactosidase activity and indicates that this G+C-rich palindrome lies within a region important for regulation as well as expression. This small deletion (Δ26) lowers dramatically the basal levels of β-galactosidase expression (Table 1); this might be due to the loss of the functional entry site of RNA polymerase to the *leu2* locus or to the appearance of abnormally initiated transcripts. The assay used in these experiments cannot distinguish between these possibilities. In a second experiment the DNA region between the *Hpa*I and *Hin*cII sites was inverted (InΔ1, Fig. 2). This inversion moves the palindromic sequence 150 bp further upstream of the transcription site. In yeast, this fusion yielded levels of β-galactosidase comparable to the wild-type fusion, but with only a twofold repression by leucine plus threonine (Table 1). These results are consistent with a possible role of this symmetric structure in the regulation of expression at the *leu2* gene.

In an additional experiment aimed at identifying sequences capable of substituting for the *leu2* upstream modulator element by restoring leucine regulated β-galactosidase expression to plasmid Δ2, various DNA sequences were inserted into the *Eco*RI site at the left end of Δ2 *leu2* DNA. One fragment, derived from Tn*9*[23], restored expression comparable to Δ1 with a twofold leucine plus threonine regulation (data not shown). Examination of the sequence of this fragment[24] revealed a 5′ GGTGCGTACCGGGTT 3′ sequence related to the *leu2* repeat region located 170 bp from the *leu2* translation initiation site. Although this region does not contain a perfect palindrome, it embraces a core sequence quite homologous to the one present in *leu2* (see above). This control sequence also appears in a similar position in the promoter of *leu1* (Yun-Pung Hsu and P. Schimmel, personal communication), another leucine biosynthetic pathway gene which is coordinately regulated with *leu2*[25]. Further mutational analysis of the *leu2* control region will be necessary to determine precisely which bases are essential for leucine regulation.

The results presented here exclude any major *cis*-acting regulatory role for the open reading frame upstream of the *leu2* gene. Plasmid HYΔ1 lacks the 3′ end of the open reading frame and still shows *leu2* regulation; on plasmid InΔ1 the open reading frame is disrupted by inversion; and the Tn*9* substitution lacks the 5′ end of the open reading frame. Furthermore, small insertions and deletions around the *Hin*cII site within the open reading frame do not eliminate regulation even in the absence

Table 2 Repression of hybrid promoter *leu2–cyc1*

Plasmid	leu2 activity					
	No amino acids	Leu+Thr	His	Raffinose	No amino acids Leu+Thr	Raffinose: glucose
pMC1876Δ1	940	320	1,000	1,100	2.9	1.2
pLG669Δ292	4,000	5,200	ND	10,400	0.8	2.6
pMC1876HYΔ1	210	30	260	340	7.0	1.6

The *cyc1–lacZ* fusion plasmid[21] pLG669Δ292 contains a 300 bp fragment of the *cyc1* gene from *S. cerevisiae*, a 2μ replicon, and a *ura3* gene as a selectable marker. Amino acids were added at 1 mM each. 2% raffinose was substituted for 2% glucose as the carbon source to determine the response of the fusions to catabolite repression.

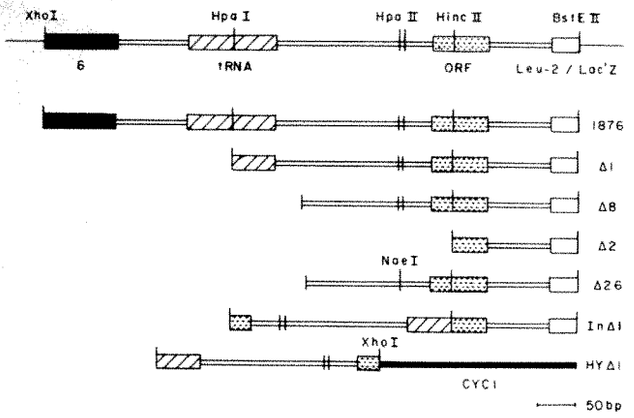


Fig. 2 Derivatives of pMC1876. The Δ1 and Δ8 fragments were obtained by cleaving pMC1876 on the left (*Xho*I side) with *Eco*RI, digestion with the exonuclease *Bal*31 followed by cleavage on the right (*Bst*EII side) with *Bam*HI and isolation in 4.5% polyacrylamide gels. Δ2 is a *Hin*cII–*Bam*HI fragment of pMC1876. The Δ1, Δ8 and Δ2 fragments were inserted into pMC1790 cleaved with *Sma*I and *Bam*HI. Δ26 is a 6 bp *Hpa*II deletion obtained by isolating the *Eco*RI–*Bam*HI fragment of Δ8, cleaving with *Hpa*II, and ligating the two large fragments from this reaction (eluted from a polyacrylamide gel) back into pMC1790 cleaved with *Eco*RI and *Bam*HI. This construction was verified by the observation that the deletion of the 6 bp between the *Hpa*II sites (5′ GCCGGAACCGGC 3′) creates a *Nae*I site (5′ GCCGGC 3′). The hybrid *leu2–cyc1* promoter was formed by cleaving the derivative of Δ1, containing an *Eco*RI–*Sma*I–*Bam*HI linker at the *Hin*cII site, with *Sma*I and *Sac*I (a unique restriction site in the middle of *lacZ*) and ligating the purified vector fragment to a fragment containing the *cyc1* transcription and translation initiation sites from pLG669Δ292[21] cleaved with *Xho*I, rendered blunt, and cleaved with *Sac*I. All clones were selected in *E. coli* strain MC1066 as ampicillin resistant, Trp^+ colonies displaying β-galactosidase activity on M63 Xgal medium. Note that the fusion points in *lazZ* of the *cyc1* and the *leu2* are different. DNA manipulations[29], strains and methods of selection used[14] have been described previously. The end points of Δ1, Δ8 and Δ2, and the centre of the palindromic repeat are located 400 ± 10, 310 ± 10, 125 and 192 bp respectively before the *leu2* initiation codon.

of the chromosomal copy of the open reading frame at the *leu2* locus (unpublished results).

Mutations at upstream modulator elements, similar to our Δ26, affect both gene regulation and expression systems that have been investigated[4,7,17]. This phenomenon has been interpreted as evidence that these sequences mediate positive regulatory mechanisms at these loci and thus may represent binding sites for specific proteins whose function is to open the chromatin structure locally, allowing RNA polymerase positioning. Alternatively these elements may be entry sites for the RNA polymerase destined to initiate transcription at the downstream TATA box.

We thank A. Andreadis and P. Schimmel for communicating unpublished results, L. Guarante for sending pLG669Δ292, and L. Rothman-Denes and C. Giroux for constructive criticism of the manuscript. This work was supported by NIH grant GM 29067. H.J.Y. was supported by The Training Program in Genetics and Regulation PHS GM 07197–08, and M.J.C. by NIH Research Career Development grant AI 00468.

Received 8 September; accepted 13 December 1983.

1. Grosschedl, R. & Birnstiel, M. C. *Proc. natn. Acad. Sci. U.S.A.* **77,** 7102–7106 (1980).
2. Grosschedl, R. & Birnstiel, M. C. *Proc. natn. Acad. Sci. U.S.A.* **79,** 297–301 (1982).
3. Pelham, H. R. *Cell* **30,** 517–528 (1982).
4. Struhl, K. *Nature* **300,** 284–287 (1982).
5. Moreau, P. *et al. Nucleic Acids Res.* **9,** 6047–6068 (1981).
6. Hen, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7132–7136 (1982).
7. Donahue, T. F., Daves, R. S., Lucchini, G. & Fink, G. R. *Cell* **32,** 89–98 (1983).
8. Breadhnach, R. & Chambon, P. *A. Rev. Biochem.* **50,** 349–383 (1981).
9. McKnight, S. L. *Cell* **31,** 355–365 (1982).
10. Beier, D. R. & Young, E. T. *Nature* **300,** 724–728 (1982).
11. Yanofsky, C. *Nature* **302,** 751–752 (1983).
12. Andreadis, A., Hsu, Y. P., Kohlhaw, G. B. & Schimmez, P. *Cell* **31,** 319–325 (1982).
13. Satyanarayana, T. *et al. J. Bact.* **96,** 2078–2079 (1968).
14. Casadaban, M. J. *et al. Meth. Enzym.* **100,** 293–308 (1983).
15. Martinez-Arias, A. & Casadaban, M. J. *Molec. cell. Biol.* **3,** 580–586 (1983).
16. Rose, M., Casadaban, M. J. & Botstein, D. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2460–2464 (1981).
17. Guarante, L. & Ptashne, M. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2460–2464 (1981).
18. Osley, M. A. & Hereford, L. *Proc. natn. Acad. Sci. U.S.A.* **79,** 7689–7693 (1982).
19. Hinnen, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **75,** 1929–1933 (1978).
20. Hall, B. D. *et al. Cell* **29,** 3–5 (1982).
21. Guarante, L., Yocum, R. & Gifford, P. *Proc. natn. Acad. Sci. U.S.A.* **79,** 7910–7914.
22. Davidson, E. H.,Jacobs, H. T. & Britten, R. *Nature* **301,** 468–470 (1983).
23. Shapira, S. K., Chou, J. Richaud, F. & Casadaban, M. J. *Gene* (in the press).
24. Alton, N. K. & Vapnek, D. *Nature* **282,** 864–869 (1979).
25. Kohlhaw, G. in *Biotechnology: Amino Acid Biosynthesis and Regulation* Vol. 1 (eds Herrman, K. H. & Somerville, R. S.) (Addison-Wesley, London, in the press).
26. Sherman, F., Fink, G. R. 8 Lawrence, C. *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, New York, 1979).
27. Ratzkin, B. & Carbon, J. *Proc. natn. Acad. Sci. U.S.A.* **74,** 487–491 (1977).
28. Miller, J. *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, New York, 1972).
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).

Expression and regulation of human low-density lipoprotein receptors in Chinese hamster ovary cells

Robert D. Sege, Karen Kozarsky, David L. Nelson & Monty Krieger*

Department of Biology, Whitaker College of Health Sciences, Technology and Management, and the Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Low-density lipoprotein (LDL), the major cholesterol transport component of human plasma, delivers cholesterol to mammalian cells via the LDL pathway of receptor-mediated endocytosis[1]. LDL receptor activity and cholesterol biosynthesis are coordinately regulated by cholesterol-mediated feedback suppression[2–4]. We have developed methods for the isolation of mutant[5,6] and revertant (M.K., unpublished data) Chinese hamster ovary (CHO) cells having alterations in cholesterol biosynthesis and in the receptor-mediated endocytosis of LDL. The defective locus of one LDL receptor-negative CHO mutant (clone 7a-1)[6] is apparently the structural gene for the LDL receptor (D. Kingsley, M. Segal and M.K., unpublished data). Here we have transfected 7a-1 cells with human DNA and selected colonies which synthesize functional human LDL receptors whose expression is regulated normally. This selection may prove useful for cloning genes required for the receptor-mediated endocytosis of LDL and other ligands and thus for elucidating the molecular defects responsible for familial hypercholesterolaemia, one of the most common genetic diseases in humans[7].

Human DNA was introduced into the LDL receptor-negative clone 7a-1 by the $CaPO_4$ method[8,9] and cells were selected in MeLoCo medium as described in Fig. 1 legend. In this selective medium the cells die from cholesterol deprivation unless they can obtain cholesterol by the endocytosis of LDL[5,6,10,11]. Colonies survived continuous selection in MeLoCo medium at a frequency of 2×10^{-7} (6 colonies per 3×10^7 cells). This frequency of survival was ~1,000-fold lower than that obtained when the neomycin analogue G418 was used to select pSV2-neo transfected cells[12] (data not shown). Two of the colonies which survived MeLoCo selection, designated LET-1 and LET-2 (LDL endocytosis transfectants), were selected for further study. These colonies contained discrete DNA sequences homologous to human repetitive DNA (*Alu* family), whereas untreated 7a-1 cells did not (Fig. 1).

The expression of the LDL receptor pathway in LET-1 and LET-2 was compared with that in parental CHO and receptor-negative 7a-1 cells by measuring the specific cellular binding,

* To whom reprint requests should be addressed.

0028–0836/84/080742—04$01.00

© 1984

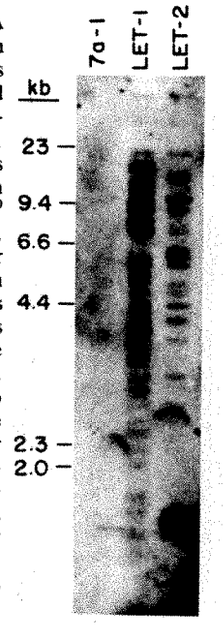


Fig. 1 Detection of human repetitive DNA sequences in CHO cells following transfection and selection. All cells were cultured in Ham's F-12 media supplemented with the indicated sera as described previously[5,6,13]. DNA, isolated from LDL receptor-negative clone 7a-1 and transfected LET-1 and LET-2 cells, was digested with *Eco*RI and analysed by Southern blot[28,29] hybridization with ^{32}P-labelled[30] cloned human *Alu* family (BLUR11)[31] DNA. Kodak XAR-5 film was exposed at −70 °C for 20 h with a Cronex Lightning Plus screen (Dupont). LDL endocytosis transfectants (LETs) were obtained as follows: 7a-1 cells were transfected with human DNA using the $CaPO_4$ technique[9] with minor modifications. DNA was extracted[32] from portions of two human fetal livers which were pulverized in the presence of liquid nitrogen[33] and further purified by sequential digestion with ribonuclease A (Sigma) and proteinase K (Boehringer-Mannheim). 24 h before transfection, the cells were plated (7.5×10^5 cells per 100 mm plate) in 10% newborn calf serum (NCS) and the DNA was sheared three times through a 21-gauge needle (final length >45 kilobases (kb) by agarose electrophoresis) and precipitated by overnight incubation with ethanol at −20 °C. The precipitate was dissolved (30 μg ml^{-1}) in HEPES-buffered saline (HBS), *p*H 7.1. The $CaPO_4$/DNA coprecipitate was allowed to form for 45 min at room temperature, and added (1.0 ml per dish) to cell monolayers from which medium had been removed. After incubation for 20 min at room temperature, fresh 10% NCS was added and the plates were incubated at 37 °C for 4.5 h. Cells were then treated with 15% glycerol in HBS; 2 days later, cells were replated (7.5×10^5 cells per 100 mm dish) in selective medium (3% newborn calf lipaprotein deficient serum (NC-LPDS) containing a mixture of mevalonate (238 μM), LDL (2.8 μg protein ml^{-1}) and compactin (38 μM), called MeLoCo)[5,6,10,11]. The compactin inhibits synthesis of mevalonate and thus inhibits synthesis of steroidal and non-steroidal isoprenoids; the trace levels of mevalonate permit the synthesis of non-steroidal but not steroidal isoprenoids; and the LDL serves as a source of sterol for cells which express the LDL pathway. Most of the cells began to die within 3 days and macroscopic colonies of survivors were visible by eye after 13–17 days of selection. These colonies (LETs) were picked[34] and subsequently maintained in selective medium. An analogous selection relying on the reversal of nutritional deficiencies by receptor-mediated delivery of inorganic ions has been suggested[35].

uptake and degradation of ^{125}I-LDL (10 μg protein ml^{-1})[13]. Relative degradation activities were: parental CHO, 100%; LET-1, 184%; LET-2, 93%; and 7a-1, 2%. The saturation kinetics of ^{125}I-LDL degradation by LET-1 (Fig. 2) and LET-2 (data not shown) cells exhibited high affinity and saturability, hallmarks of the classic LDL pathway[14]. The affinity of LET-1 cells for LDL at 37 °C (K_m 16 μg protein ml^{-1}) was twice as strong as that of parental CHO cells (K_m 35 μg protein ml^{-1}) and was essentially equal to that of human LDL receptors (K_m 17 μg protein ml^{-1})[15]. LET-1 bound twofold more LDL than CHO cells at 4 °C (data not shown), and the ratio of ^{125}I-LDL internalization and degradation to surface binding, that is, the efficiency of the LDL pathway[16], in LET-1 cells (ratio 23) was essentially identical to that in CHO cells (ratio 24, data not shown).

These results suggested that LET cells survived selection because transfection and expression of the human LDL receptor gene complemented the mutation in clone 7a-1. Therefore, synthesis of the human LDL receptor (an ~160,000-molecular weight (MW) monomeric glycoprotein)[17] was examined by immunoprecipitation and electrophoresis[18]. A previously described[19] monoclonal antibody against bovine LDL receptor (IgG C7) cross-reacted with and inhibited LDL binding to human LDL receptors (Fig. 3*a* and ref. 19) but not to LDL receptors from CHO cells (Fig. 3*b* and ref. 19). No specific immunoprecipitable protein was detected in 7a-1 cells

Table 1 Degradation of ^{125}I-LDL by LET-1 cells: inhibition by anti-human receptor antibody and regulation by LDL and sterols

	^{125}I-LDL degradation (% of control)	
	Parental CHO cells	LET-1 cells
Expt *a*		
Additions to assay medium		
None	100	100
IgG C7 (190 nM)	104	49
IgG 2001 (190 nM)	121	117
Expt *b*		
Additions to growth medium		
None	100	100
LDL (300 μg protein ml^{-1})	25	35
Cholesterol (12.5 μg ml^{-1})	72	77
Cholesterol + 25-hydroxy-cholesterol (0.5 μg ml^{-1})	24	39

Expt *a*: On day 0, parental CHO and LET-1 cells were plated in 1.5 ml of 3% NC-LPDS in 24-well plates (60,000 cells per well). On day 2, each monolayer was preincubated for 1 h with 0.5 ml of 3% NC-LPDS containing either no additions or 190 nM of anti-bovine (human) LDL receptor monoclonal antibody (IgG C7) or control antibody (IgG 2001). Each monolayer was re-fed with 5% H-LPDS containing the same additions plus 10 μg protein ml^{-1} of ^{125}I-LDL (247 c.p.m. ng^{-1}). After incubation at 37 °C for 5 h, the amount of ^{125}I-LDL degradation products released into the medium was determined. The values are expressed as a percentage of the high affinity values for the cells incubated in the absence of antibody. The 100% control values were: CHO, 1,795 ng per 5 h per mg; LET-1, 4,034 ng per 5 h per mg. Expt *b*: In a separate experiment, cells were plated on day 0 as described above. On day 1, the medium was replaced with 1 ml of 3% NC-LPDS containing the indicated additions of LDL, cholesterol in ethanol or cholesterol plus 25-hydroxycholesterol. On day 2 (23 h later), each monolayer was washed once with phosphate-buffered saline, then 0.5 ml of 5% H-LPDS containing 10 μg protein ml^{-1} of ^{125}I-LDL (446 c.p.m. per ng protein) was added and high affinity degradation was determined. The high affinity values are expressed as the percentage of the values for the same cells incubated with no additions. The 100% control values were: CHO, 1,293 ng per 5 h per mg; LET-1, 2,698 ng per 5 h per mg. The thrombin used to isolate H-LPDS was given by the Parke-Davis Division of Warner Lambert.

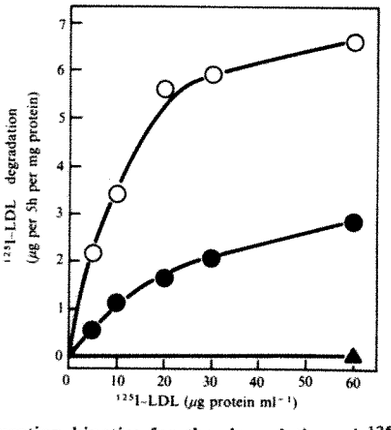


Fig. 2 Saturation kinetics for the degradation of ^{125}I-LDL at 37 °C by monolayers of parental CHO, LDL receptor-negative clone 7a-1 and LET-1 cells. On day 0, parental CHO (●), clone 7a-1 (▲) and LET-1 (○) cells were seeded in 1.5 ml of 3% NC-LPDS into 24-well plates (60,000 cells per well). On day 2, each monolayer received 0.5 ml of 5% human lipaprotein deficient serum (H-LPDS) containing the indicated amounts of ^{125}I-LDL (442 c.p.m. per ng of protein). After incubation at 37 °C for 5 h, the amounts of ^{125}I-LDL degradation products released into the media were determined as described previously[13]. Data are expressed as high affinity values for degradation.

(Fig. 3*a*, *b*). IgG C7 specifically precipitated one protein of MW ~ 160,000 from both LET-1 (Fig. 3*a*, *b*) and LET-2 (Fig. 3*b*). IgG C7 also specifically inhibited degradation of ^{125}I-LDL by LET-1 but not by parental CHO cells (Table 1, expt *a*). Thus, human LDL receptors in these hamster cells are respon-

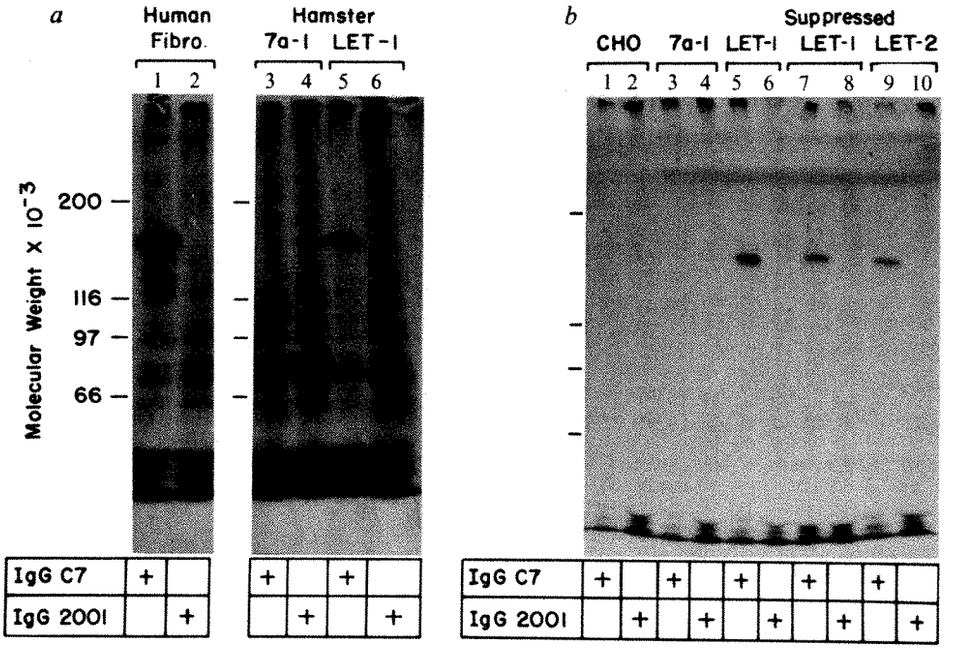


Fig. 3 Immunochemical detection of human LDL receptors in transfected hamster (LET) cells: regulation of expression by LDL. *a*, On day 0, normal human diploid fibroblasts (GM3349)[13] were plated in 3 ml of 10% fetal bovine serum in 60 mm dishes (50,000 cells per dish). On day 2, the monolayers were re-fed, then on day 5 they were washed and re-fed with 3 ml of 10% H-LPDS. In addition, LDL receptor-negative clone 7a-1 cells and human DNA transfectants of clone 7a-1 (LET-1) were plated on day 5 in 3 ml of 3% NC-LPDS (300,000 cells per 60 mm dish). On day 6, all monolayers were re-fed with either 10% H-LPDS (fibroblasts) or 3% NC-LPDS (7a-1 and LET-1 cells) prepared using methionine-free Ham's F-12 medium (K-C Biologicals) supplemented with 50 μCi ml^{-1} of ^{35}S-methionine (NEN) and 5 μM unlabelled methionine (Sigma). After 16 h, the monolayers were washed, lysed, and immunoprecipitated with either anti-bovine (and human) LDL receptor antibody (IgG C7: lanes 1, 3 and 5) or control antibody (IgG 2001: lanes 2, 4 and 6) as described[18]. Each lane represents immunoprecipitates derived from 6.4×10^7 (A) or 10^8 (B) c.p.m. of labelled cell extract. The immunoprecipitates were subjected to SDS-6% polyacrylamide gel electrophoresis[36] (7 mA per gel) for 18 h, impregnated with Autofluor (National Diagnostics), dried and subjected to autoradiography (Kodak XAR-5 film at −70 °C for 7 days with a Cronex Lightning Plus screen). *b*, Parental CHO, 7a-1, LET-1 and LET-2 cells were plated on day 5 as described above except that the LET-1 monolayers were divided into two groups. The first group (lanes 5 and 6) was plated in 3% NC-LPDS as were the other cell strains (see above). The second group, 'suppressed LET-1' (lanes 7 and 8), was plated and labelled in 3% NC-LPDS containing LDL (300 μg protein ml^{-1}). The cells were processed for immunoprecipitation with IgG C7 or IgG 2001 as indicated, and for electrophoresis and fluorography (24 h exposure) as described above.

sible for the receptor-mediated endocytosis of LDL.

A distinctive feature of the LDL pathway is the feedback suppression of receptor activity by sterols[2]. When parental CHO and LET-1 cells were incubated with regulatory sterols (cholesterol, the potent analogue 25-hydroxycholesterol[20], or esterified cholesterol delivered by LDL[2,21]), receptor activity was suppressed to essentially the same extent (Table 1, expt *b*). Receptor activity in LET-2 cells was similarly suppressed by LDL (data not shown). The sterol-mediated suppression of LDL receptor activity in human fibroblasts is due to a decrease in the number of LDL receptors[19]. Similarly, the suppression of LDL receptor activity in LET-1 cells was associated with a reduction in the amount of immunoprecipitable LDL receptor protein (Fig. 3*b*, compare lane 7 with lane 5). Thus, the expression of the human LDL receptor gene in transfected hamster cells was subject to the same sterol-mediated regulatory control as that observed in normal human[2] and hamster[13] cells.

The present studies show that selection in MeLoCo medium can be used to isolate receptor-positive transfectants from a population of receptor-negative (mutant) cells. Using the method of Shih and Weinberg[22], it should be possible to clone the LDL receptor gene and combine the techniques of *in vitro* gene manipulation (for example, see ref. 23) and MeLoCo selection to explore the mechanism by which the expression of LDL receptors is regulated. Also, it may be possible to exploit previously described selection methods[5,6] to isolate mutant LET cells having defects in human LDL receptors and compare these mutant LDL receptors with the naturally occurring mutant LDL receptors from familial hypercholesterolaemia patients[18].

Chemical modification of LDL can be used to re-target the delivery of cholesterol through other surface receptors[24,25,37]; therefore, it may be possible to use MeLoCo selection to express a wide variety of exogenous receptors (including tissue-specific receptors) in CHO cells and eventually to clone the genes for these receptors. This approach complements a previously described two-step procedure in which cells co-transfected with genomic DNA and a selectable gene were isolated and screened by flow cytometry for the expression of surface antigens (for example, the transferrin receptor[26] and histocompatibility antigens[27]). In contrast, MeLoCo medium directly selects for receptor function without immunochemical reagents. MeLoCo selection may prove useful for cloning the genes, other than those of receptors, which have been defined by somatic cell mutations to be required for the expression of LDL receptors (ref. 13 and D. Kingsley, M. Segal and M.K., in preparation).

We thank Julianna Martin and Marsha Penman for technical assistance; Kathy Thurmond for preparation of the manuscript; Drs J. Goldstein, M. Brown, W. Schneider and Y.-K. Ho for providing the monoclonal antibodies and for advice about immunoprecipitations; Dr R. Rosenberg for permitting access to his γ-spectrophotometer; Dr A. Schwartz for providing the liver specimens; Dr A. Endo for supplying compactin; S. Lewis for the gift of psv2-neo DNA; Dr D. Housman, Dr J. Cunningham and R. Cone for advice regarding transfection methodology; and D. Kingsley for a critical review of the manuscript. This research was supported by a March of Dimes Basil O'Connor Research Grant and a NIH research grant. M.K. was the recipient of a NIH Research Career Development Award and R.D.S. was supported by a NIH Medical Scientist Training Program grant (GM07753) through Harvard Medical School.

Received 20 October; accepted 2 December 1983.

1. Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. *Nature* **279,** 679–685 (1979).
2. Brown, M. S. & Goldstein, J. L. *Cell* **6,** 307–316 (1975).
3. Brown, M. S. & Goldstein, J. L. *Proc. natn. Acad. Sci. U.S.A.* **76,** 3330–3337 (1979).
4. Brown, M. S. & Goldstein, J. L. *J. biol. Chem.* **249,** 7306–7314 (1974).
5. Krieger, M., Brown, M. S. & Goldstein, J. L. *J. molec. Biol.* **150,** 167–184 (1981).
6. Krieger, M., Martin, J., Segal, M. & Kingsley, D. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5607–5611 (1983).
7. Goldstein, J. L. & Brown, M. S. in *The Metabolic Basis of Inherited Disease* (eds Stanbury, J. B., Wyngaarden, J. B., Frederickson, D. S., Goldstein, J. L. & Brown, M. S.) 672–712 (McGraw-Hill, New York, 1983).
8. Graham, F. L. & van der Eb, A. J. *Virology* **52,** 456–467 (1973).
9. Parker, B. A. & Stark, G. R. *J. Virol.* **31,** 360–369 (1979).
10. Goldstein, J. L., Helgeson, J. A. S. & Brown, M. S. *J. biol. Chem.* **254,** 5403–5409 (1979).
11. Krieger, M. *Analyt. Biochem.* (in the press).
12. Southern, P. J. & Berg, P. *J. molec. appl. Genet.* **1,** 327–341 (1982).
13. Krieger, M. *Cell* **33,** 413–422 (1983).
14. Brown, M. S. & Goldstein, J. L. *Science* **191,** 150–154 (1976).
15. Krieger, M., Brown, M. S., Faust, J. R. & Goldstein, J. L. *J. biol. Chem.* **253,** 4093–4101 (1978).

16. Goldstein, J. L., Brown, M. S. & Stone, N. J. *Cell* **12,** 629–641 (1977).
17. Schneider, W. J., Beisiegel, U., Goldstein, J. L. & Brown, M. S. *J. biol. Chem.* **257,** 2664–2673 (1982).
18. Tolleshaug, H., Hobgood, K. K., Brown, M. S. & Goldstein, J. L. *Cell* **32,** 941–951 (1983).
19. Beisiegel, U., Schneider, W. J., Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. *J. biol. Chem.* **256,** 11923–11931 (1981).
20. Krieger, M., Goldstein, J. L. & Brown, M. S. *Proc. natn. Acad. Sci. U.S.A.* **75,** 5052–5056 (1978).
21. Krieger, M., McPhaul, M. J., Goldstein, J. L. & Brown, M. S. *J. biol. Chem.* **254,** 3845–3853 (1979).
22. Shih, C. & Weinberg, R. A. *Cell* **29,** 161–169 (1982).
23. Zuniga, M. C. *et al. Cell* **34,** 535–544 (1983).
24. Murray, G. J. & Neville, D. M. *J. biol. Chem.* **255,** 11942–11948 (1980).
25. Brown, M. S., Goldstein, J. L., Krieger, M., Ho, Y.-K. & Anderson, R. G. W. *J. Cell Biol.* **82,** 597–613 (1979).
26. Newman, R., Domingo, D., Trotter, J. & Trowbridge, I. *Nature* **304,** 643–645 (1983).
27. Kavathas, P. & Herzenberg, L. A. *Proc. natn. Acad. Sci. U.S.A.* **80,** 524–528 (1983).
28. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
29. Wahl, G. M., Stern, M. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76,** 3683–3687 (1979).
30. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113,** 237–251 (1977).
31. Jelinek, W. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **77,** 1398–1402 (1980).
32. Graham, D. E. *Analyt. Biochem.* **85,** 609–613 (1978).
33. Blin, N. & Stafford, D. W. *Nucleic Acids Res.* **3,** 2303–2308 (1976).
34. Thompson, L. H. *Meth. Enzym.* **58,** 308–322 (1979).
35. Block, T. & Bothwell, M. *Nature* **301,** 342–344 (1983).
36. Laemmli, U. K. *Nature* **227,** 680–685 (1970).
37. Leichtner, A. L. & Krieger, M. *J. Cell Sci.* (in the press).

Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells

W. J. King & G. L. Greene*

Ben May Laboratory for Cancer Research, University of Chicago, 5841 South Maryland, Chicago, Illinois 60637, USA

Although it is widely accepted that specific intracellular receptor proteins are involved in the oestrogenic regulation of gene expression and growth in reproductive tissues, the precise nature of the regulation is poorly understood. Among the unresolved issues are the distribution and dynamics of the oestrogen receptor protein (oestrophilin) in target tissues in the presence and absence of oestrogens and antioestrogens. The use of radiolabelled and unlabelled receptor ligands to detect and measure oestrogen receptors in tissues has been complicated by the presence of other intracellular steroid-binding proteins[1] and by the low concentration of receptors in responsive tissues. We report here the development of an immunocytochemical procedure that is suitable for localizing oestrophilin directly in frozen tissue sections or cells from human and several non-human sources. When monoclonal antibodies to oestrophilin were used to detect receptor in various oestrogen-sensitive tissues, specific staining was confined to the nucleus of all stained cells, suggesting that both cytosol and nuclear forms of the receptor protein may reside in the nuclear compartment.

Five monoclonal rat antibodies (D547Spγ, D58P3μ, D75P3γ, H222Spγ, and H226Spγ)[2–5], which have been shown to be oestrogen receptor-specific by several criteria, were used individually to localize oestrophilin by an indirect immunoperoxidase technique[6] in frozen, fixed sections of human breast tumours (Fig. 1), human uterus, rabbit uterus (Fig. 2), and in other mammalian reproductive tissues, as well as in fixed MCF-7 cell cultures (Fig. 3). The nuclear staining observed in each case fulfilled all of the accepted criteria for specificity[7,8], including its dependence on the presence (compare Figs 1*b*, 2*b* and 3*b* with 1*a*, 2*a* and 3*a*) and concentration of monoclonal antioestrophilin and the susceptibility of such staining to competitive inhibition by highly purified oestrophilin (Figs 1*e*, 2*e*). Specific nuclear staining was either absent or limited to a few cells in receptor-poor breast cancers (Fig. 4); nontarget tissues such as colon epithelium were always negative. Specific staining for oestrophilin in MCF-7 cells, like that in many human breast tumours, was heterogeneous and also confined to the nucleus (Fig. 3). Staining heterogeneity may reflect the polyclonal origins of these specimens[9], or it may be related to the physiological status (for example, cell cycle) of the cell populations. Little or no specific cytoplasmic staining for oestrophilin has been observed in any of the tissues or tumour cells examined thus far, including those which have been deprived of exogeneous oestrogens for extended times due to postmenopausal amenorrhea or experimental intervention.

For basic studies of receptor dynamics in oestrogen-responsive tissues and cells, the immunoperoxidase technique must be applicable to animal models and cultured tumour cells. Two monoclonal rat antibodies, H222Spγ and H226pγ, which cross-react with all tested mammalian and avian oestrophilins[10], were used to test the method on oestrogen target tissues from the rabbit. A pattern of predominantly nuclear localization identical to that found in human tissues was observed in sections of rabit uterus (Figs 2*a*, 2*d*), oviduct, corpus luteum, mammary gland, pituitary and liver, but not in colon epithelium. The specificity of the staining patterns in rabbit tissues was confirmed by application of the same controls used for human breast tumours, as shown for rabbit uterus in Figs 2*b*, 2*c* and 2*e*. Animals used in this study were either sexually immature or experimentally deprived of endogenous oestrogens by ovariectomy or induction of pseudopregnancy[11]. In each case, 70% to 95% of the unoccupied oestrophilin was found in low-salt cytosols, as determined by steroid-binding assays (see legend to Fig. 2).

The nuclear localization of immunoperoxidase staining for oestrogen receptor is surprising as the generally accepted hypothesis is that receptor resides primarily in the cytoplasm of target cells in the absence of steroid[12–14]. However, early autoradiographic evidence that radioactive steroid incorporated by rat uteri on exposure to ^{3}H-oestradiol at 2 °C *in vitro* is mostly extranuclear[15] has recently been challenged on the basis of similar autoradiographic studies in which binding in the cold appeared to be predominantly nuclear[16]. Similar histochemical[17–19] and immunohistochemical[20,21] evidence for cytoplasmic receptor sites has been challenged on the basis that the oestrogen derivatives used are bound to lower-affinity intracellular binding sites other than oestrogen receptor[1] or that receptor diffuses out of unfixed frozen sections during localization[22]. A recent report[23] of cytoplasmic localization of oestrogen receptor with polyclonal antioestrophilin antibodies raised against an impure preparation of human breast tumour oestrophilin lacks supporting evidence of complete antibody specificity for oestrophilin.

It is unlikely that the absence of cytoplasmic staining in the current study is due to the duration of fixation, solubilization of the receptor protein, or to artifactual translocation of receptor during fixation or processing of tissues or cells. The uniformity of our results under a variety of conditions, including treatment of tissues and intact cells with both precipitating and crosslinking fixatives before and after sectioning or freezing, and at temperatures ranging from 4 to 37 °C, suggests that receptor is being permanently immobilized without redistribution. Extending fixation times beyond 1–2 h results only in a progressive loss of nuclear staining without any increase in cytoplasmic staining above background levels. In addition, cytosolic oestrophilin covalently labelled with ^{3}H-tamoxifen aziridine, an antioestrogen derivative[24], precipitates quantitatively in 35% aqueous ethanol and remains insoluble when exposed to PBS (unpublished observation). Receptor-containing tissue sections treated with the same fixative show only nuclear staining.

These immunocytochemical results indicate that oestrogen receptor may reside primarily in target cell nuclei of oestrogen-sensitive tissues and tumours both in the presence and absence of steroid, analogous to what has been reported for the vitamin D and thyroid hormone receptors[25–29]. The oestrophilin recovered in the cytosol fraction of a homogenate would then represent receptor that is loosely associated with the nucleus and it would follow that the binding of oestradiol to its receptor leads to a tighter association, a phenomenon previously interpreted as indicating translocation of the receptor from the

* To whom correspondence should be addressed.

© 1984 Macmillan Journals Ltd

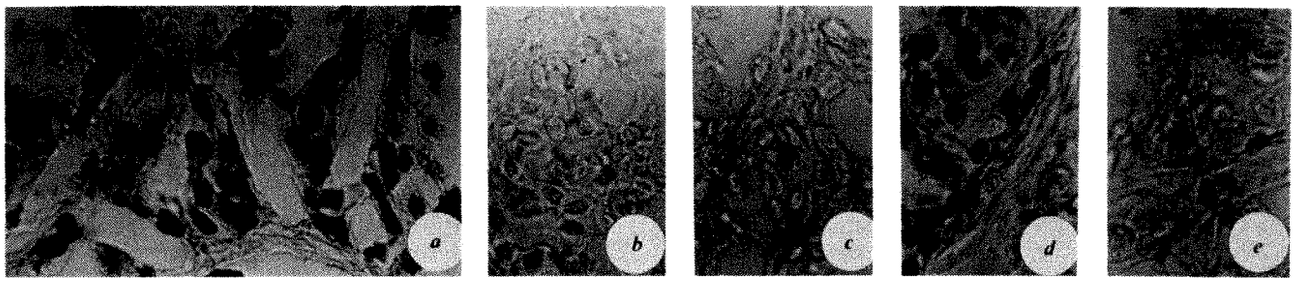


Fig. 1 Immunocytochemical localization of oestrogen receptor in a breast tumour taken from a 67-year-old postmenopausal woman, and which contained 1,546 fmol of cytosolic oestrogen receptor per gram wet weight, as measured by sucrose density gradient analysis[3]. Frozen sections (8 μm) were thaw-mounted on uncoated glass slides and fixed with picric acid-paraformaldehyde[30] for 10 min at 23 °C. After fixation, sections were rinsed with phosphate-buffered saline (PBS) and treated with a 10% (w/v) solution of ovalbumin in PBS to minimize the nonspecific binding of reagents in subsequent steps. Tissue sections were then incubated successively with one or more monoclonal antibodies, goat antibody to rat IgG, and rat peroxidase-antiperoxidase complex (PAP) for 30 min each at ambient temperature in a humidified chamber. Each incubation was followed by a 5-min wash with PBS. Specifically bound antibodies were visualized by immersing the sections for 10–30 min in a solution of diaminobenzidine (1.7 mM) and hydrogen peroxide (0.06%) in PBS. *a–e*, sections were treated identically, except for the first incubation which consisted of either (*a*) 2 μg ml^{-1} (13.4 pmol ml^{-1}) of H226Spγ antibody in PBS; (*b*) 2 μg ml^{-1} of purified normal rat IgG in PBS; (*c*) 2 μg ml^{-1} of H226Spγ in MCF-cytosol (20 pmol receptor per ml); (*d*) 2 μg ml^{-1} of H226Spγ in receptor-depleted (by selective adsorption to estradiol-Sepharose[4]) MCF-7 cytosol (2 pmol receptor per ml); or (*e*) 2 μg ml^{-1} of H226Spγ in receptor-depleted MCF-7 cytosol supplemented with 20 pmol ml^{-1} of highly purified (~90% pure) oestrophilin[10,31]. Original magnification, ×400. The specificities of the antibodies used in this study were tested extensively by Western blot, sucrose gradient[2–4], double antibody precipitation[2] and indirect enzyme immunoassay (EIA) techniques. Regardless of the technique employed, each of the antibodies appears to be completely specific for oestrogen receptor. When ^{35}S-labelled H222Spγ and H226Spγ IgGs were used to detect immunoreactive proteins on nitrocellulose blots of SDS gels after electrophoresis of low and high salt extract of MCF-7 cells, both antibodies recognized a single protein band (molecular weight, 65,000), which corresponded to highly purified receptor. The same band was obtained when crude oestrophilin was isolated by immunoadsorption to a column of D547Spγ-Sepharose and eluted with SDS.

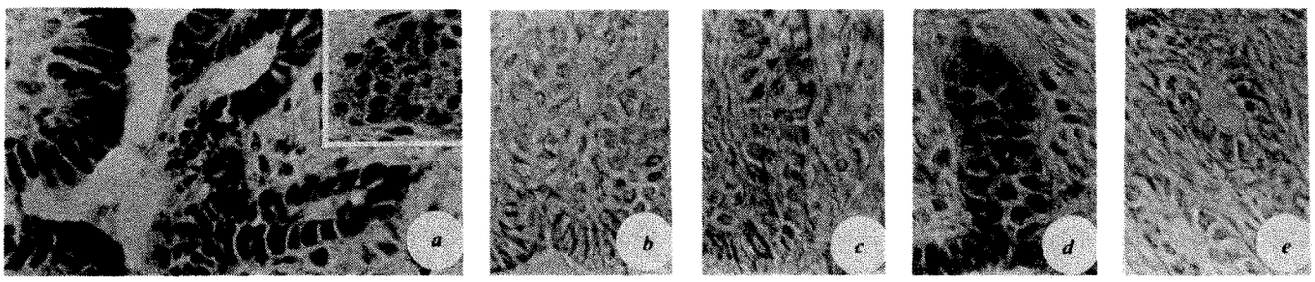


Fig. 2 All procedures were the same as Fig. 1, except that sections were prepared from a uterus taken from a rabbit ovariectomized 8 days prior to killing. Uteri from similarly treated rabbits were found to contain 3.15 ± 0.85 pmol cytosolic receptor per mg of DNA ($n = 3$) and 0.16 ± 0.01 pmol of high-salt (0.4 M KCl) extractable receptor per mg of DNA, as measured by exchange assay with ^{3}H-oestradiol[32]. The insert in the upper right corner of Fig. 2*a* represents rabbit myometrium.

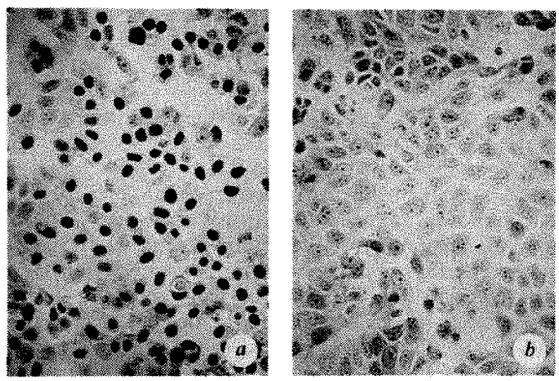


Fig. 3 Localization of oestrogen receptor in MCF-7 human breast cancer cells. Cells were grown on chambered glass slides, fixed for 10 min with picric acid-paraformaldehyde[30] and oestrogen receptor was localized as described in Fig. 1 using 10 μg ml^{-1} of H226Spγ antibody in PBS (*a*). Cells shown in (*b*) were treated identically except that 10 μg ml^{-1} of normal rat IgG was substituted for the monoclonal antibody.

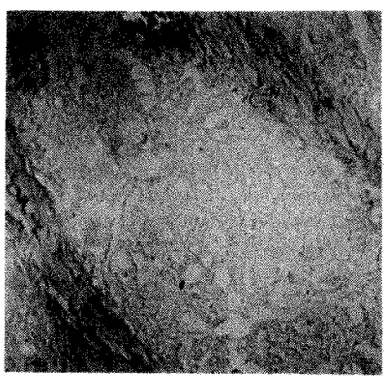


Fig. 4 Absence of nuclear staining in a human receptor negative (0 fmol g^{-1} tissue) breast tumour from a postmenopausal patient. Staining conditions were as described in Fig. 1, using 10 μg ml^{-1} of H226Spγ antibody in PBS. Original magnification, ×400.

cytoplasm to the nucleus[12]. In support of our hypothesis, we have seen little or no increase in nuclear staining intensity in MCF-7 cells and immature or ovariectomized rabbit uteri following short-term treatment of cells or animals with oestradiol. Our observations are clearly inconsistent with the concept of steroid-induced receptor translocation, suggesting that the classical 'two-step' model[12] for oestrogen action requires reinterpretation.

We acknowledge Dr Larry Miller of Abbott Laboratories, North Chicago, who developed the H226 and H222 antibodies used in this study. We were supported by research grants from the ACS (BC-86), Abbott Laboratories, NCI (CA-02897), and by the Women's Board of the University of Chicago Cancer Research Foundation. W.J.K. was supported by a postdoctoral training grant (NIH/NCI 09183) and by the NCI (CA 14599).

Received 29 July; accepted 13 December 1983.

1. Mercer, W. D. *et al. Cancer Res.* **41**, 4644–4652 (1981).
2. Greene, G. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 5115–5119 (1980).
3. Jensen, E. V. *et al. Rec. Prog. Horm. Res.* **38**, 1–34 (1982).
4. Greene, G. L. & Jensen, E. V. *J. steroid Biochem.* **16**, 353–359 (1982).
5. Miller, L. S. *et al. Fedn Proc.* **41**, Abstr. 1459 (1982).
6. Sternberger, P. H. *et al. J. histochem. Cytochem.* **18**, 315–333 (1970).
7. Childs, G. V. *J. histochem. Cytochem.* **31**, 168–176 (1983).
8. Petrusz, P. *J. histochem. Cytochem.* **31**, 177–179 (1983).
9. Poulsen, H. S. *et al. Cancer* **48**, 1791–1793 (1981).
10. Greene, G. L. in *Biochemical Actions of Hormones* Vol. 11 (ed. Litwack, G.) (Academic, New York, in the press).
11. Bender, E. M. *et al. Endocrinology* **103**, 1937–1943 (1978).
12. Jensen, E. V. & DeSombre, E. R. *Science* **182**, 126–134 (1973).
13. Gorski, J. & Gannon, F. *A. Rev. Physiol.* **38**, 425–450 (1976).
14. Muldoon, T. G. *Endocrine Rev.* **1**, 339–364 (1980).
15. Jensen, E. V. *et al. Proc. natn. Acad. Sci. U.S.A.* **59**, 632–638 (1968).
16. Martin, P. M. & Sheridan, P. J. *J. steroid Biochem.* **16**, 215–229 (1982).
17. Lee, S. H. *Am. J. clin. Path.* **70**, 197–203 (1978).
18. Pertschuk, L. P. *et al. Am. J. clin. Path.* **71**, 504–508 (1979).
19. Rao, B. R. *et al. Cancer* **46**, 2902–2906 (1980).
20. Pertschuk, L. P. *et al. Cancer* **41**, 907–911 (1978).
21. Nenci, I. *et al. J. steroid Biochem.* **7**, 505–510 (1976).
22. Underwood, J. *et al. J. clin. Path.* **35**, 401–406 (1982).
23. Raam, S. *et al. Eur. J. Cancer clin. Oncol.* **18**, 1–12 (1982).
24. Katzenellenbogen, J. A. *et al. J. biol. Chem.* **258**, 3487–3495 (1983).
25. DeGroot, L. J. & Strausser, J. L. *Endocrinology* **95**, 74–83 (1974).
26. Spindler, B. J. *et al. J. biol. Chem.* **250**, 4113–4119 (1975).
27. Oppenheimer, J. H. *et al. Rec. Prog. Horm. Res.* **32**, 529–565 (1976).
28. Lawson, D. E. M. & Wilson, P. W. *Biochem. J.* **144**, 573–583 (1974).
29. Walters, M. R. *et al. J. biol. Chem.* **255**, 6799–6805 (1980).
30. Stefani, M. *et al. Nature* **216**, 173–174 (1967).
31. Greene, G. L. in *Gene Regulation by Steroid Hormones II* (eds Roy, A. K. & Clark, J. H.) (Springer, New York, in the press).
32. Sica, V. *et al. Biochemistry* **19**, 83–88 (1980).

Nuclear localization of unoccupied oestrogen receptors

Wade V. Welshons, Mara E. Lieberman & Jack Gorski

Departments of Biochemistry, Human Oncology and Animal Science, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA

According to the current model of steroid hormone action oestrogen is thought to bind to its receptor in the cytoplasm of target cells[1,2] and the oestrogen–receptor complex is then translocated into the nucleus[2–4]. This model is based on evidence obtained in homogenized cell preparations in which free receptor is associated with the cytosol, whereas steroid-bound receptor is associated with the nuclear fraction. Some data suggest, however, that the unfilled receptor may reside in the nucleus, and that cytosolic localization represents an extraction artefact. We have now reinvestigated the subcellular distribution of unfilled oestrogen receptor using cytochalasin B-induced enucleation to obtain cytoplast and nucleoplast fractions from receptor-containing GH_3 cells derived from rat pituitary tumours. We found that cytoplasts prepared from GH_3 cells contain little oestrogen-binding activity and that most of the unfilled oestrogen receptors are associated with the nuclear fraction. We therefore suggest that the standard model is in error and that the unoccupied receptor is nuclear in the intact cell.

If unfilled oestrogen receptor is cytoplasmic, the receptor concentration in the cytoplasts should be greater than the concentration in whole cells. In fact, when cells were enucleated (Fig. 1) the concentration of receptor relative to protein was greatly reduced in cytoplasts and increased in the fraction containing cells and nucleoplasts compared with intact cells (Fig. 2). In contrast, receptor relative to DNA was similar in all cell preparations (Fig. 2), indicating that the receptor is associated with nuclear rather than cytoplasmic elements. In an experiment using cytoplasts that contained particularly few contaminating whole cells (0.25%), the concentration of receptor relative to protein was less than 10% of that found in intact cells (Table 1). In four enucleation experiments the recovery of protein, DNA or oestrogen receptor was 93, 97 or 104%, respectively. The essentially complete recovery indicates that receptor was not selectively lost from cytoplasts.

The cell + nucleoplast fraction was further fractionated by centrifugation to obtain cells from which a larger proportion of the cytoplasm had been removed. Receptor more than doubled relative protein (Fig. 3, fraction C), whereas the receptor: DNA ratio was constant.

The cytoplasts obtained following enucleation are approximately one-fifth the size of whole cells, according to protein content (Table 1) and 75% or more of the cells appear to form cytoplasts (Fig. 1). The cytoplasts exclude trypan blue (98%), incorporate ^{3}H-leucine into protein generally and prolactin specifically (data not shown). Specific oestrogen binding by whole cells (or whole cytoplasts) was saturable with K_d of 3.4×10^{-10} M by Scatchard analysis, and there were usually 20,000–30,000 oestrogen-binding sites per cell.

Incubation of whole cells in enucleation medium (Percoll, cytochalasin B, dimethyl sulphoxide (DMSO)) did not significantly change the number of receptors per cell or the distribution of receptor between the cytosol and the crude nuclear pellet in homogenized cells (data not shown). This confirms earlier studies with intact uteri in which cytochalasin had no effect on the uptake of oestradiol, the binding, or the distribution of filled receptors in homogenized tissue[5].

Our finding that the oestrogen receptor is predominantly nuclear, whether or not it is bound to the steroid, contradicts a large body of evidence which has been interpreted to indicate that the free oestrogen receptor is a cytoplasmic protein. There are, however, several earlier reports in the literature which are compatible with our findings. These include autoradiographic localization of most of hormone binding to the nucleus in 'non-translocation' conditions[6], appearance of 4S 'cytoplasmic' receptor in nuclei of some tissue before 5S 'nuclear' forms which appear after binding of the hormone[7], and evidence that some oestrogens produce full physiological effects even though the receptor appears entirely in cytosol extracts of homogenized cells[8,9]. The unfilled vitamin D receptor and thyroid hormone receptor have been reported to be nuclear in homogenized cells[10,11], and cell lines have been described in which unfilled oestrogen receptor remains nuclear in homogenized cells[12] until nuclei are washed and extracted more rigorously[13].

In contrast to earlier immunohistochemical studies which showed cytoplasmic localization of receptor[14–16], a recent immunocytochemical study indicates that antibodies to the oestrogen receptor interact only with nuclei of human endometrium or human mammary tumour cells, whether or not oestrogen is present[17].

In the present study we used GH_3 cells, a clonal strain derived from a rat pituitary tumour[18,19] that contain oestrogen receptors[20] localized in the cytosol after the usual cell disruption procedures[20,21]. GH_3 cells, including the specific strain used here, respond to oestrogen by increased prolactin synthesis[22,23]. The concentration of oestrogen receptor in cytoplasts was only 5–10% of that observed in intact cells. This was not due to degradation of receptor during enucleation, because all of the binding activity was recovered in the fraction enriched in nuclei. It is unlikely that the cytoplasts were formed from a small, atypical subpopulation of cells that were deficient in receptor,

© 1984 Macmillan Journals Ltd

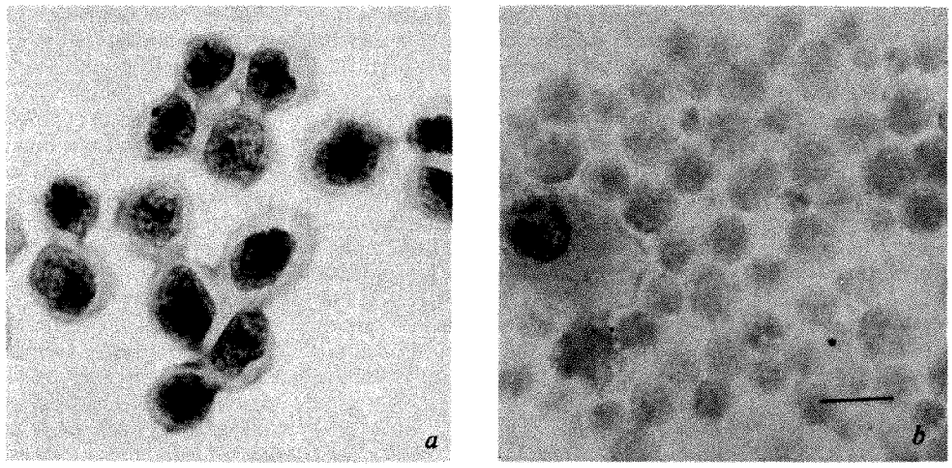


Fig. 1 Feulgen-stained whole cells (*a*) and cytoplasts (*b*) or cells + nucleoplasts (*c*) after enucleation. Enucleation of GH_3 cells yielded cytoplasts with diameters up to approximately 50% that of whole cells, and nucleoplasts plus cells with varying amounts of cytoplasm. Scale bar, 10 μm.

Methods: GH_3 cells were grown in Dulbecco's minimum essential medium (DMEM, Gibco) containing HEPES 25 mM, penicillin (Sigma) 100 U ml^{-1}, Fungizone (Gibco) 0.5 μg ml^{-1} and 17.5% horse-fetal calf serum (Gibco) 6:1. Charcoal-stripped serum was used throughout, or for the last medium change, with similar results. Cells were removed from the tissue culture dish by incubating in 1 mM EDTA in calcium–magnesium-free Hanks' balanced salt solution + 25 mM HEPES (CMFH) for 10 min at 37 °C. Cells were enucleated by the cytochalasin B-equilibrium centrifugation method of Bossart *et al.*[24], with modifications. For preparation of cytoplasts that were contaminated with few nucleated cells, a narrow density range of cells was selected before enucleation. This was accomplished by applying cells ($20–40 \times 10^6$ per 13-ml tube) to a step-gradient of Percoll (Pharmacia) in CMFH, with steps of 1.04, 1.05, 1.058, 1.063 and 1.07 g ml^{-1}. After a 20-min spin at 400*g* in a swinging bucket centrifuge, the predominant band was collected, usually at 1.058–1.063 g ml^{-1}. The cells were diluted 5× in CMFH, pelleted (125*g*, 10 min) and resuspended in Percoll of 1.075 g ml^{-1} (for cells from 1.058–1.063 g ml^{-1}), containing cytochalasin B (Aldrich) at 20 μg ml^{-1} and solvent DMSO 20 μl ml^{-1}, $5–10 \times 10^6$ cells per ml. The cells were incubated at 37 °C for 45 min, then layered onto more of the Percoll plus cytochalasin B (10–20 μg ml^{-1}), 37 °C, in a clear ultracentrifuge tube, and then spun at 64,000*g* for 45 min at 37 °C in a Beckman SW41 swinging bucket rotor. At 1×10^7 cells per gradient (12 ml total), cytoplasts could be recovered with minimal contamination by whole cells (<1%), but for higher yields, up to 40×10^6 cells were applied, which resulted in cytoplast preparations that contained up to 5% whole cells. After centrifugation, the cytoplasts were collected from a middle quarter of the tube. The very fine uppermost band of dead cells was avoided. The lowest layer of cells + nucleoplasts was also collected, and after a 5× dilution with CMFH, fractions were pelleted (125*g* 10 min for cells, 275*g* 10 min for cytoplasts). Subsequent centrifugation for cells was at 80*g*, 10 min. Generally, 10×10^6 cells applied per gradient yielded 7.5×10^6 cytoplasts. For Feulgen staining, cells were fixed in 10% formalin in CMFH overnight, dried and ethanol-fixed onto slides, and stained by the Feulgen procedure. Cells were counterstained for 30 s in 0.5% Wool green (CI 44090, Aldrich) in 0.5% acetic acid, rinsed briefly in water, air dried, and mounted in balsam.

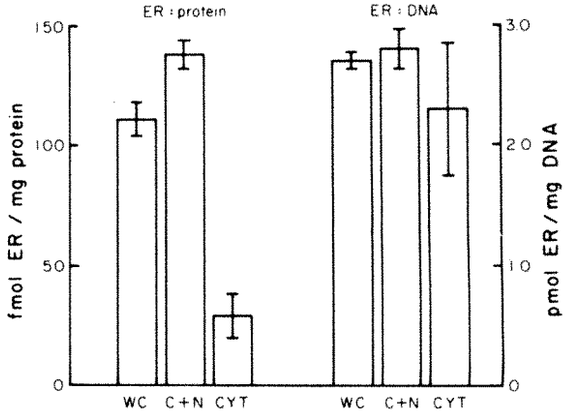


Fig. 2 Intracellular distribution of oestrogen receptor (ER). GH_3 cells were enucleated as described in the text. Oestrogen receptor, protein and DNA were measured in untreated density-selected whole cells (WC), in the cell + nucleoplast fraction (C + N), and in the cytoplast fraction (Cyt). In intact cells or cytoplasts, oestrogen receptor was measured as specific uptake of ^{3}H-oestradiol (151 Ci $mmol^{-1}$, NEN) 2 nM in culture medium + DNase (Worthington DPFF 50 μg ml^{-1}) after 30 min at 37 °C, with or without 200 nM nonradioactive oestradiol. Protein was measured with Coomassie blue staining[25] using the Bio-Rad microassay with bovine γ-globulin (Sigma) as the standard. DNA was measured using diphenylamine[26] or the fluorescent dye Hoechst 33258[27]. The figure shows the results of three separate experiments (error bar is the standard error) in which cytoplasts contained 4%, 2.5%, and 0.25% contaminating whole cells.

Table 1 Oestrogen receptor in enucleated cells

	Oestrogen receptor per mg protein (fmol)	Oestrogen receptor per mg DNA (pmol)	Protein per cell (pg)	DNA per mg protein (μg)	Oestrogen receptors per cell (molecules)
Whole cells	113	2.7	363	42	25,000
Cells + nucleoplasts	146	2.9	336	50	30,000
Cytoplasts	10	1.4	69	7.5	2,100*

One of the experiments of Fig. 3. Cytoplasts contained only 0.25% contaminating whole cells, by cell count with haemocytometer.

* As cytoplasts are approximately one-fifth the size of whole cells, by protein content, cytoplast receptors: cell equivalent was calculated as 5× the actual number of receptors per cytoplast.

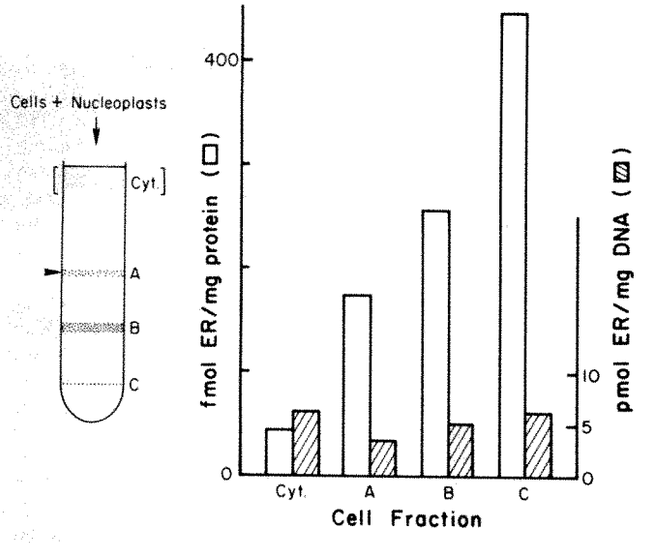


Fig. 3 After enucleation, the cytoplasts were saved separately while the cell+nucleoplast layer was further fractionated on a density step gradient, with Percoll at 1.04, 1.05, 1.07, 1.08 and 1.10 g ml^{-1}. After 20 min at 400*g*, cells were collected at interfaces between 1.05 to 1.07 g ml^{-1} (A), 1.07 to 1.08 g ml^{-1} (B), and 1.08 to 1.10 g ml^{-1} (C). The arrowhead at A indicates where the density-selected whole cells (1.058–1.063 g ml^{-1}) would have been found before enucleation, and the position that the cytoplasts would have occupied is indicated in brackets. Oestrogen receptor, DNA and protein were measured in each fraction. Of the cells+nucleoplasts that were recovered after fractionation by density, 15% were recovered at A (which includes the pre-enucleation density), 76% were recovered at B, and 9% were recovered at C.

since the majority of the cells appeared to contribute a cytoplast (Fig. 1). Furthermore, at least 85% of the cells increase in density after enucleation (Fig. 3 legend), which is consistent with this hypothesis.

Thus, the unfilled oestrogen receptor appears to be largely nuclear in the intact GH_3 cell. We propose that in the absence of steroid, the loose association of the receptor with the nuclear elements causes the receptor to partition into the cytosol when the cell is disrupted, and thus the cytosolic receptor represents an extraction artefact. This phenomenon may be limited to the GH_3 cell, but the similarity of the different steroid receptor systems suggests that these findings may be more broadly applicable. From our data and the literature cited above, we propose that in the intact cell, there is no nuclear translocation of receptor as part of the steroid response, but rather an increase in receptor affinity for nuclear elements.

We thank Rick Koch for technical assistance and Diane Mellon for help with the manuscript. This work was supported in part by the Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, and NIH grants 5 F32 HD06008 (W.W.), AM-25694 (M.E.L.) and HD08192 (J.G.).

Received 9 August; accepted 8 November 1983.

1. Noteboom, W. D. & Gorski, J. *Archs Biochem. Biophys.* **111,** 559–568 (1965).
2. Gorski, J., Toft, D., Shyamala, G., Smith, D. & Notides, A. *Recent Prog. Horm. Res.* **24,** 45–80 (1968).
3. Jensen, E. V. *et al. Proc. natn. Acad. Sci. U.S.A.* **59,** 632–638 (1968).
4. Stumpf, W. E. *Endocrinology* **83,** 777–782 (1968).
5. Gorski, J. & Raker, B. *Endocrinology* **93,** 1212–1216 (1973).
6. Sheridan, P. J., Buchanin, J. M. & Anselmo, V. C. *Nature* **282,** 579–582 (1979).
7. Siiteri, P. K. *et al. Adv. exp. Med. Biol.* **36,** 97–112 (1973).
8. Jordan, V. C., Gosden, B. & Tate, A. C. *Endocrinology* **112,** abstr. 407 (1983).
9. Muldoon, T. *J. biol. Chem.* **255,** 1358–1366 (1980).
10. Walters, M., Hunziker, W. & Norman, A. *J. biol. Chem.* **255,** 6799–6805 (1980).
11. Samuels, H. & Tsai, J. *Proc. natn. Acad. Sci. U.S.A.* **70,** 3488–3492 (1973).
12. Zava, D. & McGuire, W. *J. biol. Chem.* **252,** 3703–3708 (1977).
13. Edwards, D., Martin, P. M., Horwitz, K. B., Chamness, G. C. & McGuire, W. L. *Expl Cell Res.* **127,** 197–213 (1980).
14. Morel, G. *et al. Expl Cell Res.* **132,** 249–257 (1981).
15. Jensen, E., Greene, G., Closs, L., DeSombre, E. & Nadji, M. *Recent Prog. Horm. Res.* **38,** 1–40 (1982).
16. Raam, S., Nemeth, E., Tamura, H., O'Briain, D. & Cohen, J. *Eur. J. Cancer clin. Oncol.* **18,** 1–12 (1982).
17. King, W. & Greene, G. *Nature* (in the press).
18. Tashjian, A., Bancroft, F. & Levine, L. *J. Cell Biol.* **47,** 61–70 (1970).
19. Tashjian, A. & Hoyt, R. in *Molecular Genetics and Developmental Biology* (ed. Sussman, M. 353–387 (Prentice-Hall Englewood Cliffs, 1972).
20. Haug, E., Naess, O. & Gautvik, K. M. *Molec. cell. Endocr.* **12,** 81–95 (1978).
21. Noteboom, W. D., Durham, J. B. & Mitra, R. *J. Steroid Biochem.* **16,** 633–638 (1982).
22. Haug, E. & Gautvik, K. M. *Endocrinology* **99,** 1482–1489 (1976).
23. Tate, A. C., Lieberman, M. & Jordan, V. C. *J. Steroid Biochem.* (in the press, 1984).
24. Bossart, W., Loeffler, H. & Bienz, K. *Exp Cell Res.* **96,** 360–366 (1975).
25. Bradford, M. *Analyt. Biochem.* **72,** 248–254 (1976).
26. Burton, K. *Biochem. J.* **62,** 315–323 (1956).
27. Labarca, C. & Paigen, K. *Analyt. Biochem.* **102,** 344–352 (1980).

Novel κ light-chain gene rearrangements in mouse λ light chain-producing B lymphocytes

Jeannine Durdik, Mark W. Moore & Erik Selsing

Rosenstiel Basic Medical Sciences Research Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02254, USA

The genes that encode the immunoglobulin proteins made by B lymphocytes are made up of segments that are separately encoded in the germ-line genome and brought together by recombination during B-cell ontogeny[1]. There are two types of immunoglobulin light chain, κ and λ, but only a single type is expressed in individual B cells. It is thought that κ gene recombination precedes λ gene recombination during B-cell ontogeny[2,3]. We describe here unusual recombinations that have occurred in two λ-producing B-cell lines and suggest that they are involved in the developmental switch from κ to λ gene expression in maturing B cells. These recombinations involve the J_κ–C_κ introns of *V–J* joined but nonfunctional κ genes and a sequence that in the germ line occurs downstream of the C_κ exon (called RS, for recombining sequence).

We first detected κ DNA segments having unusual hybridization characteristics in Southern blot analyses[4] of two λ-producing cell lines, CH2 and MOPC315. In these cells, we found segments of DNA that hybridized with J_κ probes and intron probes but not with C_κ probes or probes representing sequences 5′ of the J_κ region (Fig. 1). These results indicated that J_κ-containing DNA segments in CH2 and MOPC315 had apparently undergone two recombinational events leading to the removal or replacement of DNA on both the 5′ and 3′ sides of the germ-line J_κ region. These recombinational events have resulted in J_κ regions having no associated C_κ exons in CH2 and MOPC315 cells. Indeed, both CH2 and MOPC315 have no C_κ exons within their genomes (Fig. 1); this is often the case in λ-producing cell lines[2,5,6].

We isolated and sequenced portions of the unusual J_κ-containing DNA segments from CH2 and MOPC315 cells to determine their structures (Fig. 2). Our data show that the two recombinational events generating the unusual DNAs in CH2 and MOPC315 have occurred within the J_κ locus and within the J_κ–C_κ intron. The J_κ-associated recombinations in CH2 and MOPC315 cells represent *V–J* joining events similar to those observed in all immunoglobulin-producing B cells (sequence data not shown). Alignment of the DNA sequences, using the invariant amino acid positions of mouse V_κ proteins[7] and the DNA sequences of the germ-line J_κ locus[8], shows that these *V–J* joining events are nonfunctional due to misalignments and frameshifts similar to others observed in several recombined antibody genes[9–11]. On the other hand, the J_κ–C_κ intron-associated recombinations that we find in the M315-rs1 and CH2-rs3 clones (Fig. 2) do not resemble any previously reported immunoglobulin gene recombinations. It is striking that our restriction map and sequence data indicate that the recombined

© 1984 Macmillan Journals Ltd

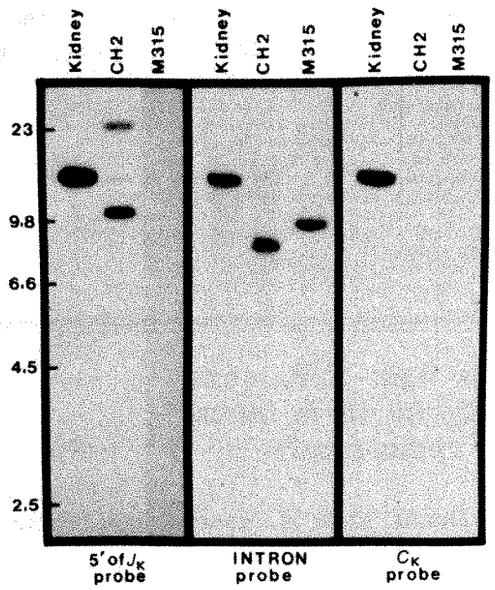


Fig. 1 Recombination of κ-gene DNA in CH2 and MOPC315 myelomas. Southern blots of ~30 μg of the indicated genomic DNAs, digested with *Bam*HI, were hybridized with the probes indicated. The probes are described in Fig. 2. Sizes of marker λ phage *Hin*dIII restriction fragments are indicated. RS DNA recombinations are detected as DNA fragments that hybridize with intron probes (and with J_κ probes, not shown) but not with C_κ probes or probes 5′ of J_κ. The DNA fragments in CH2 cells that are detected by 5′ of J_κ probes but not by intron or C_κ probes have been reported previously[21]. These DNA fragments seem to be related to κ gene *V–J* joining and will be described in detail elsewhere (E.S., J. Voss and U. Storb, in preparation).

RS DNA segments in CH2 and MOPC315 cells are identical. Our sequences also localize the joining sites for the intron-associated RS DNA recombinational events in rs3 and rs1 to within three nucleotides of each other (Fig. 2).

Because the recombined RS DNA segments found in CH2 and MOPC315 cells seem identical, we prepared unique hybridization probes for RS sequences from our rs1 and rs3 clones to ascertain the status of RS DNA in other cell lines. Figure 3 shows that RS sequences appear to be present in one copy per haploid genome in normal mouse kidney cells as judged by the intensity of a single germ-line hybridization band. In various murine B-cell tumour lines, however, RS DNA is often recombined (Fig. 3). In some cell lines, only a recombined RS segment is observed, reinforcing the notion that RS DNA is a single-copy DNA segment. In total, we found RS recombinations in four of six κ-producing plasmacytomas tested and also in all five λ-producing cell lines screened (see Fig. 3 legend). Of the 10 recombined RS segments that we observed in B-cell tumours, only the two in CH2 and MOPC315 hybridized with J_κ or intron probes (Fig. 1) indicating that many RS recombinations apparently involve sequences other than the J_κ–C_κ region. No RS recombinations were present in 12 T-cell tumour lines (data not shown). When we screened antibody-producing hybridomas we found that, in 14 κ-producing hybrids, only the recombined RS sequences contributed by the myeloma parent were present; no recombined RS DNA segments from the normal B-cell partner were detected (data not shown). In contrast, out of 10 λ-producing hybridomas, 8 displayed one or more recombined RS sequences apparently coming from the normal B-cell fusion partner (Fig. 3). We discuss the contrasting data from the normal and transformed B cells below; the results from the hybridomas, however, indicate that RS recombination is frequently linked to λ light-chain gene expression.

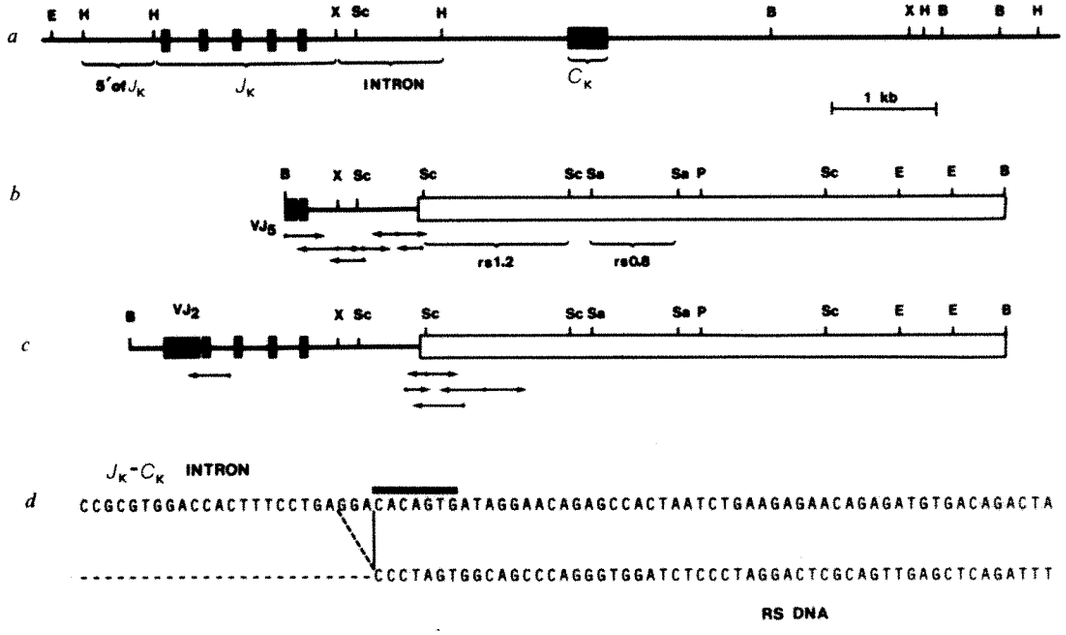


Fig. 2 Restriction enzyme maps of RS DNA clones. Restriction sites within the germ-line J_κ–C_κ locus and hybridization probes that were used to detect RS DNA recombination in MOPC315 and CH2 cells are shown in *a*. *b* And *c* show restriction maps of recombined RS DNAs (CH2-rs3 and M315-rs1) in CH2 and MOPC315 cells, respectively. Segments of rs3 and rs1 that comprise V_κ and J_κ sequences are indicated as lines or solid bars. Portions of rs3 and rs1 that derive from RS DNA are indicated as open bars. The positions of the rs1.2 and rs0.8 probes are also indicated. Chain termination DNA sequence analyses[22] of rs3 and rs1 *Sau*3A, *Hae*III or *Bam*HI, *Xba*I restriction fragments using M13 sequencing vectors[23] are indicated by arrows in *b* and *c*. All maps are drawn to scale and aligned at the *Xba*I site within the J_κ–C_κ intron. Restriction enzymes are: *Eco*RI (E), *Hin*dIII (H), *Xba*I (X), *Sac*I (Sc), *Sau*3A (Sa) and *Bam*HI (B). No *Hin*dIII sites are present in CH2-rs3 or M315-rs1. Not all *Sau*3A sites have been mapped in the rs3 and rs1 clones. *d* Shows nucleotide sequences surrounding the RS DNA recombination sites in rs3 and rs1. The diagonal dashed line represents RS recombination in CH2; the vertical solid line is RS recombination in MOPC315. An immunoglobulin recognition site heptamer, localized near CH2 and MOPC315 RS DNA recombination sites within the germ-line J_κ–C_κ intron region[8,14], is indicated by a heavy bar. Our J_κ–C_κ intron sequence differs at a few positions from that reported by Max *et al.*[8]; we find the same sequences in the rs3 and rs1 clones and presume that the previous sequence is in error. The horizontal dashes represent sequences 5′ of unrecombined RS DNA; these have not yet been determined.

Sequences near the sites of RS DNA recombination in CH2 and MOPC315 demonstrate an intriguing feature. In the J_κ–C_κ intron segment of DNA which is replaced by RS sequences, an immunoglobulin recognition site heptamer is present (Fig. 2). This heptamer represents one portion of the bipartite conserved recognition sequences that are found next to immunoglobulin V, D and J segments and that have been proposed to be binding sites for proteins involved in V–J and V–D–J antibody gene recombination[12,13]. Thus, the RS DNA recombinations in CH2 and MOPC315 bear a remarkable resemblance to the immunoglobulin gene recombinations that occur in normal differentiating B cells. This suggests that some enzymatic activities involved in normal B-cell antibody gene recombination could also have a role in RS DNA recombination. The recognition site heptamer located near RS DNA recombinations in CH2 and MOPC315 has previously been shown to be involved in an aberrant V_κ–C_κ recombination event in the myeloma, MPC11 (ref. 14).

Using Chinese hamster–mouse fusion cell lines, we have localized RS sequences to chromosome 6 in the mouse (J.D., D. Pravtcheva, F. Ruddle and E.S., unpublished). The structures of CH2-rs3 and M315-rs1 (Fig. 2) suggest that RS DNA may lie downstream of the C_κ exon on chromosome 6 and that the V_κ–J_κ–RS recombinations in CH2 and MOPC315 cells have resulted in deletions of C_κ (Fig. 1). Using various probes we can detect no homology between our RS DNA clones and a germ-line J_κ–C_κ clone (Charon 4A·Sp101) described previously[15]. Thus, RS sequences seem to be localized more than 10 kilobases (kb) downstream of the C_κ exon.

It is unknown which sequences recombine with RS in those cell lines where RS recombination does not involve the J_κ–C_κ intron. However, because, the RS recombinations in CH2 and MOPC315 cells involve a DNA sequence that resembles an immunoglobulin gene recognition site, it is possible that some RS DNA recombinations represent joining with V_κ genes. If RS sequences are downstream of the C_κ exon then such putative V_κ–RS recombinations would presumably also result in the deletion of C_κ (and J_κ) regions. These observations suggest that we have detected, for the first time, the molecular events responsible for the deletions of C_κ and J_κ exons that have frequently been observed in mouse λ-producing myelomas[5,6]. It will be interesting to determine whether sequences similar to RS are found in human cells because human λ-producing B-cell lymphomas also commonly have lost the J_κ–C_κ region[2].

Do the RS DNA recombinations that we have found have functional significance during B-cell ontogeny? The apparent lack of RS recombinations in two λ-producing hybridomas (Fig. 3), although possibly due to chromosomal loss during the cell fusion and selection process, does indicate that recombined RS segments are not essential for continued λ-chain production in cells that have functional, V–J joined λ genes. It could be that RS recombinations specifically act as mechanisms to remove C_κ regions from the genomes of B lymphocytes. It has been suggested that C_κ deletion might serve to remove nonfunctional V–J joined κ genes from the genomes of B lymphocytes and thus eliminate aberrant transcripts and prevent the synthesis of incomplete light chains[16]. Alternatively, RS recombination might have a positive role in B-cell differentiation. The structures of CH2-rs3 and M315-rs1 place RS DNA directly downstream from potentially active immunoglobulin gene promoters associated with recombined V_κ regions[17,18]. The putative V_κ-RS recombinations mentioned above would also exhibit structures with RS downstream from V_κ gene promoters. It is possible that RS DNA contains a gene that could be transcriptionally activated by recombination. Our finding of RS recombinations only in λ-producing hybridomas and not in κ-producing hybridomas suggests that such a gene might be involved in a switch from κ recombination to λ recombination in maturing B lymphocytes. In this model, C_κ exon deletion would be a side effect of RS recombination events.

We have sequenced ~900 base pairs of RS DNA adjacent to the recombination site in M315-rs1 (see Fig. 2). Within this sequence there are several short potential protein-encoding

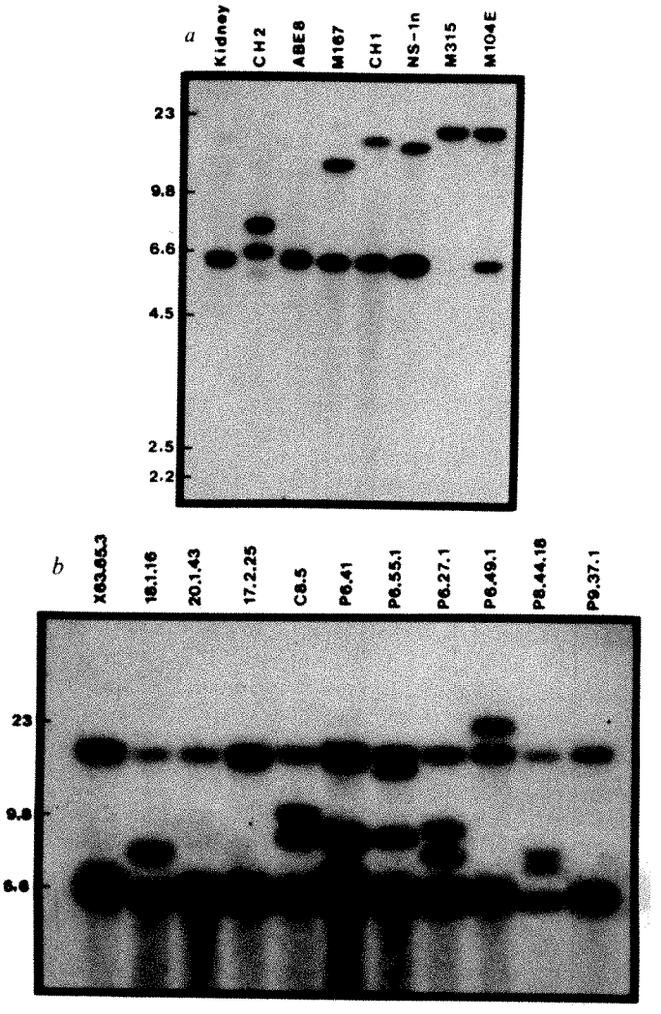


Fig. 3 RS recombination in B-cell lines. *Eco*RI-digested genomic DNAs were probed with the rs0.8 fragment (see Fig. 2). In *a*, recombined RS segments are seen in the κ-producing myelomas, MOPC167 (Litton) and NS-1n[20]; in the λ-producing myelomas, MOPC315 and MOPC104E (Litton); and in the λ-producing B lymphomas, CH1 and CH2 (ref. 24). In the Abelson murine leukaemia virus-transformed cell line, ABE8 (ref. 25), only a germ-line RS segment is evident. In other experiments we have detected recombined RS segments in the κ-producing myelomas MOPC511, MOPC321 and MOPC21 (Litton) and in the λ-producing myeloma, S178 (Litton). Only germ-line RS segments were found in the κ-producing myelomas, MOPC41 and J606 (Litton). In the 12 T-cell lines, SL1-SL9 (ref. 26), BALENTL-5 (Litton), EL-4 and YAC-1 (Salk), we found only germ-line RS segments. In *b*, recombined RS segments are seen in 8 of 10 anti-NP λ-producing hybridomas (ref. 27 and M. Borsch-Supan and T. Imanishi-Kari, unpublished) compared with the myeloma partner (X63.65.3) used in the cell fusions. In the hybridoma, 17.2.25, a recombined RS band at ~13 kb can be resolved from the X63.65.3 parental RS band in the original autoradiogram. An analogous experiment with 14 κ-producing hybridomas showed no recombined RS segments other than those from the myeloma fusion partner. κ Hybridomas included the six anti-PC hybridomas, 101.3C2.1, 101.3G8.4, 101.6G6.2, 100.6F9.1, 137.5G6 (ref. 28) and HP16 (ref. 29) and eight anti-(T,G,A)-L hybridomas (J. Press, unpublished).

open reading frames (40–80 amino acids long) but no clearly relevant RNA transcription or processing signals. We have not yet found any RNA transcription products from recombined RS segments using Northern blots of MOPC315 poly(A)$^+$ RNA hybridized with the rs0.8 and rs1.2 probes shown in Fig. 2 (J.D., S. Ogden, U. Storb and E.S., unpublished). To rule out a potential role of RS transcription in λ gene recombination, however, it will be necessary to probe cells actually undergoing immunoglobulin gene recombination.

The RS DNA recombinations that we observe in four κ-producing myelomas do not seem to be consistent with a regulatory role for RS in λ gene recombination. However, in two of these κ-producing myelomas (MOPC511 and MOPC21) recombined λ genes are also found (ref. 19 and our unpublished results). In addition, RS recombination may not be completely repressed in myeloma cells. In support of this notion, we find different RS DNA recombination products in the myeloma, MOPC21, and in a tissue culture variant, NS-1n, derived from MOPC21 myeloma cells[20]. Southern blots of MOPC21 DNA digested with *Eco*RI show a recombined RS band at 12.5 kb and an intense germ-line RS band at 6.5 kb. NS-1n, on the other hand, has a recombined RS band at 13.5 kb together with a germ-line 6.5-kb band. It is possible that a chromosomal loss of one recombined RS segment and a separate RS recombination on a different chromosome may have occurred during the generation of the NS-1n variant. Thus, RS recombinations may not be stable in myelomas maintained for long periods by serial passage. We believe, therefore, that it would be premature to discount a role for RS in controlling λ gene recombination on the basis of two κ-producing tumours.

Apparently, we have discovered molecular recombination events involved in the C_κ-deletion phenomenon commonly observed in λ-producing B lymphocytes. Our results raise the possibility that these recombination events have an active role in switching from κ to λ recombination in B-cell precursors. Clearly, further studies will be required to ascertain the relationships between RS recombination and the normal processes of immunoglobulin gene recombination in developing B lymphocytes.

We thank M. Potter, G. Haughton, N. Warner, R. Nowinski, M. Borsch-Supan, M. White-Scharf, T. Imanishi-Kari, J. L. Claflin, C. Berek, J. Gawoski and J. Press for cell lines and D. Persiani for help with the DNA sequencing. Initial experiments were performed in the laboratory of Dr Ursula Storb and were supported by NIH grants CA/AI 25754 and DE 02600 and NSF grant PCM 78-13205 to Dr Storb. This work was also supported by NSF grant PCM 83-02110 and by a grant from the American Cancer Society, Massachusetts Division, to E.S. J.D. was supported by a fellowship from the Cancer Research Institute/Benjamin Jacobson and Sons Foundation.

Received 5 September; accepted 1 December 1983.

1. Tonegawa, S. *Nature* **302,** 575–581 (1983).
2. Hieter, P. A., Korsmeyer, S. J., Waldmann, T. A. & Leder, P. *Nature* **290,** 368–372 (1981).
3. Lewis, S., Rosenberg, N., Alt, F. & Baltimore, D. *Cell* **30,** 807–816 (1982).
4. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
5. Alt, F. W., Enea, V., Bothwell, A. L. M. & Baltimore, D. *Cell* **21,** 1–12 (1980).
6. Coleclough, C., Perry, R. P., Karjalainen, K. & Weigert, M. *Nature* **290,** 372–378 (1981).
7. Kabat, E. A., Wu, T. T. & Bilofsky, H. in *Sequences of Immunoglobulin Chains* (National Institutes of Health, Bethesda, 1979).
8. Max, E. E., Maizel, J. V. & Leder, P. *J. biol. Chem.* **256,** 5116–5120 (1981).
9. Altenburger, W., Steinmetz, M. & Zachau, H. G. *Nature* **287,** 603–607 (1980).
10. Walfield, A., Selsing, E., Arp, B. & Storb, U. *Nucleic Acids Res.* **9,** 1101–1109 (1981).
11. Hozumi, N. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 7019–7023 (1981).
12. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. *Cell* **19,** 981–992 (1980).
13. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. *Nature* **286,** 676–683 (1980).
14. Seidman, J. G. & Leder, P. *Nature* **286,** 779–783 (1980).
15. Walfield, A. M., Storb, U., Selsing, E. & Zentgraf, H. *Nucleic Acids Res.* **8,** 4689–4707 (1980).
16. Korsmeyer, J. *et al. J. exp. Med.* **156,** 975–985 (1982).
17. Kelley, D. E., Coleclough, C. & Perry, R. P. *Cell* **29,** 681–689 (1982).
18. Bentley, D. L., Farrell, P. J. & Rabbitts, T. H. *Nucleic Acids Res.* **10,** 1841–1856 (1982).
19. Storb, U. & Arp, B. *Proc. natn. Acad. Sci. U.S.A.* **80,** 6642–6646 (1983).
20. Storb, U., Arp, B. & Wilson, R. *Nucleic Acids Res.* **8,** 4681–4687 (1980).
21. Selsing, E. & Storb, U. *Nucleic Acids Res.* **9,** 5725–5735 (1981).
22. Sanger, F., Nicklen, S. & Coulson, A. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
23. Messing, J., Crea, R. & Seeburg, P. *Nucleic Acids Res.* **9,** 309–321 (1981).
24. Lynes, M. A., Lanier, L. L., Babcock, G. F., Wettstein, P. J. & Haughton, G. *J. Immun.* **121,** 2352–2357 (1978).
25. Warner, N. L., Harris, A. W. & Gutman, G. in *Membrane Receptors of Lymphocytes* (eds Seligmann, M., Preud'Homme, J. L. & Kourilsky, F. M.) 203–216 (North Holland, Amsterdam, 1975).
26. Nowinski, R. & Hayes, E. *J. Virol.* **27,** 13–18 (1978).
27. White-Scharf, M. E. & Imanishi-Kari, T. *Eur. J. Immun.* **11,** 897–904 (1981).
28. Andres, C. M., Maddalena, A., Hudak, S., Young, N. M. & Claflin, J. L. *J. exp. Med.* **154,** 1584–1598 (1981).
29. Berek, C. *et al. Eur. J. Immun.* **10,** 258–263 (1980).

A variant translocation places the λ immunoglobulin genes 3′ to the c-*myc* oncogene in Burkitt's lymphor

G. F. Hollis, K. F. Mitchell, J. Battey, H. Potter, R. Taub, G. M. Lenoir* & P. Leder

Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA
* International Agency for Research on Cancer, Lyon, France

Most translocations that occur in Burkitt's lymphoma[1,2] inv movement of part of chromosome 8, containing the c-*myc* g from its normal position to the immunoglobulin heavy-c locus on chromosome 14[3–7]. The genes are often joined at 5′ ends in opposite transcriptional directions[4–13]. Howev significant minority of Burkitt translocations[8] involve the l chain loci on chromosome 2 (κ)[14] or 22 (λ)[15]. We characterized one of these from a European-derived cell (IARC-BL37) that carries an 8;22 translocation. Here translocation has joined the 5′ portion of the λ light-chain l to the 3′ portion of the c-*myc* gene at a position about 7 kilol from the normal c-*myc* promoters. The translocatio reciprocal and relatively conservative, involving the loss of 21 base pairs from the site of recombination. This transloc allows us to orient the λ genes with respect to the centro of chromosome 22 and to predict the orientation of c translocations involving these chromosomal segments. Tl translocation is accompanied by an increased level of c- transcripts, especially that derived from a normally under- c-*myc* promoter.

The Burkitt's lymphoma line BL37 is a European-der Epstein–Barr virus (EBV) positive cell line that carries a 22 translocation and produces immunoglobulin μ and κ ch In our hands, this line is the only one for which the previ described correlation between type of translocation and l chain expression[8] is not observed, and the first line with t 22 translocation to show a detectable rearranged c-*myc* Comparison of *Eco*RI restriction fragments derived fron genomic DNA of the Burkitt cells with those from normal t (Fig. 1, lanes 1, 2) showed that both tissues contain a kilobase (kb) *Eco*RI fragment corresponding to the germ c-*myc* gene. The Burkitt cell contained an additional fragi 19.5 kb in length, presumably corresponding to the rearra c-*myc* gene. If this rearranged fragment contains the imm globulin λ light-chain genes[16], as suggested by the fact tha translocation involves chromosome 22, we might further e to find a correspondingly rearranged *Eco*RI fragment of that hybridizes with a probe derived from the λ constant re The relevant experiment is also shown in Fig. 1 (lanes 3, which a λ constant region probe detects a single rearra fragment, also 19.5 kb in length, in addition to the normal l at 5, 8, 14 and 16 kb. (The 5 kb *Eco*RI fragment seen i middle lanes corresponds to a processed λ pseudogene unl to the λ locus[17].) Note that these λ *Eco*RI fragments mai their germ-line configuration, as would be expected κ-producing B cell[18,19].

These data suggest that the c-*myc* and the immunoglo λ gene sequences now exist on a 19.5 kb *Eco*RI fragme DNA. While any of the six λ genes represented in this hapl could have been involved in this rearrangement, syste studies (data not shown) with uniquely hybridizing p derived from the flanking sequences 5′ to the first three λ suggested that the rearrangement occurred 5′ to the λ_1 (see Fig. 1). Evidence that the recombination betwee chromosomes is a reciprocal event was obtained using a from the region 5′ to the cross-over point of recombin (Fig. 1). The experiment is shown in the lanes 5 and 6 of

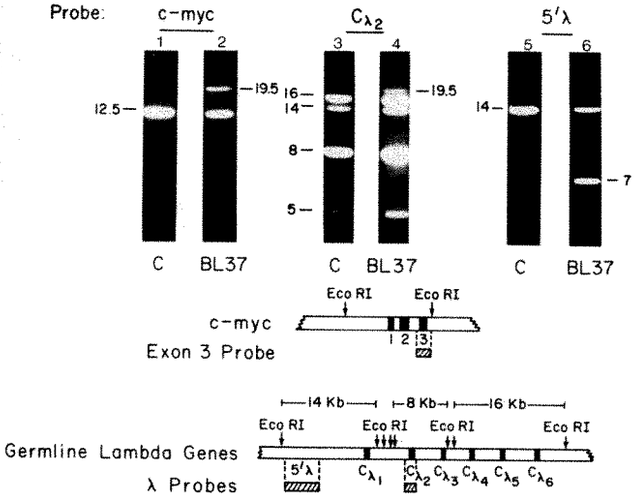


Fig. 1 The arrangement of c-*myc* and immunoglobulin λ gene segments in normal cells and the Burkitt's lymphoma BL37. Southern transfers[20] of *Eco*RI-digested genomic DNAs (10 μg per lane) from normal human white blood cells, C, and a Burkitt's lymphoma cell line containing a t(8; 22), BL37, were hybridized to ^{32}P-labelled DNA probes corresponding to exon 3 (*Cla*I–*Eco*RI) of the human c-*myc* gene (lanes 1, 2), human λ constant region 2 (*Bam*HI–*Bam*HI) (lanes 3, 4), and 5′ flanking region of λ constant region 1 (*Eco*RI–*Hin*dIII) (lanes 5, 6). The probe locations used are shown in the diagram. The transfers were washed in 0.1×SSC, 0.1% SDS at 52 °C and visualized by autoradiography. In addition to the 12.5-kb germ-line *Eco*RI band, the c-*myc* probe identifies a 19.5 kb band in the BL37. The λ constant region 1 probe identifies a non-germ-line 19.5 kb *Eco*RI fragment, as well as the 5, 8, 14 and 16 germ-line bands. The 19.5 kb and 7 kb non-germ-line *Eco*RI fragments were cloned into the λ vector Charon 4A (ref. 20) using the exon 3 c-*myc* probe and 5′ flanking λ probe respectively.

and the appearance of the expected 7 kb reciprocal recombination product in the lane 6 indicates that the cross-over point lies between this 5′ probe and the λ_1 gene. Both rearranged fragments were isolated by preparative gel electrophoresis and cloned in the λ phage vector Charon 4A[20].

The orientation of the two genes and the cross-over point of their recombination was established by comparing their structures with those of known germ-line c-*myc* and immunoglobulin genes[9,16] by both heteroduplex mapping and restriction fragment map comparison. The heteroduplex structure shown in Fig. 2*a* was formed between the cloned 19.5-kb *Eco*RI fragment containing the rearranged c-*myc* gene and the germ-line c-*myc* gene. The structure has a 12.5-kb region of homology (representing virtually the entire 12.5-kb *Eco*RI germ-line DNA c-*myc* segment, see map) and a single-stranded substitution loop approximately 7 kb in length. The position of the substitution loop indicates that rearrangement has occurred 3′ to the c-*myc* gene. When the same rearranged fragment is hybridized to a germ-line fragment of DNA derived from the λ_1 gene (maps in Figs 1, 2), a 7 kb length of homology is seen, as are two single-stranded regions of non-homology, 7.0 and 12.5 kb in length (Fig. 2*b*). Comparison of subclones derived from the cross-over region from the two germ-line genes and the products of their reciprocal recombination showed that the 12.5-kb portion of the 19.5 kb rearranged fragment has a restriction map identical to that of the germ-line c-*myc* gene and is then identical for 7 kb to the λ_{C1} immunoglobulin fragment (data not shown).

The orientation and breakpoints were finally established by determining the nucleotide sequence of the cross-over region in its germ line and rearranged configurations and the sequence of the cross-over region is shown in Fig. 3. With the exception of a 21 base pair (bp) segment of DNA derived from the λ_1 germ-line locus which is missing from the recombinants, the sequences at the breakpoints are entirely conserved over the sequenced regions. The data are all consistent with a translocation between a position 400 bases 3′ to the poly(A) addition site of the *myc* gene and a point 5 kb 5′ of the sequence

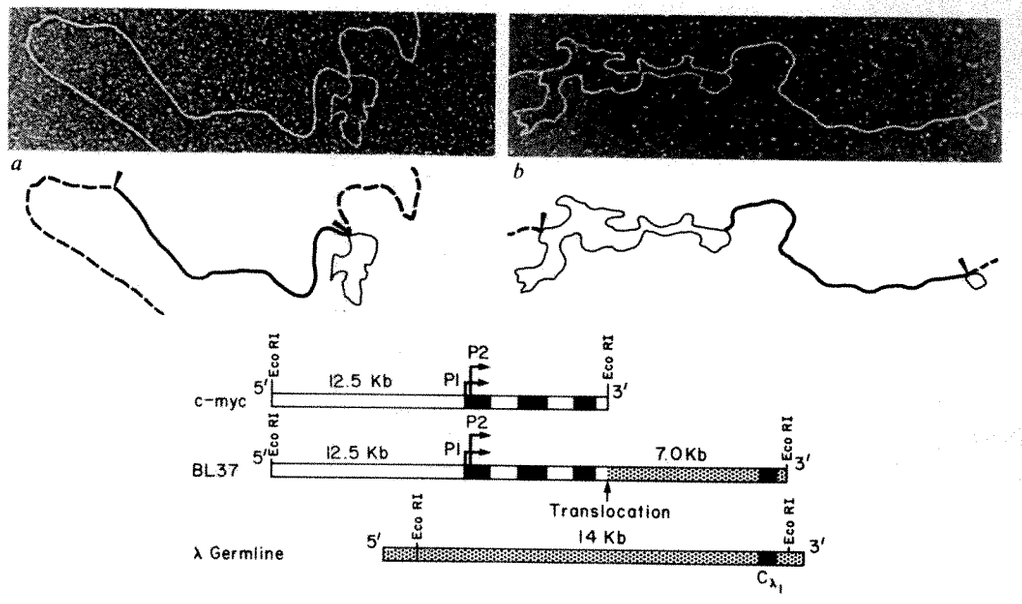


Fig. 2 Heteroduplex comparison of the rearranged c-*myc* gene derived from BL37 and germ-line c-*myc* and immunoglobulin λ genes. *a*, A recombinant phage clone containing the 19.5 kb *Eco*RI restriction fragment identified in Fig. 1 was heteroduplexed to a recombinant phage containing the 12.5 kb *Eco*RI c-*myc* fragment using alkali denaturation followed by renaturation in 50% formamide, 12.5 mM EDTA, 50 mM NaCl and 100 mM Tris *p*H 8.5 at 37 °C for 15 min. In addition to the heteroduplex formed between the vector phage arms, there is a region of homology (see arrows in graphic) that corresponds to nearly the entire 12.5 kb *Eco*RI c-*myc* DNA fragment. At a position 3′ to the c-*myc* gene a 7 kb region of non-homology is seen. This heteroduplex structure is consistent with the 19.5-kb recombinant DNA phage clone containing nearly all of the 12.5 kb *Eco*RI fragment, including the three exons of c-*myc* that are normally located on chromosome 8. The structures of this 19.5 kb clone as well as the germ-line c-*myc* fragment are shown schematically. *b*, The phage containing the rearranged 19.5 kb *Eco*RI fragment BL37 was heteroduplexed to a phage containing a 15 kb segment of DNA that encodes the λ_1 gene that was derived from human fetal liver. The latter clone includes the 14 kb *Eco*RI fragment carrying λ constant region 1 as shown in the map. In this heteroduplex, a 7 kb homology is seen, as well as two single-stranded regions 12.5 kb and 7.0 kb in size. The position of the homology in this heteroduplex indicates that this library clone and the 19.5 kb clone share sequence homology for 7 kb 5′ to λ constant region 1. The two clones diverge at this point, the region where the 19.5 kb clone carries sequence from the c-*myc* locus of chromosome 8. A small region of non-homology is seen (see arrow to the right), which corresponds to the distance the library clone extends 3′ to the *Eco*RI site. These results are shown schematically.



Fig. 3 Sequences of the regions involved in the 8; 22 translocations in BL37 were determined by dideoxy sequence analysis[26]. Four aligned sequences are shown, each of which represents the DNA strand which bears the coding sequences of the genes. The upper sequence represents germ-line chromosome 8. The second sequence is the recombinant which bears the *myc* and λ genes. The third sequence is the reciprocal translocated product and the fourth sequence represents the germ-line chromosome 22. The 21 bases deleted in the translocation event are indicated separated from the adjacent sequence. The CCAAACCC sequence described in the text is boxed with a solid line and the YAGGTTG sequence boxed with a dashed line.

that has been identified as the J region of λ_{C1} (N. Berliner, unpublished).

In the other translocations investigated thus far, the c-*myc* gene is often joined to one of the heavy-chain switch region signals[4–6,9–13]. It is, therefore, attractive to think that the switch recombination system catalyses the Burkitt-type translocation. Analysis of the structure of the cross-over point of the BL37 translocation has allowed us to compare the structure of the substrates and products of the recombination event. Several hundred nucleotides of sequence in this region from both germline c-*myc* and immunoglobulin genes have been examined and extensive regions of switch-like homology have not been found. Therefore, the situation in the BL37 cell line, in which neither partner provides an extensive switch signal, makes such a mechanism less likely. It is, therefore, worth noting that the sequence, YAGGTTG, which has been found 8–16 nucleotides 5′ of many normal heavy-chain switching events[21], occurs in 6/7 consensus form 11 bases away from one of the breakpoints on chromosome 22. This sequence does not appear in the products of the translocation since it occurs within the 21 bp segment that is deleted (Fig. 3). Also, an octanucleotide, CCAAACCC, occurs very close to the cross-over point in the c-*myc* (chromosome 8) segment (Fig. 3). This sequence also flanks the recombination site in the translocation that occurs 5′ to the c-*myc* gene in the Burkitt line BL22[9]. We note that a sequence which differs by only a single base from this is present in reverse complement form adjacent to the breakpoint in a murine plasmacytoma reported recently[22]. It will require the characterization of further examples before we will be able to evaluate the significance of these observations.

One clue to a potentially important regulatory feature of the c-*myc* gene comes from comparative studies of its normal structure[9]. The c-*myc* gene is encoded in three exons, the first of which is a 550 bp untranslatable leader sequence that is tightly conserved in sequence between mouse and human species. This region encodes two promoter sequences that define two transcription initiation sites about 160 bp from one another (map,

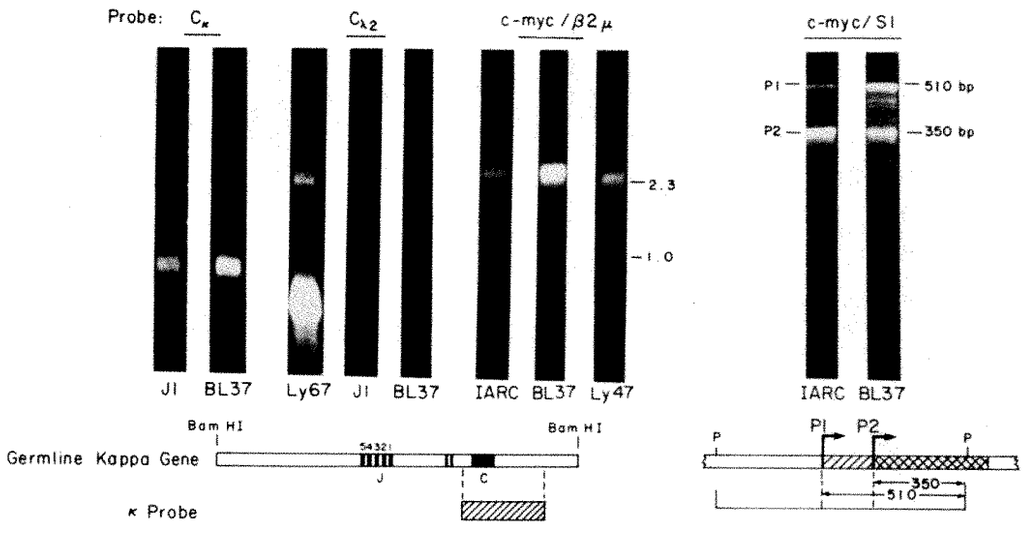


Fig. 4 Comparative analysis of the mRNA derived from BL37 and other Burkitt's lymphoma and lymphoblastoid cell lines. Total RNA was made[27] from Burkitt's lymphomas BL37, J1, Ly67 and Ly47 and the lymphoblastoid cell line IARC 100 and used for RNA blotting and S_1 nuclease analysis. RNA blots[28] from Burkitt's lymphomas J1 and BL37, both κ light-chain producers, were probed with a DNA (*Eco*RI–*Eco*RI) probe containing the κ constant region sequence. RNA from both cell lines hybridized specifically to give one band on the blot (see C_k probe). When J1 and BL37 RNA, as well as RNA from a λ-producing Burkitt's lymphoma, Ly67, was probed with a λ constant region, only the RNA from the Ly67 hybridized to the probe (see $C_{\lambda 2}$ probe). Transcription of the c-*myc* gene was determined in BL37 as well as IARC-100 and Burkitt's lymphoma Ly47, a λ producer, by probing with the second coding exon of c-*myc* (see Fig. 1). All three cell lines produce a 2.3 kb c-*myc* specific RNA, but the amount of c-*myc* RNA in BL37 appears higher than in IARC 100 (see c-*myc* probe). To control for the amount of RNA loaded in each lane, the same RNA blot was rehybridized with a β_2-microglobulin probe[29]. All three cell lines produce a 1.0 kb β_2-microglobulin specific RNA. By using the β_2-microglobulin specific hybridization as a control for RNA concentration, densitometric scans showed that BL37 produces 15-fold more c-*myc* RNA than IARC 100. To determine the point of initiation of transcription of the c-*myc* gene in BL37, an 860-bp *Pvu*II–*Pvu*II M13 mp9 clone containing the two promoters of transcription was labelled *in vitro* with ^{32}P-dATP by primer extension[9]. The single-stranded probe was allowed to anneal to 10 μg of tRNA and 10 μg of total RNA from BL37 and IARC 100 and then digested by 750 units of S_1 nuclease essentially as described by Berk and Sharp[23]. The digestion products were electrophoresed on an 8 M urea 5% acrylamide and visualized by autoradiography (see c-*myc*/S1), tRNA alone showed no protection of the probe (data not shown).

Fig. 4). Studies carried out in EBV-immortalized lymphoblastoid cell lines and non-transformed fibroblasts indicate that products derived from the use of the second promoter, P_2 (Fig. 4), are consistently represented among the stable c-*myc* transcripts of these cells at a higher level than transcripts derived from the first promoter, P_1. The fixed, approximately fourfold excess of P_2 transcripts in untransformed cells suggests the existence of a regulatory programme that maintains this ratio[24].

With these features in mind, we have used RNA blot hybridization to examine the immunoglobulin light chain and c-*myc* transcripts in several Burkitt's lymphoma cell lines including BL37 and a lymphoblastoid cell line. The results are shown in Fig. 4. The first two sets of panels in Fig. 4 show that the BL37 cell line produces large amounts of κ mRNA, but only trace amounts of λ RNA. Control Burkitt cell lines that produce only κ (J1) and only λ (Ly67) mRNAs are shown for comparison. The third set of three panels compares the c-*myc* transcript present in a lymphoblastoid cell line (IARC-100) with that in the BL37 cell line and another Burkitt cell line, IARC-Ly47. The level of β_2-microglobulin mRNA is determined in each cell line to control for the amount of mRNA added to each lane. Although the amount of β_2-microglobulin is approximately equivalent in each lane, the level of c-*myc* transcript is about 15-fold higher in the BL37 cell line. In contrast, the c-*myc* RNA is only slightly increased (1.4-fold) in the Burkitt line Ly47 when compared with the non-malignant IARC-100 cell line.

A more revealing analysis is one that distinguishes between the products of the two c-*myc* promoters. Such an experiment, carried out using the S_1 nuclease technique[23], is shown in lanes 9 and 10 of Fig. 4 (lanes c-*myc*/S1). As with other lymphoblastoid cell lines, the IARC-100 cell line has a predominant transcript that arises from the second promoter, P_2, which is represented in about fourfold excess over that which arises from the first promoter, P_1. In contrast, the two c-*myc* promoters P_1 and P_2 are both extensively used in BL37 indicating an activation of the *myc* gene in this cell line similar to that we have observed in other Burkitt-derived lines[24].

The orientation of the c-*myc* gene on chromosome 8 may be deduced from the data presented by Dalla-Favera *et al.*[25] to be centromere–(5′-c-*myc*-3′)–telomere. The genes on the recombinant chromosome in BL37 are arranged 5′-c-*myc*-3′-5′-λ1-3′ (tail-to-head). Since the breakpoint on chromosome 8 is 3′ to the c-*myc* gene, the gene itself must be associated with its original centromere in the recombinant chromosome. Therefore, the germ-line orientation of the λ genes is established by the structure of the translocation product and must be centromere-(5′-λ_1-3′)–telomere. Since all the λ genes are encoded on the same strand of DNA, one can predict that all simple translocations between chromosomes 8 and 22 will place the c-*myc* and λ immunoglobulin genes in a tail-to-head orientation. It is possible that some chromosomes will rearrange such that the λ gene will in fact be 5′ to the c-*myc* gene in a tail-to-head orientation and the *myc* gene could be affected by upstream λ gene promoters or enhancers.

We thank Ms T. Broderick and Ms A. Jacobs for assistance in the preparation of this manuscript, M. Vuillaume and C. Bonnardel for their technical assistance, E. I. duPont de Nemours and Co. and the American Business for Cancer Research Foundation for supporting portions of this work. Portions of this work was supported by NIH grant GM17088. J.B. is supported in part by American Cancer Society, R.T. by the Cancer Research Institute and H.P. is supported in part by the Anna Fuller Fund.

Received 17 October; accepted 15 December 1983.

1. Manolov, G. & Manolova, Y. *Nature* **237,** 32–33 (1972).
2. Zech, L., Haglund, U., Nilsson, K. & Klein, G. *Int. J. Cancer* **17,** 47–56 (1976).
3. Kirsch, I. R. *et al. Science* **216,** 173–175 (1981).
4. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7837–7841 (1982).
5. Dalla-Favera, R., Martinotti, S., Gallo, R., Erikson, J. & Croce, C. *Science* **219,** 963–967 (1983).
6. Hamlyn, P. H. & Rabbitts, T. *Nature* **304,** 135–139 (1983).
7. Adams, J. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1982–1986 (1983).
8. Lenoir, G. *et al. Nature* **298,** 474–476 (1982).
9. Battey, J. *et al. Cell* **34,** 779–787 (1983).
10. Crews, R., Barth, R., Hood, L., Prehn, J. & Calame, K. *Science* **218,** 1319–1321 (1982).
11. Shen-Ong, G. L., Keath, E. J., Piccolli, S. P. & Cole, M. D. *Cell* **31,** 443–452 (1982).
12. Marcu, K. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 519–523 (1983).
13. Stanton, L. W., Watt, R. & Marcu, K. B. *Nature* **303,** 401–406 (1983).
14. Malcolm, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 4957–4961 (1982).
15. McBride, O. W. *et al. J. exp. Med.* **155,** 1480–1490 (1982).
16. Hieter, P. A., Hollis, G. F., Korsmeyer, S. J., Waldmann, T. A. & Leder, P. *Nature* **294,** 536–540 (1981).
17. Hollis, G. F., Hieter, P. A., McBride, O. W., Swan, D. & Leder, P. *Nature* **296,** 321–325 (1982).
18. Hieter, P., Korsmeyer, S. J., Waldmann, T. A. & Leder, P. *Nature* **290,** 368–372 (1981).
19. Korsmeyer, S. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 7096–7100 (1981).
20. Blattner, F. *et al. Science* **196,** 161–169 (1977).
21. Marcu, K. B., Lang, R. B., Stanton, L. W. & Harris, L. J. *Nature* **298,** 87–89 (1982).
22. Neuberger, M. S. & Calabi, F. *Nature* **305,** 240–243 (1983).
23. Berk, A. J. & Sharp, P. A. *Cell* **12,** 721–732 (1977).
24. Taub, R. *et al. Cell* (in the press).
25. Dalla-Favera, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7824–7827 (1982).
26. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
27. Chirgwin, J., Aeybyle, A., McDonald,R. & Rutter, W. *Biochemistry* **18,** 5294–5299 (1979).
28. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5201–5205 (1977).
29. Suggs, S. V., Wallace, R. B., Hirose, T., Kawashima, E. H. & Itakura, K. *Proc. natn. Acad. Sci. U.S.A.* **78,** 6613–6617 (1981).

Novel mannosidase inhibitor blocking conversion of high mannose to complex oligosaccharides

Ulrike Fuhrmann*, Ernst Bause†, Günter Legler† & Hidde Ploegh*§

Institute for *Genetics and †Biochemistry, University of Cologne, Weyertal 121, 5000 Cologne 41, FRG

Many secretory and membrane proteins are glycoproteins carrying asparagine-linked (*N*-linked) oligosaccharides. There are two types of *N*-linked glycans, referred to as high-mannose and complex type, respectively. Biosynthesis of *N*-linked glycans of the complex type proceeds via a high-mannose intermediate. After the initial transfer of a high-mannose oligosaccharide with the composition $(Glc)_3(Man)_9(GlcNAc)_2$ from a lipid carrier to the nascent polypeptide chain, trimming reactions take place. Trimming glucosidases remove the glucose residues quantitatively and mannosidases IA/B and II can remove all but three mannose residues. After trimming, terminal sugars such as *N*-acetylglucosamine, galactose, sialic acid and fucose may be added and result in the conversion to a glycan of the complex type[1]. Because suitable inhibitors were lacking, it was difficult to assess the importance of the trimming reactions for proper intracellular traffic, modification reactions other than the addition of terminal sugars, or as regulatory steps in glycoprotein processing. Here we describe the action of 1-deoxymannojirimycin (1,5-dideoxy-1,5-imino-D-mannitol, dMM; Fig. 1) on the biosynthesis of IgM and IgD. dMM is the mannose analogue of 1-deoxynojirimycin (dNM; Fig. 1), itself a glucosidase inhibitor[2–4]. We present evidence that dMM is a mannosidase inhibitor. *In vivo* dMM inhibits the equivalent of the mannosidase IA/B activities and blocks conversion of high-mannose to complex oligosaccharides. It is the first such inhibitor to be reported. Interference with the biosynthetic pathway of *N*-linked glycans could prove to be a powerful way to manipulate carbohydrate structure *in vivo*.

The experimental system consists of hybridoma cells producing IgM[5] and IgD[6] specific for the hapten (4-hydroxyl-3-nitrophenylacetyl, NP). After preincubation with inhibitor, these cells were pulse labelled for 10 min with ^{35}S-methionine and then chased with non-radioactive methionine in the continued presence of inhibitor. The inhibitors used were dMM and, for comparative reasons, the mannosidase II inhibitor swainsonine[7,8] (SW; Fig. 1). At different times of chase samples were taken and immunoglobulin was isolated from detergent lysates of cells (cytoplasmic immunoglobulin) or culture supernatants

§ To whom reprint requests should be sent.

© 1984 Macmillan Journals Ltd

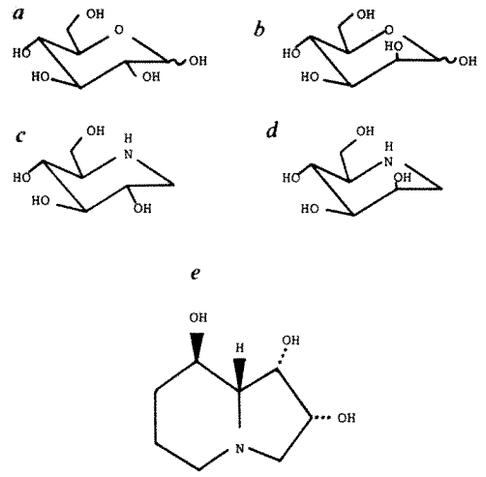


Fig. 1 Structures of glucose, *a*, mannose, *b*, the corresponding sugar analogues 1-deoxynojirimycin, *c* and 1-deoxymannojirimycin, *d* and the mannosidase II inhibitor[8] swainsonine, *e*.

(secretory immunoglobulin) by incubation with an affinity support (Fig. 2). For control cells and SW-treated cells the cytoplasmic immunoglobulin shows a steady decrease in apparent molecular weight (M_r) with time in pulse-chase experiments of this type[3,9], due to oligosaccharide trimming (Fig. 2*a*). On secretion an observed increase in M_r is caused by the addition of terminal sugars[9] (Fig. 2*a*). The M_r of cytoplasmic immunoglobulin from SW-treated and control cells is indistinguishable at each corresponding chase time as reported earlier[3]. Immunoglobulin from dMM-treated cells has a higher M_r than that from control cells, suggesting the inhibition of trimming reactions. In Fig. 2*b* these differences in M_r can be clearly demonstrated in a mixing experiment. dMM does not inhibit secretion of IgM or IgD (Fig. 3), as opposed to dNM which was shown to inhibit secretion of IgD but not IgM[3]. The analysis of IgD precursors is complicated by the fact that differentially *N*-glycosylated membrane and secretory IgD are present simultaneously[6,9]. This explains the multitude of δ-specific bands in Figs 2 and 3. In dMM-treated cells the secreted δ chains are indistinguishable in M_r from the middle two bands present in the cytoplasmic samples (Fig. 2*a*). Similarly, no difference in M_r was observed for secretory IgM and cytoplasmic IgM at 60 min in dMM-treated cells (Fig. 2*c*). It is possible that in the presence of dMM, terminal modifications can no longer occur because removal of mannose residues is incomplete. In SW-treated cells terminal modifications do take place, but to a lesser extent than in control cells, as evidenced by a lower M_r of secreted immunoglobulin. This observation is consistent with the inhibitory action of SW on mannosidase II which does not allow all oligosaccharide antennae to be terminally modified[3,10].

The results obtained with dMM suggest that no terminal modifications occur and that the target of dMM inhibition *in vivo* acts before that of SW. Such a situation would be consistent with inhibition of mannose removal, that is, inhibition of the mannosidase IA/B activity. If correct, a $(Man)_9(GlcNAc)_2$ structure, which is endoglycosidase H (EndoH) sensitive[11], would be expected to accumulate and be present on immunoglobulin secreted by dMM-treated cells. Therefore, no incorporation of terminal sugars such as galactose and sialic acid should occur. Moreover, the oligosaccharides should be of a size consistent with this hypothesis and should remain sensitive to EndoH. Therefore the following experiments were carried out.

IgM- and IgD-producing cells were mixed and preincubated with inhibitor for 4 h. These cells were then labelled with ^{3}H-galactose, and a small aliquot was labelled in parallel with ^{35}S-methionine. Immunoglobulin was prepared from the culture supernatants and analysed by SDS–polyacrylamide gel electrophoresis (PAGE). Secreted immunoglobulin from SW-treated and control cells was readily labelled with ^{3}H-galactose (Fig. 3*a*), whereas no label was incorporated into immuno-

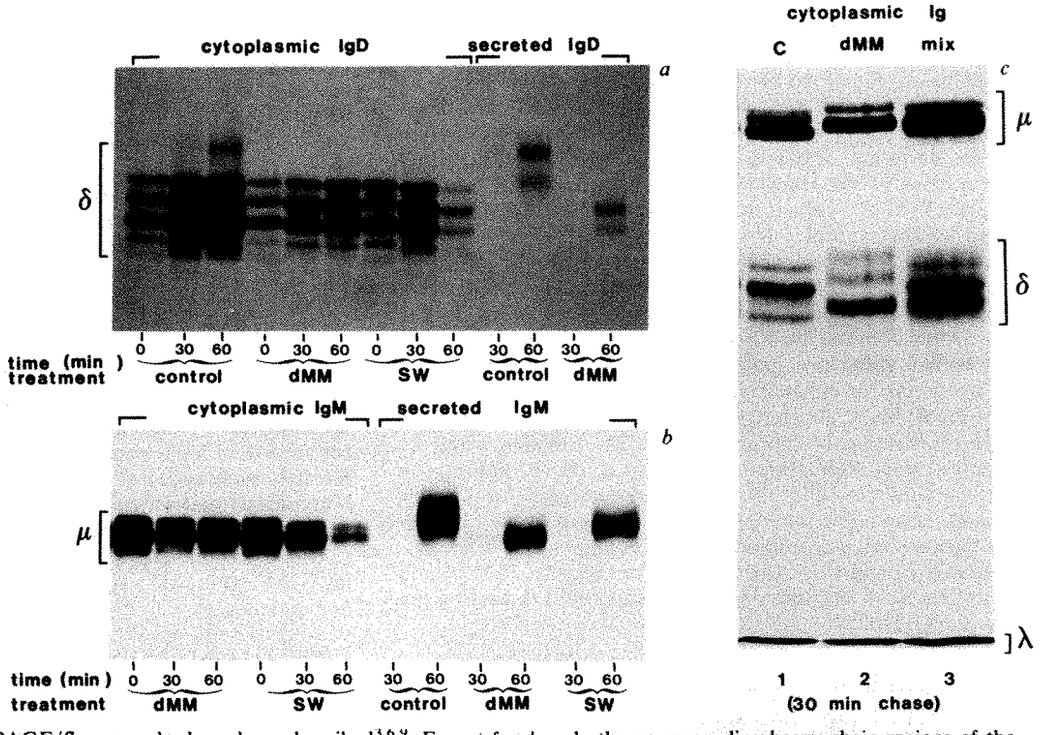


Fig. 2 Pulse-chase analysis of the biosynthesis of IgD (*a*, *b*) and IgM (*b*, *c*). Cells (6×10^6 per sample) were preincubated with inhibitor (dMM or SW, at 1 mM or 10 μg ml^{-1} respectively) for 1 h, pulse-labelled with 50 μCi of ^{35}S-methionine per sample and chased with non-radioactive methionine in the continued presence of inhibitor. SW was a gift of Drs Vosbeck and Elbein, dMM was a gift of Drs Kinast and Truscheit; a novel scheme for the synthesis of dMM has been developed and will be published elsewhere[15]. Samples were taken for isolation of immunoglobulin at the times indicated in the figure. Detailed procedures for labelling of cells isolation of NP-binding immunoglobulin on affinity matrices and analysis by SDS–PAGE/fluorography have been described[3,6,9]. Except for *b*, only the corresponding heavy chain regions of the autoradiograms are shown. Immunoglobulin was isolated or from detergent extracts of cells (cytoplasmic immunoglobulin) or from culture supernates (secreted immunoglobulin). C, control; dMM, 1-deoxymannojirimycin; SW, swainsonine. For *b*, approximately equal amounts of IgM and IgD biosynthetic precursors were mixed and co-electrophoresed. Cytoplasmic immunoglobulin for 30 min is shown for control cells (lane 1), dMM-treated cells (lane 2) and a mixture of the samples from lanes 1 and 2 (lane 3).

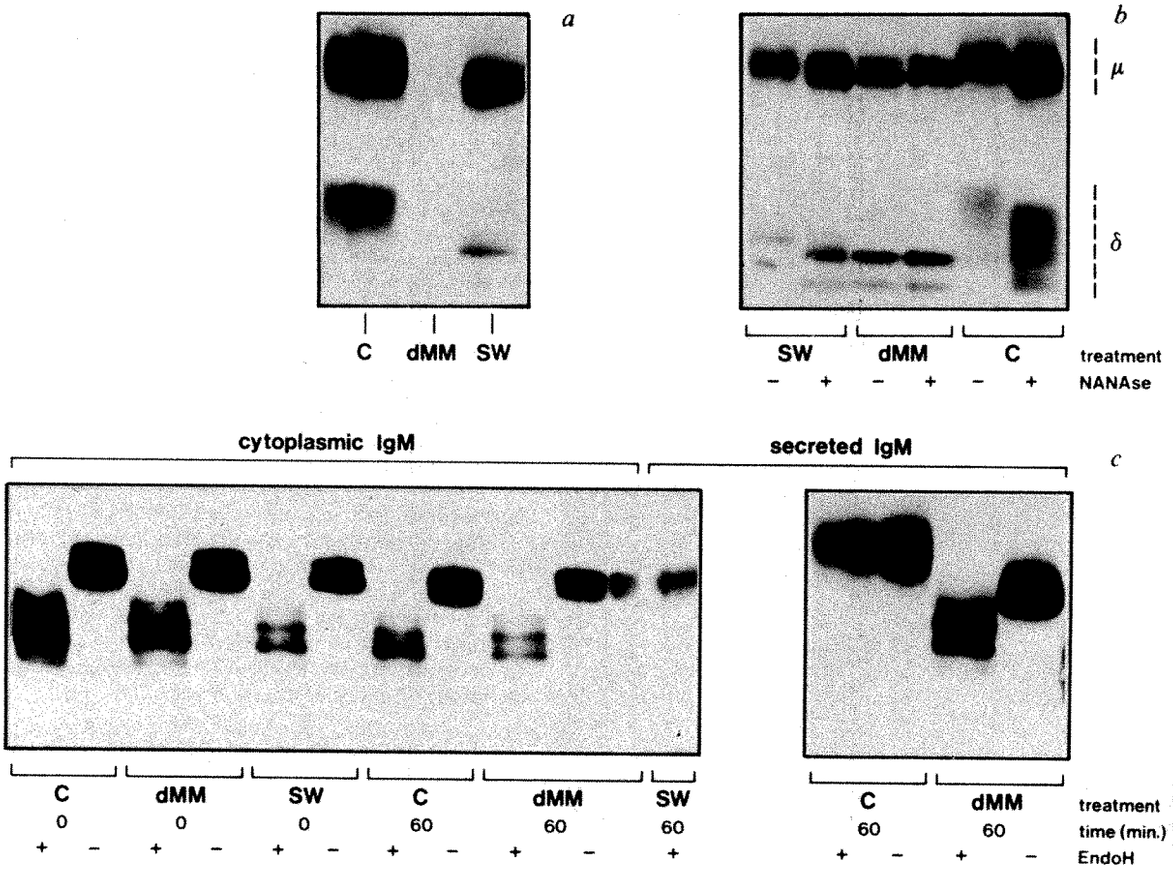


Fig. 3 Analysis of IgM and IgD biosynthesis by sugar labelling, neuraminidase and endoH treatment. *a*, A mixture of IgD- and IgM-producing cells (8×10^6 cells ml^{-1}, final volume 1.25 ml) were preincubated with dMM (1 mM) or SW (10 $\mu g\ ml^{-1}$) for 4 h. A 1-ml aliquot was then labelled with ^{3}H-galactose as described elsewhere[3]. Immunoglobulin was isolated from the culture supernatant and displayed by SDS–PAGE as in Fig. 2. *b*, The remainder of the cells (250 μl) were labelled with ^{35}S-methionine after transfer into methionine-free medium containing inhibitor. Immunoglobulin was isolated from the supernatant and analysed as in Fig. 2. Neuraminidase digests were prepared as described elsewhere[9] and were run on the same gel. *c*, Samples obtained from ^{35}S-methionine-labelled samples prepared as described in Fig. 2, were taken at the times indicated and digested with EndoH as described elsewhere[9].

globulin secreted by dMM-treated cells. Synthesis and secretion of immunoglobulin were not inhibited, as is evident from the ^{35}S-methionine labelling experiment carried out in parallel: similar quantities of immunoglobulin, as verified by quantitation of trichloroacetic acid precipitable material (data not shown) were secreted by SW-treated, dMM-treated and control cells (Fig. 3*b*). The ^{35}S-methionine-labelled samples were subjected to digestion with neuraminidase (NANAse). Removal of sialic acid residues is accompanied by a decrease in M_r (ref. 9), as is observed for both control and SW-treated samples. Immunoglobulin from dMM-treated cells was not susceptible to NANAse (Fig. 3*b*). Further proof of inhibition of conversion of high mannose to complex type oligosaccharides was derived from digestion with EndoH of ^{35}S-methionine-labelled samples. Cytoplasmic immunoglobulin precursors, regardless of time or treatment, were sensitive to EndoH (Fig. 3*c*). Immunoglobulin secreted by SW-treated and control cells was resistant to EndoH, whereas IgM secreted by dMM-treated cells was as sensitive to EndoH as cytoplasmic IgM (Fig. 3*c*).

Conclusive evidence for the inhibition of high mannose to complex type oligosaccharide conversion comes from the analysis of EndoH-released oligosaccharides, obtained from dMM-treated cells labelled with ^{14}C-mannose. After digestion of the labelled immunoglobulin with EndoH the size of the released oligosaccharides was determined by gel filtration in comparison with standards of known structure. The elution profile of these oligosaccharides on a Biogel P4 column, as well as that of marker oligosaccharides, is shown in Fig. 4. The major peak of EndoH-released IgM oligosaccharides (III, Fig. 4*b*) eluted at a position identical to that of a $(Man)_9(GlcNAc)$ marker (I, Fig. 4*a*). The absence of radioactivity in the void volume again indicates that all oligosaccharides on IgM from dMM-treated cells were fully susceptible to EndoH. Approximately one-quarter of the released oligosaccharides was found to elute at a position corresponding to that of $(Man)_8(GlcNAc)$ (IV, Fig. 4*b*), probably the consequence of incomplete inhibition of mannose removal in our experimental conditions. The peaks from the Biogel P4 column were recovered and analysed in parallel by TLC in conditions that allow resolution of the various oligosaccharides (Fig. 4*c*). The major peak (III) possessed an R_F identical to that of the $(Man)_9(GlcNAc)$ marker (II). The minor peak had a mobility consistent with a $(Man)_8(GlcNAc)$ structure (IV). Thus the combined data show that dMM treatment results in the retention of the high mannose configuration.

1-Deoxymannojirimycin is the first inhibitor to be described that inhibits *in vivo* conversion of high mannose to complex type oligosaccharides by blocking the equivalent of a mannosidase IA/B activity. Substances have now been identified that inhibit *in vivo* trimming glucosidases[3,4,12,13], mannosidase II[7,8], and, here, also mannosidase IA and/or IB. Earlier results obtained with tunicamycin[3,9] and 1-deoxynojirimycin[3] showed that elimination of *N*-linked glycosylation altogether or manipulation of glucosidase trimming may have different effects on secretion of immunoglobulin. However, we found no evidence for an inhibitory effect of dMM on secretion of IgM or IgD. The observation that dMM, the mannose analogue of dNM, itself a potent glucosidase inhibitor, is a mannosidase inhibitor suggests that the reaction mechanisms for trimming glucosidases and mannosidase reactions are similar. Both may be postulated to proceed through a cationic reaction intermediate[2].

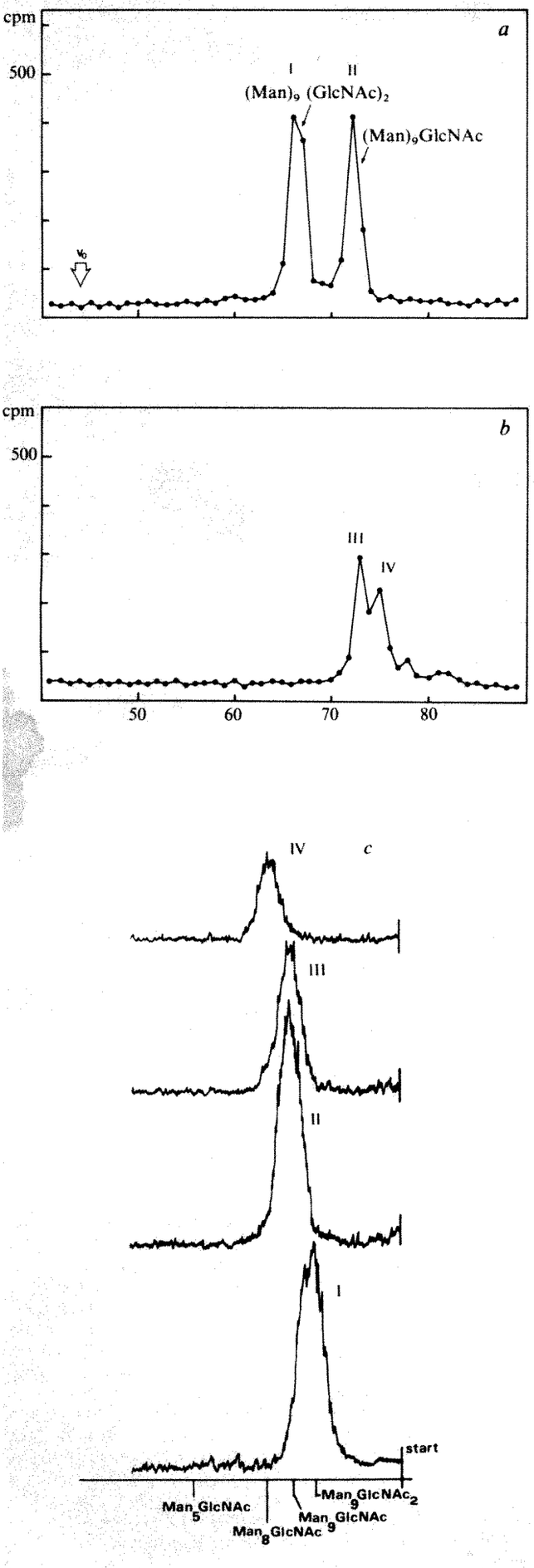


Fig. 4 Gel filtration of ^{14}C-mannose-labelled oligosaccharides obtained from IgM secreted by dMM-treated cells. IgM-producing cells were labelled with ^{14}C-mannose (50 μCi) for 8 h in the presence of 1 mM dMM. IgM was isolated from the culture supernatant as in Fig. 2 and digested with EndoH as described elsewhere[9] but with the inclusion of 0.1% SDS after 3 h digestion. The digest was applied directly to a Biogel P4 (200–400 mesh) column (180 × 1.2 cm) and eluted with 1 M acetic acid. Fractions of 1.32 ml were collected and 200 μl aliquots were counted by liquid scintillation counting. Recovery was better than 90%. The included volume of the column, as determined by chromatography of ^{14}C-glucose, was at fractions 98–102. The void volume is indicated by an open arrow. *a*, Elution profile of marker oligosaccharides $(Man)_9(GlcNAc)_2$, I, and $(Man)_9(GlcNAc)$, II, prepared as described elsewhere[4]. *b*, Elution profile of IgM-derived oligosaccharides after digestion with EndoH. *c*, TLC of: I (fractions 66–67); II (fractions 72–74); and IV (fractions 75–76) from *b* in silica gel. The plate was developed twice with *n*-propanol : acetic acid : water (3 : 3 : 2). Radioactivity profiles obtained by scanning are shown. Peaks II and III have identical R_F values ($(Man)_9(GlcNAc)$).

Carbohydrate moieties have been implicated in a variety of recognition phenomena, ranging from cellular aggregation[14] to participation in embryonic development and immune recognition. Such problems can now be addressed in a more refined manner by manipulation of carbohydrate structure through use of oligosaccharide trimming inhibitors.

This work was supported by the Deutsche Forschungsgemeinschaft through Sonderforschungsbereich 74.

Received 20 June 1983; accepted 5 January 1983.

1. Hubbard, C. S. & Ivatt, R. J. *A. Rev. Biochem.* **50,** 555–583 (1981).
2. Lalegerie, P., Legler, G. & Yon, J. *Biochimie* **64,** 977–1000 (1982).
3. Peyrieras, N. *et al. EMBO J.* **2,** 823–832 (1983).
4. Hettkamp, H., Bause, E. & Legler, G. *Biosci. Rep.* **2,** 899–906 (1982).
5. Reth, M., Hämmerling, G. & Rajewsky, K. *Eur. J. Immun.* **8,** 393–400 (1978).
6. Neuberger, M. J. & Rajewsky, K. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1138–1142 (1981).
7. Elbein, A. D., Solf, R., Dorling, P. R. & Vosbeck, K. *Proc. natn. Acad. Sci. U.S.A.* **78,** 7393–7397 (1981).
8. Tulsiani, D. R. P., Harris, T. M. & Touster, O. *J. biol. Chem.* **257,** 7936–7939 (1982).
9. Vasilov, R. G. & Ploegh, H. L. *Eur. J. Immun.* **12,** 804–810 (1982).
10. Gross, V., Tran-Thi, T.-A., Vosbeck, K. & Heinrich, P. C. *J. biol. Chem.* **258,** 4032–4036 (1983).
11. Kobata, A. *Analyt. Biochem.* **100,** 1–14 (1979).
12. Saunier, B., Kilber, R. D., Tkazc, J., Quaroni, A. & Herscovics, A. *J. biol. Chem.* **257,** 14155–14161 (1982).
13. Datema, R., Romero, P. A., Legler, G. & Schwarz, R. T. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6787–6791 (1982).
14. Edelman, G. M. *Science* **219,** 450–457 (1983).
15. Legler, G. & Jülich, E. *Carbohyd. Res.* (in the press).

A new myosin fragment: visualization of the regulatory domain

Donald A. Winkelmann, Sheri Almeda, Peter Vibert & Carolyn Cohen

Rosenstiel Center, Brandeis University, Waltham, Massachusetts 02254, USA

Many of the functional domains of the myosin molecule have been defined by the use of proteolytic enzymes. Major fragments that retain enzymatic or assembly properties have been prepared by cleavage in the rod to form heavy meromyosin (HMM) and light meromyosin (LMM)[1] or at the head–rod junction to form S-1 and rod[2,3]. Limited tryptic digestion of vertebrate skeletal myosin S-1[4] indicates that the head contains three major regions: an amino-terminal nucleotide binding domain of molecular weight (MW) 25,000[5,6], a central domain of MW 50,000 and a carboxyl domain MW 20,000; the latter two are both able to bind to actin[7,8]. Tryptic digestion of scallop S-1 has also been used to isolate a head fragment MW 14,000 associated with both types of scallop light chains[9]. Here we report that myosin from vertebrate (chicken and rabbit skeletal) and molluscan (scallop adductor) striated muscles is cleaved in an unusual way with an enzyme from *Pseudomonas aeruginosa*[10–12]. This bacterial protease (designated Ps-1) does not cleave myosin at the head–rod junction or in the rod;

0028-0836/84/080758—03$01.00

© 1984 M

instead, Ps-1 splits the myosin heavy chain within the head, yielding a complete rod joined to the 20,000-MW head domains. The scallop regulatory and essential light chains remain associated with this fragment. We examined this new fragment by electron microscopy; the rods bear two 'nubs' about 100 Å long, which appear to correspond morphologically to the neck region of the myosin molecule[13,14].

In contrast to trypsin[1], chymotrypsin[3] or papain[2], Ps-1 cleaves myosin initially at the 75,000–20,000 junction in the head so that a new, larger rod fragment (MW 140,000) and a head fragment (MW 75,000) are produced (Fig. 1). The rod contains the complete rod heavy chain (MW 120,000) together with an additional 20,000-MW peptide derived from the head (Figs 2, 3). The reactive sulphydryls (SH-1 and SH-2)[15] are retained on this 140,000 fragment (data not shown). The associated myosin light chains also show limited cleavage by Ps-1: the two larger light chains (LC1 and LC2) of the vertebrate myosins and the regulatory light chain (RLC) of scallop myosin each lose fragments with molecular weight of about 1,000 (Fig. 1).

The 75,000-MW head fragment is further cleaved by Ps-1 to 25,000 and 50,000 fragments which correspond respectively to the nucleotide[5,6] and actin[7] binding domains identified by tryptic digestion of S-1 (Fig. 2). These head domains from vertebrate striated myosins are then stable even after long incubation periods with Ps-1 (48 h). This selectivity of Ps-1 contrasts with that of trypsin which splits myosin heads into similar domains, but degrades these fragments on longer incubation[16]. Scallop myosin appears, however, to be particularly sensitive to proteolysis, since the head fragments are further digested by Ps-1 (Fig. 1). This property does not appear to be related to the light chain-dependent regulation of this molecule[17]. Vertebrate smooth muscle myosin (turkey gizzard) also exhibits a form of myosin-linked regulation[18], and upon digestion with Ps-1 yields stable head domains of slightly differing molecular weights (47,000 and 28,000) and a 140,000 rod fragment (data not shown).

The sensitivity of the scallop head to digestion permits the new rod fragment to be isolated from this myosin. Most of the degraded scallop head fragments remain soluble at low ionic strength, whereas the scallop Ps-1 rod fragment, with both the regulatory and essential light chains still associated, precipitates in these conditions (Fig. 3*b*). In contrast, all of the heavy and light chain fragments remain associated in Ps-1-digested chicken and rabbit myosin on low ionic strength precipitation or on binding to F-actin.

Electron microscopy of this new scallop rod fragment using rotary shadowing shows predominantly a population of rods terminating in two nubs, each about 100 Å long (Fig. 4*b*). Some variability is found in the appearances of the nub structures, which may reflect the small size and flexibility of this region in myosin[14,19]. Although the head fragments remain associated with the rod in Ps-1-digested vertebrate myosins, shadowed preparations occasionally show molecules with one-nub/one-head and even two nubs (Fig. 4*d*, *e*).

These nubs appear to correspond morphologically to the 'neck' region of the myosin head. Myosin light chains have been localized in this region of both scallop adductor myosin[14] and chicken pectoralis myosin[20]. Moreover, it is likely that the 14,000-MW light chain binding fragment recently isolated from scallop S-1[9] corresponds to a portion of this region. These results, together with the findings presented above, suggest that the neck region of myosin is comprised of the 20,000-MW domain with the associated light chains. We have called this region the 'regulatory domain' because the functional significance of this part of the molecule is established both for scallop[21,22] and vertebrate smooth muscle myosin[18,23,24], and has recently also been suggested for vertebrate striated myosin[25]. In this connection, it is likely that the precise digestion pattern

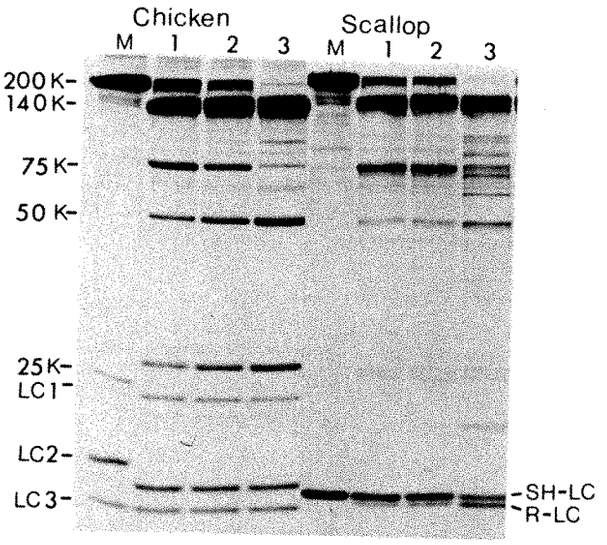


Fig. 1 Myosin fragments obtained by *Pseudomonas* protease (Ps-1) digestion. Digests of chicken pectoralis and scallop adductor myosin were analysed by Laemmli[27] SDS-polyacrylamide gel (12.5%) electrophoresis. M, myosin; lane 1, 1/200 dilution of protease; lane 2, 1/100; lane 3, 1/10. Note the formation of the stable fragments of MW 140,000 for both chicken and scallop myosin. The 75,000-MW fragment is cleaved to 25,000 and 50,000 fragments which are stable fragments from chicken pectoralis myosin; however, these fragments from scallop myosin are further degraded. LC1 and LC2 of the vertebrate myosins and the scallop regulatory light chain (RLC) each lose fragments of about MW 1,000. The rabbit psoas myosin digest pattern was identical to the chicken myosin pattern. Ps-1 is a serine protease with a narrower specificity than trypsin which was isolated from *Pseudomonas aeruginosa*[12]; it has been purified and is being characterized (S.A. and C.C., in preparation). Myosin (5 mg ml^{-1}, 0.5 M NaCl, 0.02 M NaH_2PO_4 *p*H 7.5, 1 mM $MgCl_2$) was digested for 24 h (4 °C) with various dilutions of purified Ps-1 stock (0.3 mg ml^{-1}).

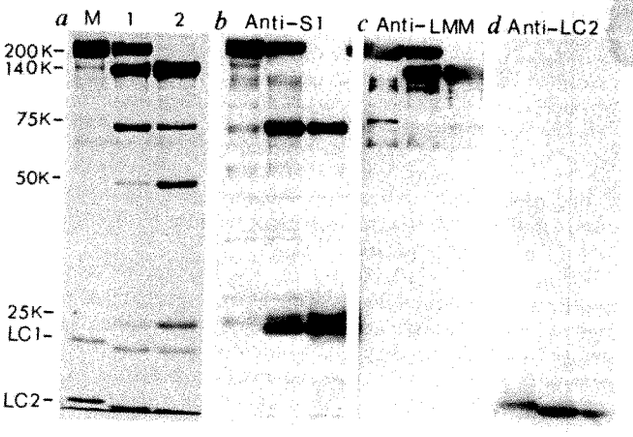


Fig. 2 Immunochemical identification of the fragments obtained by Ps-1 digestion of chicken myosin. *a*, Myosin, M, and the Ps-1 fragments obtained by digestion at two protease concentrations (lane 1, 1/100 dilution; lane 2, 1/10) were electrophoresed on a Laemmli SDS-polyacrylamide gel (11%). This pattern was transferred to nitrocellulose paper and these replicas were probed for specific antibody binding to the myosin fragments using monoclonal antibodies which react with defined regions of the myosin heavy and light chains[20]. *b*, Anti-S-1 (12C5.3) recognizes an epitope in the amino-terminal fragment (MW 25,000) of S-1. This antibody identifies the band (MW 75,000) as an S-1 fragment which breaks down to the amino-terminal 25,000-MW fragment and a 50,000-MW fragment. *c*, Anti-LMM (5C3.2) identifies an epitope that maps to the carboxyl-terminus of the myosin rod. This antibody identifies the 140,000-MW fragment as a larger form (~20,000) of the myosin rod. *d*, Anti-LC2 (7C10.2) identifies the LC2 fragment obtained by Ps-1 digestion. Antibody binding to the nitrocellulose replicas was detected with ^{125}I-labelled rabbit anti-mouse $F(ab')_2$ and autoradiography. The SH–LC and RLC fragment produced by Ps-1 cleavage of scallop myosin have been similarly identified using polyclonal rabbit antisera[28] (data not shown).

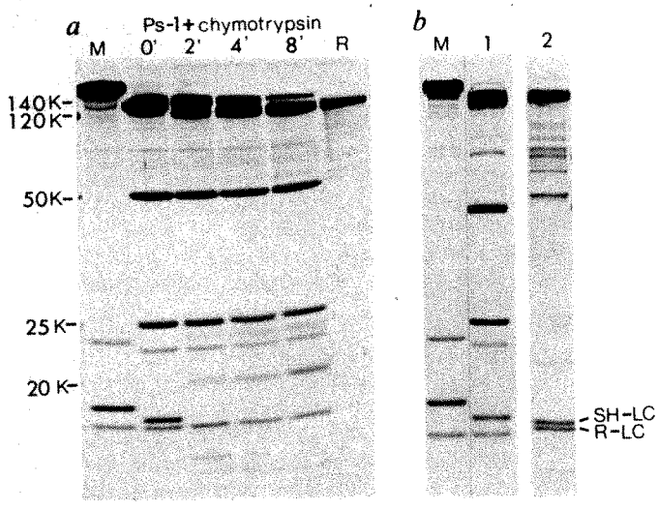


Fig. 3 *a*, The 20,000-MW head fragment can be released from the 140,000-MW Ps-1 fragment by chymotryptic digestion of Ps-1 cleaved myosin. After Ps-1 cleavage, chicken pectoralis myosin filaments (5 mg ml^{-1}, 0.12 M NaCl, 0.02 M NaH_2PO_4 *p*H 7.0, 1 mM EDTA) were treated with α-chymotrypsin (50 μg ml^{-1}) for 0–8 min in conditions that selectively cleave the head–rod junction[3]. The 140,000-MW Ps-1 rod is reduced by chymotryptic digestion to a 120,000-MW fragment which co-migrates with the papain rod fragment (R), and the 20,000-MW S-1 peptide appears simultaneously. This 20,000-MW peptide has been shown to contain the reactive sulphydryl (SH-1) by selective labelling of this cysteine with IAEDANS[16] (data not shown). *b*, SDS-gel electrophoresis of Ps-1-digested chicken and scallop myosin after precipitation by dialysis against low-salt buffer. M, chicken pectoralis myosin; lane 1, Ps-1-digested chicken myosin heavy and light chain fragments remain associated in the washed pellet; lane 2, washed low-salt pellet of scallop digest; bands identified immunochemically as the scallop light chains remain associated with the rod fragment. Variable amounts of the head fragments were present in the rod pellet in different experiments, but in all cases the nub appearance predominated. Digested chicken and scallop myosin were precipitated by dialysis against low salt buffer (40 mM NaCl, 5 mM NaH_2PO_4 *p*H 6.7, 1 mM $MgCl_2$, 1 mM dithiothreitol), and the washed pellets were analysed by Laemmli SDS-polyacrylamide gel (12.5%) electrophoresis.

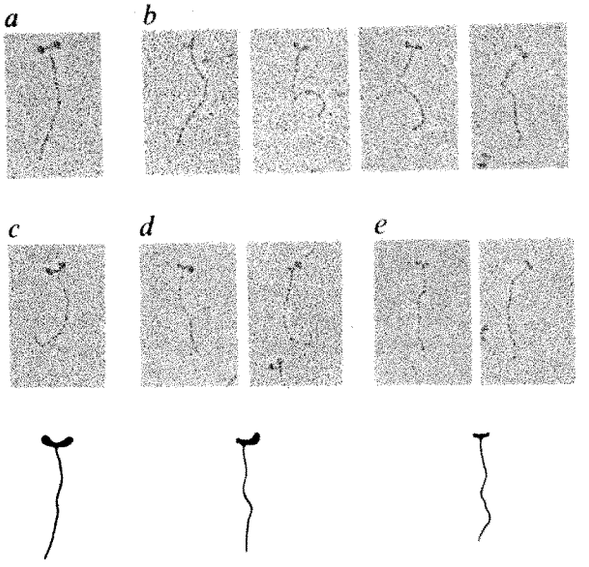


Fig. 4 Electron micrographs of myosin and its Ps-1 rod fragments. *a*, Scallop adductor myosin; *b*, a gallery of scallop Ps-1 rod fragments with associated light chains showing the projecting 100 Å nubs (100 ± 20 Å, $n = 86$); *c*, chicken pectoralis myosin; *d*, chicken Ps-1 rod fragments with one-nub/one-head and *e*, two nubs. The chicken rod fragments were taken from fields of Ps-1-digested myosin in which most of the molecules (>90%) appeared like normal myosin. Tracings of myosin and one-nub/one-head and two-nubbed rod fragments are shown below *c*–*e*. Replicas were prepared by rotary shadowing with platinum[14,20]. Molecules were contrasted with ~12 Å platinum coating in *a* and *b* and ~6 Å platinum coating in *c*–*e*. Magnification ×90,000.

of this region may depend on the physiological state of the myosin molecule (for example, with or without phosphorylation, bound Ca^{2+} and/or nucleotide).

We need a detailed picture of this domain to understand myosin–actin interactions as well as the mechanism of Ca^{2+} control by myosin light chains. The elongated shape of the nub that we have visualized by rotary shadowing is in general agreement with three-dimensional reconstruction results of Vibert and Craig[13] that show a long neck region for myosin heads containing light chains. The precise length and shape of the regulatory domain in the intact molecule are not known, but these results suggest that this domain may span about half the length of the head[14,19]. We have yet to determine the relation between this picture of the head and cross-linking experiments with myosin S-1 and actin[7,8] which suggest that there is a second weak actin-binding site localized in the 20,000-MW domain of the head. A second site has, in fact, been tentatively identified as a 'finger' of low density near an adjacent actin monomer in three-dimensional reconstructions of decorated thin filaments[26]. Studies are in progress to determine whether the nubs can bind to actin.

Visualization of the detailed structure of this region and its relation to the other domains in the head will require X-ray quality crystals of S-1. Selective hinge-cutting enzymes, such as Ps-1, may also prove useful in providing stable intact domains of the myosin head for crystallization. Digestion of native myosin filaments could allow us to examine aspects of the structure of these assemblies which were previously obscured by the size of the myosin heads.

We thank A. G. Szent-Györgyi, E. M. Szentkiralyi and S. Lowey for helpful discussions. Grant support was provided by the Muscular Dystrophy Association, NIH (AM 17346, C.C. and AM 17350, S.L.) and NSF (PCM 82-02516, C.C. and PCM 82-04125, S.L.). D.A.W. is a fellow of The Medical Foundation, Inc.

Received 1 September; accepted 22 November 1983.

1. Lowey, S. & Cohen, C. *J. molec. Biol.* **4,** 293–308 (1962).
2. Lowey, S., Slayter, H. S., Weeds, A. G. & Baker, H. *J. molec. Biol.* **42,** 1–29 (1969).
3. Weeds, A. G. & Pope, B. *J. molec. Biol.* **111,** 129–157 (1977).
4. Balint, M., Wolf, I., Tarcsafalvi, A., Gergely, J. & Sreter, F. *Archs Biochem. Biophys.* **190,** 793–799 (1978).
5. Szilagyi, L., Balint, M., Sreter, F. A. & Gergely, J. *Biochem. biophys. Res. Commun.* **87,** 936–945 (1979).
6. Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. *EMBO J.* **1,** 945–951 (1982).
7. Mornet, D., Bertrand, R., Pantel, P., Audemard, E. & Kassab, R. *Nature* **292,** 301–306 (1981).
8. Sutoh, K. *Biochemistry* **21,** 4800–4804 (1982).
9. Szentkiralyi, E. M. *Biophys. J.* **37,** 39a (1982).; *J. Muscle Res. Cell Motility* (in the press).
10. Tooney, N. M. & Cohen, C. *Nature* **237,** 23–25 (1972).
11. Cohen, C. & Tooney, N. M. *Nature* **251,** 659–660 (1974).
12. Tooney, N. M. & Cohen, C. *J. molec. Biol.* **110,** 363–385 (1977).
13. Vibert, P. & Craig, R. *J. molec. Biol.* **157,** 299–319 (1982).
14. Flicker, P. F., Wallimann, T. & Vibert, P. *J. molec. Biol.* **169,** 723–741 (1983).
15. Reisler, E., Burke, M. & Harrington, W. F. *Biochemistry* **13,** 2014–2022 (1974).
16. Hozumi, T. *Biochemistry* **22,** 799–804 (1983).
17. Szent-Györgyi, A. G., Szentkiralyi, E. M. & Kendrick-Jones, J. *J. molec. Biol.* **74,** 179–203 (1973).
18. Sherry, J. M. F., Gorecka, A., Askoy, M. O., Dabrowska, R. & Hartshorne, D. J. *Biochemistry* **17,** 4411–4418 (1978).
19. Elliott, A. & Offer, G. *J. molec. Biol.* **123,** 505–519 (1978).
20. Winkelmann, D. A., Lowey, S. & Press, J. *Cell* **34,** 295–306 (1983).
21. Stafford, W. F. III, Szentkiralyi, E. M. & Szent-Györgyi, A. G. *Biochemistry* **18,** 5273–5280 (1979).
22. Hardwicke, P. M. D., Wallimann, T. & Szent-Györgyi, A. G. *Nature* **301,** 478–482 (1983).
23. Sellers, J. R., Pato, M. D. & Adelstein, R. S. *J. biol. Chem.* **256,** 13137–13142 (1981).
24. Kendrick-Jones, J., Jakes, R., Tooth, P., Craig, R. & Scholey, J. in *Basic Biology of Muscles: A Comparative Approach* (eds Twarog, B. M., Levine, R. J. C. & Dewey, M. M.) 255–272 (Raven, New York, 1982).
25. Wagner, P. D. & Stone, D. B. *Biochemistry* **22,** 1334–1342 (1983).
26. Amos, L. A., Huxley, H. E., Holmes, K. C., Goody, R. S. & Taylor, K. A. *Nature* **299,** 467–469 (1982).
27. Laemmli, U. K. *Nature* **227,** 680–685 (1970).
28. Wallimann, T. & Szent-Györgyi, A. G. *Biochemistry* **20,** 1176–1187 (1981).

New and Forthcoming Books

Biochemistry of Steroid Hormones

Edited by H.L.J. Makin MA, PhD

Since the first edition of this book was published in 1975, many advances in knowledge of the biochemistry of steroid hormones have been made and a new steroid hormone, vitamin D, has been added to the repertoire. This second edition has been extensively revised and the result is a definitive reference text for all involved in the wide field of steroid hormones.

Second Edition, Early 1984. 640 pages, 314 illustrations. About £45.00

Viral Pathogenesis and Immunology

C.A. Mims MD, FRCPath and **D.O. White** MD, PhD, MSc, FRCPA

This book surveys the pathogenesis and immunology of viral diseases, with the emphasis on those of medical or veterinary importance. The broad yet central coverage of the subject will appeal to senior undergraduates and research workers in the fields of microbiology, cell pathology, immunology and virology.

Spring 1984. 300 pages, 50 illustrations. Paper, about £10.80

Molecular Biology at a Glance: from Cells to Atoms

A.R. Rees MA, DPhil and **M.J.E. Sternberg** MA, MSc, DPhil

Undergraduates in the biological sciences are provided in this text with the essential elements of the molecular aspects of cell biology and biochemistry. The selection of topics range from the description of whole cells and their organelles to the shapes of biological molecules, and several advanced and topical subjects are included, such as the principles and applications of genetic manipulation.

Spring 1984. 96 pages, 35 illustrations, Paper, about £5.00

Behavioural Ecology: an Evolutionary Approach

Edited by J.R. Krebs, *Wolfson College, Oxford* and **N.B. Davies,** *Pembroke College, Cambridge.*

The second edition of this popular text consists of a completely new set of chapters written by some of the most active research workers and teachers in the field of behavioural ecology. The book will serve both as a course text and as an important, up-to-date reference work for research workers in the fields of behavioural ecology, animal behaviour and evolutionary biology.

Second Edition, Spring 1984. 500 pages, 71 illustrations. Cloth, about £18.00, paper, about £10.50

A Synoptic Classification of Living Organisms

Edited by R.S.K. Barnes BSc, MA, PhD

Designed for reference at every level in biological and environmental sciences, this book contains an outline classification of all living organisms from bacteria, through protists, to fungi, plants and animals. The book is intended as a concise pocket dictionary of the diversity of living organisms which will be useful to all biologists.

1984. 276 pages, 67 illustrations. £7.50

Introduction to Modern Mycology Basic Microbiology, Vol 7.

J.W. Deacon BSc, PhD

This book gives a general introduction to the subject aimed at undergraduate students of microbiology, botany and biology, although experienced mycologists will also find it of interest.

'It is clearly written, compact, explanatory account of fungal structure, growth and development, coupled to consideration of the role of fungi in nature ... Dr Deacon's book is currently the best, short undergraduate introduction to fungi on the market.' *Nature* (on the first edition)

Second Edition, Early 1984. 272 pages, 79 illustrations. Paper, about £9.80

Developmental Control in Animals and Plants

Edited by C.F. Graham MA, DPhil and **P.F. Wareing** DSc, FRS

The second edition of this acclaimed text, previously entitled The Developmental Biology of Plants and Animals, includes much new material and extensive revision of the remainder to take account of significant advances in knowledge, especially in the structure of eukaryotic genome and the control of gene expression. This is the ideal textbook for advanced courses in developmental biology.

Second Edition, Early 1984. 520 pages, 257 illustrations. Paper, about £14.80

Human Evolution at a Glance

R. Lewin BSc, PhD

Human Evolution at a Glance is an up-to-the-minute presentation of the fast-moving field of palaeoanthropology, touching on primatology, palaeontology, palaeoecology and geology, taphonomy, molecular biology, archaeology and prehistory. The book gives a concise account of the current status of information and ideas in these pertinent areas and is designed for university introductory courses.

Early 1984. 128 pages, 120 illustrations. Paper, about £5.80

Microbiological Sciences

Recent Trends in Bacteriology, Mycology and Virology

Editor-in-chief: Professor C.M. Brown, *Heriot-Watt, University, Edinburgh*
Editor: Dr. I.W. Dawes, *University of Edinburgh*

Microbiological Sciences is a new monthly periodical for the whole of the microbiological sciences and will be published for the International Union of the Microbiological Societies by Blackwell Scientific Publications. Its contents will include news reports, features and opinions. Most of the articles will be specially commissioned to report and consider all recent developments in such fields as bacteriology, virology and mycology.

Subscription Information

Microbiological Sciences will be published monthly from April 1984, and the subscription rates will be *Institutional:* £75.00 (UK), £90.00 (overseas), US$162.00 (USA and Canada). *Individual:* £25.00 (UK), £30.00 (overseas), US$54.00 (USA and Canada).

BLACKWELL SCIENTIFIC PUBLICATIONS LTD
Osney Mead, Oxford OX2 0EL, England. Telephone: Oxford (0865) 240201

Circle No.28 on Reader Service Card.

Alan R. Liss, Inc.

CULTURE OF ANIMAL CELLS
A Manual of Basic Technique

by **R.I. Freshney,** *Department of Clinical Oncology, Cancer Research Campaign Laboratories, University of Glasgow*

At last there is a comprehensive self-explanatory guide to the basic techniques of tissue culture. *Culture of Animal Cells: A Manual of Basic Technique* is the first laboratory manual that enables both the novice and experienced researcher to apply the basic techniques of tissue culture.
The manual is a remarkable source of information illustrating and describing, step by step, how to culture cells in vitro, with lucid explanations of the biological principles involved. This readily accessible and extremely informative manual will allow immediate implementation of basic tissue culture techniques for sophisticated research and routine classroom exercises.
It is intended for use by Researchers, Technicians, and Graduate Students in Cell and Organ Physiology; Biochemistry; Microbiology; Genetics; Biomedical Research; Cellular, Developmental, and Molecular Biology; Virology; Pharmacology; Zoology; Anatomy; Histology; Oncology; Pathology; and Neurobiology.

November'83 — 310pp
08451 0223 0 — £33.00/$67.75

MODERN CELL BIOLOGY, Volume 1
edited by **B.H. Satir**

Modern Cell Biology is a new series providing researchers in cellular and molecular biology with concise articles on the biology of the cell. The intent of the series is to review the current status of research in the field and to provide a base from which future investigations may develop. *Modern Cell Biology* will consistently keep its multidisciplinary audience abreast of the ongoing research on cell biology.
Modern Cell Biology publishes critical reviews of specific areas in cell biology, including personal perspectives and general reviews related to the boundaries of the field or the application of new methods to cell biological problems. From time to time, symposia concerning vital topics in cell biology will appear as special volumes.

Modern Cell Biology Series Volume 1; Series Editor **B.H. Satir**

Contents: Receptor-Mediated Endocytosis; Structure and Function of the Adhesive Glycoprotein Fibronectin; Membrane Biogenesis, Enveloped RNA Viruses and Epithelial Polarity; The Acetylcholine Receptor-Ion Channel Complex: Linkage Between Binding and Response; Index.

June'83 — 216pp
08451 3300 4 — £26.00/$46.80

MODERN CELL BIOLOGY, Volume 2
Spatial Organization of Eukaryotic Cells
edited by **J.R. McIntosh**

Modern Cell Biology, Volume 2: Spatial Organization of Eukaryotic Cells is the proceedings of a symposium honoring retired scientist Keith R. Porter.
It examines current knowledge concerning the mechanisms by which cell structure is established. In addition, this volume reviews structural cell biology in the context of tissue and organ structure by looking at the factors that govern pancellular organization in multicellular organisms.

Modern Cell Biology Series Volume 2; Series Editor **B.H. Satir**

Contents: Organization of Cell Membranes and the Compartmentalization of Cytoplasm; Organisation of the Fibrous Components of Cytoplasm; Cytoskeletal Action in Cytoplasmic Organization; Organization of Nuclear Structure and the Control of Gene Action; Global Organization of Cells and Extracellular Space; Summary; Index.

September'83 — 598pp
08451 3301 2 — £38.00/$68.40

NERVOUS SYSTEM REGENERATION
edited by **B. Haber, J.R. Perez-Polo, G.A. Hashim** and **A.M.G. Stella**

Nervous System Regeneration discusses new approaches to the problem of elucidating the causes of success or failure in axonal regeneration. The book contains original data as well as reviews of ongoing research and is divided into six main sections. These sections discuss some of the newest techniques available to man and include discussions on the nerve growth factor, neuronotrophic substances, trophic factors, neuronal sprouting, neuronal differentiation and regeneration, and control of motor localization.

Birth Defects: Original Article Series, Volume 19, Number 4,
September'83 — 586pp
08451 1055 1 — £75.00/$135.00

DEVELOPING AND REGENERATING VERTEBRATE NERVOUS SYSTEMS
edited by **P.W. Coates, R.R. Markwald,** and **A.D. Kenny**

This book presents integrated approaches to problems that now exist in neurobiology, that can be applied to clarifying a wide variety of neurobiological functions and dysfunctions. It provides an in-depth look at recent viewpoints on a number of frontiers in neural development and regeneration.

Series: Neurology and Neurobiology, Volume 6
January'84 — 284pp
08451 2705 5 — £43.00/$77.40

MEMBRANE BIOPHYSICS II
Physical Methods in the Study of Epithelia
edited by **M.A. Dinno, A.B. Callahan** and **T.C. Rozzell**

This volume reviews new research methods and their use in the study of biological systems, providing insights into epithelial membranes at the microscopic level. The methods presented offer good examples of interactions between scientific disciplines and the state of the art of innovative theoretical and technological advances.

Series: Progress in Clinical and Biological Research, Volume 126
November'83 — 388pp
08451 0126 9 — £36.50/$65.70

All books available from your usual bookseller or in case of difficulty from Wiley. If ordering from Wiley please make cheques payable to ***John Wiley and Sons Limited****. You may also telephone your credit card order – dial 100 and ask for FREEFONE 3477 (UK only).*

Marketed in the U.K. and Europe by
John Wiley & Sons Limited
Baffins Lane · Chichester · Sussex PO19 1UD · England

Circle No.37 on Reader Service Card.

The elephant and the flea

R.V. Jones

Rutherford: Simple Genius. By David Wilson.
Hodder & Stoughton/MIT Press: 1984. Pp.639. £14.95. (*April publication, $25.)*

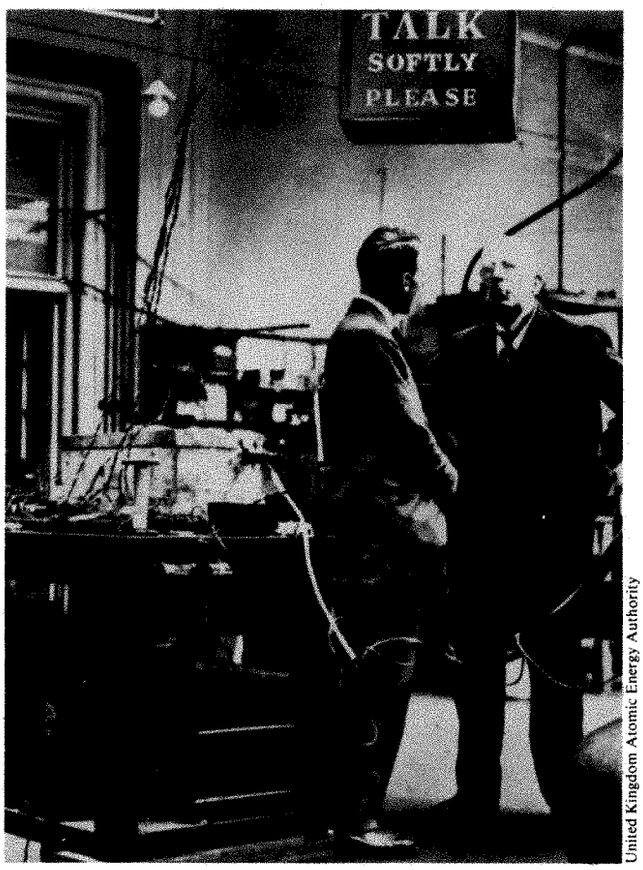
ERNEST Rutherford's towering stature in the heroic age of twentieth-century physics has made him the subject of many books, memoirs and lectures. And yet, in this new and comprehensive biography David Wilson has illuminated fresh facets of the man and his activities.

First there is Rutherford's work in the First World War for the Royal Navy, which has only been sketchily outlined by earlier biographers. In fact, Rutherford was once described as "lacking in public spirit" because, for example, he went on for a visit to his native New Zealand after the British Association meeting in Australia in 1914, while H.G.N. Moseley and H.T. Tizard rushed by ship across the Pacific, by train across the United States, and then by ship again across the Atlantic, so that they could offer their services in Britain (only, alas, for Moseley to be killed at Gallipoli). But David Wilson has discovered in the papers of the late A.B. Wood much about Rutherford's efforts to assist the Navy on his return — from which it incidentally transpires that credit for the invention of Asdic (or Sonar) should be ascribed to Rutherford as much as to his French colleague, Pierre Langevin. And it was not only by invention that Rutherford contributed: he made many visits to establishments and he joined in trials at sea. In 1917 he was pressing the Navy to bring the scientists much more into the operational picture for "in scientific research it was found to be essential that the researcher should have the widest knowledge and personal experience of the difficulties to be solved" — to which a senior naval officer objected "the only information necessary to be given was that enemy submarines were in the sea, and that means were required to detect their presence".

It was to take the experiences of the Second World War, led by the example of Henry Tizard, to convince the Services of the folly of such a view. And Tizard's second contribution, the famous Tizard Mission to the United States in 1940, was by coincidence anticipated in 1917 by a joint Anglo-French Mission led by Rutherford to acquaint American scientists with developments made in Britain and France.

After the war, his campaign for scientific support for the Navy led to the foundation of the Admiralty Research Laboratory.

When, in 1919, Rutherford became Cavendish Professor at the University of Cambridge, his qualities as a scientific administrator were frequently called upon by the British Government, and David Wilson believes that he has made the first study of Rutherford in this capacity. His activities ranged from dealing with individual problems, such as the future of the Artillery College at Woolwich, to being President of the Royal Society and Chairman of the Scientific Advisory Council of the Department of Scientific and Industrial Research, in both of which offices he stimulated much action and prompted many forecasts of national needs; and in 1933 he headed the Academic Assistance Council with the objective of aiding academic refugees from the Nazis.



United Kingdom Atomic Energy Authority

Rutherford (right) at the Cavendish — "spreading tobacco ash and noise, [ruling] the laboratory as a tribal leader".

Wilson has drawn on many sources to depict Rutherford's temperament and character. "Brash and noisy all his life", or more accurately, quoting Henry Tizard:

> He had a boisterous sense of fun and a loud laugh. By precept and by example he helped keep our minds free from cant. He hated pomposity and artificiality. He loved simple people and simple ways, and lived a simple life. . . . lived a life of feverish intellectual activity relieved by short periods of magnificent idleness. When he took a holiday it really was a holiday.

Wilson's carefully detailed account of Rutherford's interactions with the many physicists who worked with and around him provides an illuminated cavalcade through forty of the most exciting years in physics: with Soddy, Moseley, Geiger, Marsden, C.G. Darwin, the Braggs, Langevin, C.R.T. Wilson, von Hevesy, Bohr, Chadwick, Blackett, Cockcroft and Kapitza among its members. A whole chapter, in fact, is sympathetically devoted to Kapitza who, Wilson says, came closest to being the son whom Rutherford never had. "The Crocodile" was the nickname given by Kapitza to Rutherford; and this is why, when the Mond Laboratory was built (in Cambridge), Kapitza asked Eric Gill to carve a crocodile over its main door. Unfortunately, the Laboratory had scarcely been opened when Kapitza was irrevocably detained while on a visit to Russia, despite the efforts of Rutherford and many others to persuade the Soviet authorities to release him.

One item which Wilson records has, incidentally, corrected a long standing misjudgment of my own concerning F.A. Lindemann (later Lord Cherwell). When I was in his laboratory in the 1930s I felt that it was unfortunate for physics in Britain that he had not come to an understanding with Rutherford about the division of interests between the Clarendon in Oxford and the Cavendish, instead of the competition exemplified by the 1932 race to liquefy helium and so steal the Mond Laboratory's thunder before it opened. Thanks to Wilson's delving, I now learn that on his election to the Clarendon in 1919 Lindemann wrote to Rutherford in the following terms: "I am anxious to work in collaboration with Cambridge and the other schools of physics, both to prevent overlapping . . .". Unhappily, relations turned out otherwise.

As background to Rutherford's life and work we are given excellent descriptions of the opening up of New Zealand and of the development of science in Cambridge in the nineteenth century, and in particular of the establishment and achievements of the Cavendish Laboratory in the first seventy years of its existence, occasionally spiced with such episodes as the refusal of the

seventh Duke of Devonshire to allow his sons to have a scientific education despite his own accomplishments of Second Wrangler and Smith's Prizeman, and his generous foundation of the Cavendish. Wilson has of course had a great deal of material to draw upon from previous biographies and memoirs, but he has gone through these with a critic's eye and resolved discrepancies between them, and has dug further into the background.

As a result we have a biography which not only exposes Rutherford's contributions in fields which have hardly been treated before, such as his work for the Navy, but also a careful, almost blow-by-blow, account of his researches in Cambridge, Montreal, Manchester and then back again in Cambridge, starting with his magnetic detector for radio waves and his work with J.J. Thomson, leading on to the discoveries which proved him to be the greatest experimental physicist of this century: radon, the alpha-particle, the nuclear atom and nuclear disintegration. Radon, or "emanation", was discovered by the curious observation that the electrical leakage in an air-filled box containing thorium oxide was greater when the door was closed. Both the observation and the remorseless pursuit of its explanation were typical Rutherford.

Just how difficult the pursuit of explanation could be was exemplified by the time it took him to formulate the concept of the nuclear atom after the crucial observation had been made by Geiger and Marsden, in the spring of 1909, that while nearly all alpha-particles passed through a thin foil as though it were completely transparent, a few were deflected through large angles. "Every schoolboy" ever since has known the explanation: but at the time all the physicists concerned, Rutherford included, were baffled. "It was as though", he said, "you had fired a fifteen-inch shell at a piece of tissue paper and it had bounced back at you." More than eighteen months were to pass before Rutherford could give the news in a letter of December 1910 to a colleague: "I think I can devise an atom much superior to J.J.'s for the explanation of and stoppage of alpha- and beta-particles. . . . It will account for the reflected alpha-particles observed by Geiger. . . .".

David Wilson, a science journalist, is modest about his own command of physics, but I found only one small point where his account might have been helpfully expanded. It concerns Hertz's failure in 1883 to deflect cathode rays electrostatically: when such a superb observer and experimenter failed to find any deflection, the discovery of the electron was probably held up by several years, for it seemed that cathode rays were uncharged. Wilson says that J.J. Thomson suspected that the negative result was due to pressure of gas in the discharge tube, and so repeated the experiment (with the better vacuum obtainable in 1897) when deflection was immediately observed. It was not the residual pressure of gas in the discharge tube which had directly produced the negative result, but the fact that the gas became ionized, and the ions had been attracted to the deflecting plates until they built up a space-charge which neutralized the original deflecting field. The incident is worth remarking for its lesson in showing how one of the greatest of all experimental physicists, with radio waves and the photoelectric effect to his credit, could for once be let down by imperfection of technique.

If I miss anything in this magnificent biography, it is the multitude of anecdotes and apothegms which Rutherford's name immediately recalls. I myself remember, for example, how at a lecture in Oxford in the 1930s, Rutherford squashed E.A. Milne when the latter asked him how he knew that the heavy particles were in the nucleus at the centre of the atom, and not the electrons with the heavy particles orbiting the periphery as in a centrifuge. "Well", boomed Rutherford, who was a big man, over Milne, who was a small one, "when you have an elephant and a flea you assume it is the flea that jumps!".

Those with a taste for more anecdotes can doubtless dig for them in the seventeen pages of notes and five pages of bibliography with which David Wilson concludes his account. It is simply one of the best. □

R.V. Jones is Emeritus Professor of Natural Philosophy at the University of Aberdeen.

ADVERTISEMENT

Public Information

factual and informative material on Nuclear Energy

write for free catalog



American Nuclear Society
555 N. Kensington Ave.
La Grange Park, IL 60525
(312) 352-6611

Circle No.20 on Reader Service Card.

One objective for physicists

Alan H. Guth

Unity of Forces in the Universe.
By A. Zee.
World Scientific (distributed by Wi 1983. Two volumes, pp. 1,076. E volume hbk £40, $57; pbk £16.5(

To a particle physicist, the 1960s no like the Dark Ages. Of the four physical interactions, electromag was the only one for which a successful theory existed. Howev last years of the decade marked the ning of a period of enormous activi since the 1920s had physicists mad rapid and exciting progress in standing the fundamental laws of The work of Weinberg, Salam, G and 't Hooft was synthesized perfectly acceptable theory of the we electromagnetic interactions. Mea the theory of quantum chromody emerged, and it gradually came to garded as the correct theory of the interactions. Thus, by the early three of the four interactions were stood, with only gravity (at the qu level) remaining a deep mystery.

With morale bolstered by triumphs, particle theorists then be attempt to unravel the physics of scales far beyond those that can be r in available or even fores accelerators. The watchword of th movement in particle theory is *unit* as physicists of the nineteenth centu covered that electricity and magnet inextricably linked, their twentieth-c heirs are now trying to show that interactions in nature are simply manifestations of a single unifying

The movement towards unity in has given rise to a vast number of pu papers. For example, there are no 1,300 which cite the original work Georgi and S. Glashow on the SU(5 unified theory. In preparing hi volume collection, A. Zee has ca chosen 78 papers to illustrate the im developments in the quest for uni papers cover many aspects of the theme, and thereby reflect admira exciting developments in particle over the past decade. In addition, provided about 100 pages of summary and explanation to ser companion and guide to the re papers. The reader is assumed familiar with the basics of quantu theory, including non-Abelian theories. For prospective readers w this background, the author has inc short list of suggested sources.

The two-volume set is divided chapters, each of which contains pages of explanatory text and

reprinted articles. The explanations are concise yet clear, summarizing the important developments and unsolved problems in a given area. Although the information is densely packed, the style maintains a flavour of informality. With liberal use of quotation marks, Zee writes about the "see-saw mechanism" of generating neutrino masses, and of the "lopsided" versus the "orthodox" pattern of symmetry breaking in the SO(10) grand unified theory. He also informs us, in a footnote attributed to Howard Georgi, that the SO(10) theory was actually discovered half an hour before the SU(5) theory. I am still trying to imagine how breathtaking a day that must have been.

Volume I is concerned mainly with the basics of grand unification, founded on the groups SU(5), SO(10) and E(6). This volume also includes a two-part "Instant Review of Group Theory", as well as chapters on proton decay, and on attempts to understand the existence of three families of fermions and to determine the fermion masses. Volume II begins with five chapters on cosmology, emphasizing the impact of recent developments in particle physics. Writing primarily for particle physicists, Zee begins with a short course on basic cosmology. The explanatory notes will be particularly useful to the beginner, and the reprints include many of the classic papers of George Gamow and his collaborators. Subsequent chapters deal with the generation of the matter–antimatter imbalance, the theory of galaxy formation (with or without massive neutrinos) and the inflationary universe model. The remainder of Volume II includes accounts of dynamical symmetry breaking and composite models, as well as a brief discussion of the attempts to include gravity in the programme of unification.

A quality of the books which I particularly admire is the author's frank and unassuming tone. Zee points out that "the selection [of papers] assuredly reflects the author's idiosyncracies and the fact that while he has certainly not read every paper in the field, he has definitely read his own papers". (In fact, the books contain only six of Zee's articles, all of which seem quite appropriate to me.) In an effort to encourage the student who might be frightened by the vast volume of literature in this field, Zee points out that "it is not even necessary to read all the papers reprinted in this volume. This writer certainly hasn't". Nor, I confess, have I. □

Alan H. Guth is an Associate Professor of Physics at the Massachusetts Institute of Technology.

Basic methods

Roger Patient

Techniques in Molecular Biology.
Edited by John M. Walker and Wim Gaastra.
Croom Helm/Macmillan, New York: 1983. Pp.333. Hbk £19.95, $29.95; pbk £9.95.

THE success of a textbook, like that of a lecture, is dependent upon a clear idea of the likely audience: the authors and editors of *Techniques in Molecular Biology* appear to have largely satisfied this important criterion. The main readers will be final-year undergraduates (especially project students) and postgraduates embarking on research. Established research workers will find most of the book familiar, but will value its presence in the laboratory when employing a new technique or when suffering one of those irritating failures of a technique which has been working for years. In addition useful tables and titbits of information are sprinkled throughout.

The scope of the book is fairly comprehensive, covering the separation and *in vitro* synthesis of proteins and nucleic acids, their transfer to solid supports and detection, determination of their amino acid or nucleotide sequences, and molecular cloning. Very little is considered too basic for inclusion: nick translation, for instance, gets a whole chapter. The information is well-organized and concisely presented, with a lot of internal cross-references. In relation to other literature available, this publication intercalates quite nicely between *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, 1982), which contains an enormous amount of practical detail, and the *Genetic Engineering* series, published by Academic Press, which contains none.

Criticisms of the book centre on a very inconvenient way of listing references and a number of omissions or incomplete treatments of particular techniques. For instance, there is no good description of the direct application of nucleic acids to nitrocellulose and subsequent hybridization (the so-called "dot-blot" technique), and only cursory treatment of *in situ* immunoassay techniques for screening bacterial recombinants. Occasionally, the information provided is actually misleading, as in the statement that ribonuclease-free deoxyribonuclease is available commercially (in practice, further purification is usually necessary before use). Finally, the problems associated with particular techniques are sometimes glossed over, as in the cases of M13 nucleotide sequencing, which has taken a number of laboratories a long time to establish, and assays of *m*RNA solutions using ^{3}H polyuridylic acid, where problems with variability of polyadenylic acid tail length and reproducibility are often experienced. □

Roger Patient is a Lecturer in the Department of Biophysics at King's College, University of London.

Bangosomes: the past and the present

J.A. Lucy

Liposome Letters.
Edited by A.D. Bangham.
Academic: 1983. Pp.421. £17.50, $29.
Liposomes.
Edited by Marc J. Ostro.
Marcel Dekker: 1983. Pp.400. SwFr.157, $58.75.

Liposome Letters is a most unusual if not unique book, but that will not surprise those who are personally acquainted with the editor. In 1982 Alec Bangham wrote to some fifty scientists who have been concerned in one way or another with liposomes to suggest they send him informal letters, poems etc., that chronicle an anecdote, observation, hypothesis or comment about liposomes. His intention was that their offerings should recapitulate the developments and diversification of the liposome "membrane" model from its early days up to the present. In the preface he says that this formulation derived from a feeling that too much of our scientific writing is stereotyped, banal, platitudinous and devoid of all sensibility to the fabric of life in scientific research, success or failure. After just twenty years, I now realize why Alec considered my initial draft of a joint paper with him to be "pretty turgid stuff".

It is surprising what one can find in this volume. For example I see that in the preface by Papahadjopoulos, I and three other named villains are pilloried for apparently considering that he and Bangham were simply wasting their time in the early stages of work on liposomes. Then, Racker's chapter is punctuated by five Lessons, the last of which reads:

> After you publish rejoice when other scientists don't believe what you know to be true. It will give you extra time to work on the phenomenon in peace. Whey they start claiming that they have discovered it before you, look for a new project.

His chapter ends with full-page photocopies of two letters, one from Racker to Bangham that reads "The answer to your question in your letter of March 1, 1982, is yes", and the other from Bangham to Racker replies "Thanks". Scherphof has contributed a witty three-page dissertation on how to pronounce the word liposome that includes a "Light-macrograph of the essential part of a Dutch technician caught in the act of preparing [blowing] an animal-size hemilamellar liposome (ASH-L). Bar is 1,0cm". The text says "For anybody watching this picture there cannot remain a shadow of doubt that a liposome is a LIP′əsōm′, can there? From Groningen, with LUV, P.S. You should see her in full colour!".

Most papers are, however, primarily serious but with lighter touches, such as the Molecular Trojan Horse that illustrates the article by Dimitriadis on the transformation of eukaryotic cells, and perhaps most of the contributions would not seem particularly out of place in a more conventional book. (Each contribution has been photographically reproduced from the original typescript, but none is difficult to read.) There are some 40 articles in all, from which much can be learned about liposome lore. Anyone who has been personally involved with liposomes, or who knows workers in the field, will also be interested by the anecdotes. Those who have not might find them slightly tedious. The unusual format has, however, encouraged authors to be more forthright than usual, and this is a valuable feature in itself. For example, to quote finally from George Poste:

> I was highly skeptical (and remain so) about the feasibility of targeting liposomes to tumor cells in solid neoplasms and their metastases *in vivo*. . . . My criticisms are directed only to those proponents of targeting who chose to ignore the substantial problems created by anatomic and physiologic factors *in vivo*. . . . Overselling always carries the danger that real opportunities may be lost because of skepticism elicited by earlier extravagant claims which have failed to come to fruition.

Although *Liposomes* edited by Ostro is of comparable size to *Lipsome Letters*, and was published at about the same time, it appears to have taken longer to produce perhaps because it is not based on camera-ready copy. There are eight contributions, five of which are from the pens of authors who also appear in Bangham's volume. In Ostro's book, however, their contributions are basically more serious (influenced by Bangham, I nearly wrote more prosaic), or rather all of them except Bangham's own short historically-orientated chapter. The editor has attempted to assemble reviews of various areas of liposome research in such a way that the volume represents a compilation of the major work in the field since 1965 (up to, as far as I can see, fairly early in 1982).

In the preface Ostro (whose own affiliation is The Liposome Company) comments that 30% of those attending a recent meeting on liposomes were affiliated to commercial laboratories. This is a useful laboratory reference book and it similarly reflects current interest in the practical and commercial applications of the targeting of liposomes as delivery systems. Thus the three chapters (liposomes as a tool in molecular biology; immunologic aspects of liposomes; therapeutic applications of liposomes), which are most concerned with various aspects of this topic, occupy considerably more than half of the volume. □

J.A. Lucy is Professor of Biochemistry at the Royal Free Hospital School of Medicine, London.

Catastrophe, chaos and delay

Athel Cornish-Bowden

Mathematical Models in Molecular and Cellular Biology.
Edited by Lee A. Segel.
Cambridge University Press: 1983. Pp.768. Pbk £15, $29.95.

WHY is the story of the mathematician who opened a lecture with the words "Consider a spherical elephant" not funny? Biologists need to understand this before they can appreciate the value of mathematics and overcome their suspicion of it. Some caution is justified, as there are many who seem to regard numbers and equations as a convenient shield for the poverty of their ideas, but mathematics has made considerable contributions to biology and deserves to be taken seriously.

To give one example: a valid theory of metabolic control cannot be developed without a mathematical framework that extends beyond the elementary kinetics of isolated enzymes; in consequence, biochemists who reject a mathematical approach continue to be misled by oversimplified ideas, such as "bottleneck enzymes", "controlling steps" and so on. In some areas of biology it is easy to check by experiment whether a mathematical theory makes correct predictions, even if one does not understand the mathematics. Metabolic control, however, is difficult to study by experiment (other than by computer simulation), and so one cannot easily convince the sceptic that metabolic fluxes must be largely unaffected by moderate changes in the concentration of almost any enzyme. In such cases scientists who deny themselves all but the most elementary mathematics are also denying themselves understanding.

Anyone genuinely interested in learning how mathematics is being applied to research problems in biology can hardly do better than begin with *Mathematical Models in Molecular and Cellular Biology*, a book first published in 1980 and now available in paperback. Dr Segel has taken his responsibilities as editor seriously and has produced a real book, not just a haphazard collection of papers. Indeed, the individual authors are given so little prominence that their names do not appear at the beginnings of their chapters.

I particularly liked the obvious efforts taken to support the mathematics with a readable text and to make it believable. For example, the information that the equation $x_{t+1} = rx_t(1-x_t)$ can generate chaotic (aperiodic) behaviour is accompanied by the instructive suggestion that "the reader simulate the difference equation on a hand calculator to see this". After doing just that, I found it much easier to accept the important biological message that "phenomena that look chaotic may come from simple underlying laws". As the author of that chapter remarks, "the implications . . . are most unsettling"!

I was disappointed to see that population genetics is mentioned only in passing, but this was the only major omission that I noted. Some other topics, such as elementary thermodynamics and enzyme kinetics, are also dealt with briefly or not at all, but there would be little point in filling a book of this kind with the staples of general biochemistry texts.

Catastrophe, chaos, delay. . . . It will be evident by now that the title of this review is not a comment on the book: it is inspired by the editor's list of some of the mathematical concepts to be discussed in a biological context, but there is much else to interest anyone with a lively mind. □

Athel Cornish-Bowden is a Lecturer in the Department of Biochemistry at the University of Birmingham, UK, and the author of Basic Mathematics for Biochemists *(Chapman & Hall, 1981).*

Sex in society

Stuart Sutherland

Men and Women: How Different Are They?
By John Nicholson.
Oxford University Press: 1984. Pp.193. Hbk £9.95, $19.95; pbk £2.50.

ONE OF the more curious aspects of social research is that it so rarely produces definitive results. This is especially true in the area of sexual differences and their origins: *Men and Women* is a rather cursory survery of the evidence on this topic. It is intended as a popular book and rarely cites primary sources or gives enough details of experiments and surveys to allow the reader to judge for himself — or, one should perhaps add in this context, herself. John Nicholson appears to be bent on showing first that sex differences are either non-existent or small, and second that where they exist they are caused by socialization not by genetic effects. His arguments are often cunning, but that does not prevent many of them from being fallacious. Here are some examples.

In attempting to disprove the existence of an innate maternal drive, he uses as a counter-argument baby-battering by mothers. This is like arguing that hunger is not largely innate because some people are anorexic. Again, he argues that even in animals the maternal drive is a weak one, because it often disappears if the mother is separated from her offspring for a short time. But the development and maintenance of many innate drives are known to depend on the presence of the appropriate stimulus. Given the importance of maternal behaviour for the survival of the species it would be remarkable if no geneti

RECENT MAJOR TITLES IN THE BIOLOGICAL SCIENCES

The Neutral Theory of Molecular Evolution

MOTOO KIMURA

'This is the book we have been waiting for . . . a milestone in evolutionary biology . . . it expresses an original viewpoint which from now on will be part of the way we see the world.'
John Maynard Smith, *Nature*

0521231094 **£35.00 net/ $69.50**

Evolution from Molecules to Men

Edited by **D.S. BENDALL**

Containing papers given at the 1982 Darwin Centenary Conference in Cambridge, this book provides the most comprehensive and authoritative survey possible of the history of evolutionary thought and the current state of knowledge.

0521247535 **£18.00 net/ $29.95**

Fossil Invertebrates

U. LEHMANN and **G. HILLMER**

An essential, beautifully-illustrated introduction to the anatomy of the most important groups of fossil invertebrates, equally suitable for professionals, students and fossil collectors.
Cambridge Earth Science Series.

0 521 24856 6 **Hard covers £20.00 net/ $39.50**
0521270286 **Paperback £7.50 net/ $13.95**

Paleobotany and the Evolution of Plants

WILSON N. STEWART

A lively textbook, illustrated with numerous reconstruction drawings and charts, which explains the origin and evolution of algae, fungi, bryophytes and vascular plants as revealed in the fossil record from early pre-Cambrian times to the present.

0521233151 **£17.50 net/ $29.95**

An Atlas of Past and Present Pollen Maps of Europe: 0-13,000 Years Ago

B. HUNTLEY and **H.J.B. BIRKS**

This book is the first to synthesise European pollen data for the past 13,000 years. It includes maps at 500- or 1,000-year intervals and provides insights into the directions and rates of migration of the European trees.

0521237351 **£85.00 net/ $174.50**

Elm

R.H. RICHENS

'This is an extensive and penetrating account of the whole biology of the elm and of the tree's place in the folklore and art of Britain . . . the marriage of art and science, united in praise of elm . . . is remarkably successful.'
New Scientist

0521249163 **£35.00 net/ $69.50**

Plants and Microclimate

A Quantitative Approach to Environmental Plant Physiology

HAMLYN G. JONES

Designed to provide a soundly-based introduction to the effect of microclimate on plant physiology, this book examines those features of the atmospheric environment that particularly affect plants, and provides a lucid overview of the physical and physiological principles involved.

0521248493 **Hard covers £27.50 net/ $54.50**
0521270162 **Paperback £12.50 net/ $21.95**

Plant Biotechnology

Edited by **S.H. MANTELL** and **H. SMITH**

This straightforward introduction to plant biotechnology examines the subject's main areas of activity and assesses the future potential of each.

Society for Experimental Biology Seminar Series 18

0521245508 **£35.00 net/ $69.50**

Yeasts: Characteristics and Identification

J.A. BARNETT, R.W. PAYNE and **D. YARROW**
with 500 photomicrographs by **LINDA BARNETT**

The most complete and up-to-date survey of its kind ever published, this volume describes the mode of reproduction and principal characteristics of some 470 species of yeast.

0521252962 **£75.00 net/ $150.00**

The Biochemistry of Natural Pigments

G. BRITTON

This textbook describes the structures and properties of the main groups of natural pigments, their distribution in nature, their biosynthesis and functions and their associated biology and chemistry.

0521243922 **£30.00 net/ $59.50**

The Lively Membranes

SIR RUTHERFORD ROBERTSON

This book summarises the principles of structure and functions of membranes at the molecular level and uses examples drawn from the entire range of living organisms.

0521237475 **Hard covers £12.50 net/ $29.95**
0521282020 **Paperback £4.95 net/ $11.50**

The Physiology of Trematodes

Second Edition

J.D. SMYTH and **D.W. HALTON**

An entirely re-written edition which takes account of recent advances in our understanding of ultrastructure, cytochemistry, biochemistry and immunology.

0521222834 **Hard covers £30.00 net/ $59.50**
0521294347 **Paperback £11.95 net $23.95**

Comparative Vertebrate Endocrinology

Second Edition

P.J. BENTLEY

'Bentley . . . has reflected the many advances made in endocrinology in the six years since the first edition was published. Retaining the same framework and approach, each section has been updated, many new figures added . . .'
Trends in Biochemical Sciences

0521246539 **Hard covers £32.50 net/ $49.50**
0521288789 **Paperback £12.50 net/ $19.95**

Mate Choice

Edited by **PATRICK BATESON**

'Final year undergraduates specializing in evolution or animal behaviour will find that many of the review chapters are essential reading.'
The Times Higher Education Supplement

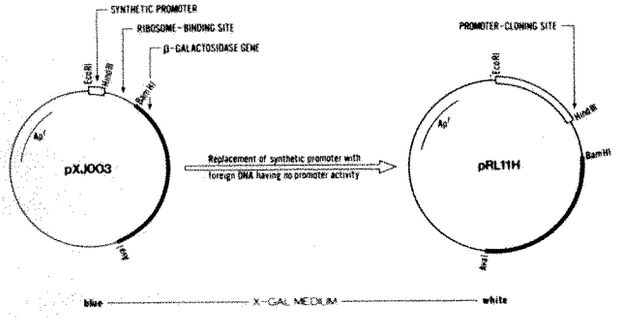
0521251125 **Hard covers £30.00 net/ $59.50**
0521272076 **Paperback £12.50 net/ $19.95**

CAMBRIDGE UNIVERSITY PRESS

The Edinburgh Building, Shaftesbury Road, Cambridge CB2 2RU — 32 East 57th Street, New York, NY 10022

Circle No.32 on Reader Service Card.

Macroinformation for the Microbiologist



Molecular Genetics of the Bacteria-Plant Interaction

Editor: **A. Pühler**

1983. 154 figures. XIV, 393 pages
(Proceedings in Life Sciences)
Cloth DM 114,–; approx. US $ 42.60. ISBN 3-540-12798-4

This book contains the most recent research results in the field of symbiotic nitrogen fixation, plant tumorgenesis and plant pathogenic bacteria.

Advanced Molecular Genetics

Editors: **A. Pühler, K. N. Timmis**

1984. 98 figures. Approx. 320 pages
Cloth DM 89,–; approx. US $ 33.20. ISBN 3-540-12740-2

Contents: Basic Methods. – Mutagenesis. – Gene Cloning. – Gene Expression. – DNA Sequencing. – Electron Microscopy. – Transcription. – DNA Replication.

L. A. Osterman

Methods of Protein and Nucleic Acid Research

Volume 1:
Electrophoresis, Isoelectric Focusing, Ulracentrifugation

1984. 102 figures. X, 342 pages
Cloth DM 156,–; approx. US $ 58.20. ISBN 3-540-12735-6

L. A. Osterman

Methods of Protein and Nucleic Acid Research

Volume 2:
Immunoelectrophoresis – Application of Radioisotopes

1984. 61 figures. Approx. 220 pages
Cloth DM 98,–; approx. US $ 36.60. ISBN 3-540-13094-2

Phytopathogenic Bacteria

Editor: **M. P. Starr**
Selections from the **Prokaryotes – A Handbook on Habitats, Isolation, and Identification of Bacteria**
Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, H. G. Schegel

1983. 17 figures. VII, 168 pages
DM 69,–; approx. US $ 25.80. ISBN 3-540-90880-3

Current Topics in Microbiology and Immunology

Editors: **M. Cooper, W. Henle, P. H. Hofschneider, H. Koprowski, H. Melchers, R. Rott, H. G. Schweiger, P. K. Vogt, R. Zinkernagel**

Volume 100

T Cell Hybridomas

A workshop at the Basle Institute for Immunology
Organized and edited by: **H. V. Bochmer, W. Haas, G. Köhler, F. Melchers, J. Zeuthen**
With the collaboration of S. Buser-Boyd

1982. 52 figures. XI, 262 pages
Cloth DM 48,–; approx. US $ 18.00. ISBN 3-540-11535-8

Volume 103

Retroviruses 1

Editors: **P. K. Vogt, H. Koprowski**

1983. 16 figures. V, 146 pages
Cloth DM 96,–; approx. US $ 35.90. ISBN 3-540-12167-6

Volume 106

Mouse Mammary Tumor Virus

Editors: **P. K. Vogt, H. Koprowski**

1983. 12 figures. VII, 103 pages
Cloth DM 58,–; approx. US $ 21.70. ISBN 3-540-12828-X

Volume 107

Retroviruses 2

Editors: **P. K. Vogt, H. Koprowski**

1983. 26 figures. VII, 180 pages
Cloth DM 96,–; approx. US $ 35.90. ISBN 3-540-12384-9

Volume 109

The Molecular Biology of Adenoviruses 1

30 Years of Adenovirus Research 1953–1983
Editor: **W. Doerffler**

1984. 69 figures. XII, 232 pages
Cloth DM 118,–; approx. US $ 44.10. ISBN 3-540-13034-9

Circle No. 58 on Reader Service Card.

Current Microbiology

An International Journal

Section Editors: **A. Balows,** Atlanta (medical microbiology); **P. Baumann,** Davis (general microbiology, physiology)

Featuring concise yet thorough reports written by leading researchers from all over the world, this international journal presents the most up-to-date information available in the entire field of microbiology. Each issue contains up to 20 short papers which discuss medical, non-medical, pure, applied, taxonomic, historical, theoretical, practical, methodological, and conceptual advances now being made. (ISSN 0343-8651, Title No. 284).

Archives of Microbiology

Editors-in-Chief: **G. Drews,** Freiburg; **J. L. Ingraham,** Davis; **H.-G. Schlegel,** Göttingen

Known as a forum for announcing discoveries of new bacteria, the **Archives of Microbiology** publishes results of basic research on bacteria and other mircoorganisms, including fundamental aspects of ecophysiology, physiological genetics, and biotechnology. Results of special interest can be rapidly published as short communications.
(ISSN 0302-8933, Title No. 203)

Applied Microbiology and Biotechnology

(formerly European Journal of Applied Microbiology and Biotechnology)

Editor-in-Chief: **H. J. Rehm,** Münster

Applied Microbiology and Biotechnology is an international journal presenting research articles on topics including:

- biotechnology (applied genetics and gene technology in relation to biotechnology)
- industrial microbiology (e. g., fermentation)
- environmental microbiology (such as sewage purification)
- applied microbiology (particularly food microbiology)

(ISSN 0171-1741 Title No. 253)

European Biophysics Journal

(formerly Biophysics of Structure and Mechanism)

Managing Editor: **P. Bayley,** London

The **European Biophysics Journal** presents the latest research findings in a wide range of areas employing the principles of physical science to understand biological structure and function. Topics include molecular structure and interactions, membrane and receptor biophysics, thermodynamics and energetics of biological processes, and theoretical biophysics.
(ISSN 0340-1057 Title No. 249)

For more information on these books and journals, or to order a free sample copy of a journal, please write to:

Springer-Verlag
Berlin Heidelberg New York Tokyo

Heidelberg: Heidelberger Platz 3, D-1000 Berlin 33, Tel. (030) 8207-1, Telex 01-85411
New York: 175 Fifth Ave., New York, NY 10010, USA
Tokyo: 37-3, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan

Circle No. 59 on Reader Service Card.

verlag chemie

Collection of Methods for the Microbiological Examination of Foods

Edited by W. Schmidt-Lorenz

There is a greater need for the microbiological examination of foods because of the recent, almost world-wide, increased emphasis on the hygiene, safety and quality of foods. Microbiological standards or specifications for foods can only be established when methods which give reproducible results are readily available. Difficulties are sometimes encountered by laboratory personnel because official and unofficial methods appear in many different publications. To help solve this problem, members of several scientific societies have compiled a comprehensive collection of methods for testing the various groups of products. This collection of methods will be useful – amongst others – to food processors throughout the world if they wish to ship their products to Germany because they must examine their products by the methods described here.

The collection is to a large extent bilingual (German and English). In particular the 15 page introductory text is presented in its entirety in both English and German.

The collection comes complete with two loose-leaf binders. In addition, the publication of „special issues" which each deal with only one product-group is being prepared. If you wish to receive further information please write to the address given below.

I wish to receive further information about „Schmidt-Lorenz, Collection of Methods for the Microbiological Examination of Foods".

☐

verlag chemie

Please write to:
Verlag Chemie GmbH
P.O. Box 1260/1280
D-6940 Weinheim
Federal Republic of Germany

Customers in U.S.A., Canada, Central and South America please write to:
Verlag Chemie International Inc.
303 N.W. 12th Avenue
Deerfield Beach, FL 33441, U.S.A.

Circle No. 40 on Reader Service Card.

ELSEVIER SCIENCE PUBLISHERS

Publications in Microbiology

Antiviral Research
Diagnostic Microbiology and Infectious Disease
FEMS Microbiology Letters
Gene
International Journal of Food Microbiology
Journal of Biotechnology
Journal of Microbiological Methods
Journal of Virological Methods
Molecular and Biochemical Parasitology
Trends in Biotechnology
Trends in Biochemical Sciences
Virus Research
Antimicrobic Newsletter
Clinical Microbiology Newsletter
Infectious Diseases Newsletter
IRCS – Microbiology, Parasitology and Infectious Diseases
Excerpta Medica Abstract Section 47 – Virology
Excerpta Medica Abstract Section 4 – Microbiology: Bacteriology, Mycology and Parasitology
Drug Information Service – Antibiotics, Chemotherapeutics and othe Anti-Infective Drugs

Sample copies of the all the above journals will be available at the Elsevier stand at the A.S.M. meeting. Booth No. 406

ELSEVIER

For further information and/or free sample copy please write to:

In the U.S.A. and Canada:
JOURNAL INFORMATION CENTER
Elsevier Science Publishing Co. Inc.,
52 Vanderbilt Ave., New York, NY 10017, U.S.A.

In all other countries:
ELSEVIER SCIENCE PUBLISHERS
P.O. Box 211, 1000 AE Amsterdam,
The Netherlands

Please send your orders to the Amsterdam address

The Dutch guilder price is definitive. US $ prices are subject to exchange rate fluctuations. Prices are excl. B.T.W. for Dutch customers

Circle No.53 on Reader Service Card.

provision had been made for the appropriate behaviour along with the appropriate physical apparatus for nurturance.

Nicholson's treatment of differences in aggression is equally odd. Having quoted a mass of evidence that suggests that men are more aggressive than women, he writes "In the absence of evidence to the contrary, I suggest we assume that men *are* more aggressive than women". Elsewhere he tries to dispose of the argument that the greater tendency of males to be aggressive is — at least in part — caused by the presence of higher levels of testosterone. He cites the case of girls who were exposed as fetuses to exceptionally high levels of androgens and notes that their behaviour tends to be tomboyish, but he fails to draw the obvious conclusion.

Despite his bias against biological explanations, Nicholson writes clearly though without much wit or charisma. Some of the results he provides may titivate. Half of American fathers have never changed a nappy — not even a disposable one; the difference in the athletic records of men and women has been enormously reduced over the past 50 years (one hopes this has nothing to do with steroids); women's scores on examinations are not influenced by the point in the menstrual cycle at which they take them; men and women do not differ in self-esteem, ambition or ability, but men are the more confident sex.

Since Nicholson virtually rules out innate factors as responsible for the differences between men and women, he is not unnaturally puzzled that all known societies are patriarchal: men occupy the prestigious jobs, and in encounters between members of the opposite sex, both men and women almost invariably feel that the man is dominant. One of his proposed solutions is that men's aggression and desire to dominate stems from their shame that they cannot have babies, a proposal that could surely be disposed of fairly quickly by a social survey — unless of course the hurt goes so deep that men are ashamed to admit it. There is another possibility that he does not consider. Perhaps men and women are innately disposed to act in different ways to members of the opposite sex. This would account for much of the evidence he quotes, for example, the fact that both men and women treat boy and girl babies differently even when the have been misled about the sex of the baby. The initial bias would determine the process of socialization, which would in turn increase the bias. The explanation would also account for one of Nicholson's recurrent themes: despite the feminist movement, it is hard to demonstrate any significant changes over the past 20 years in attitudes or practices relating to the interaction of the sexes. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition, University of Sussex.

Histochemistry and cytology

Robert Olby

History of Staining, 3rd Edn.
By George Clark and Frederick H. Kasten.
Williams & Wilkins: 1983. Pp.304. $29.95, £22.25.

A SERIES of articles published in the journal *Stain Technology* between 1928 and 1933 formed the text of the first edition of this book, which was compiled by H. J. Conn and appeared in 1933. With the additions to the second (1948) and present (third) editions, the book is now virtually twice its original size. Successive editions have followed Conn's intention, which was to cover the field broadly rather than going deeply into specialized topics. As is typical of collections of reprinted papers, there was in the original book a lack of any overall thesis which might have acted as an organizing principle; unhappily, this feature has been perpetuated rather than overcome in the later editions.

The subject matter in this new edition has been organized in terms of, first, the different sorts of dyes, second, the several kinds of biological materials — bacteria, blood, connective tissue, nerve tissue, antibody and antigen — and, finally, selected major figures such as Sir John Hill, Joseph von Gerlach, Rudolf Heidenhain, Paul Mayer, Paul Unna, Paul Ehrlich, Walther Flemming, John Belling, Gustav Mann, Frank Mallory and Ralph Lillie. It is not clear why Rudolf Heidenhain was chosen rather than his son Martin, author of the iron-haematoxylin staining procedure and pioneer in the use of aniline dyes, but the other figures are reasonable choices.

The early history of biological staining centres on the use of carmine from the cochineal insect and haematoxylin from extract of logwood. Next came the aniline dyes, following upon Perkin's production of "mauve" in 1856 — safranin, methyl violet, aniline blue, eosin, methyl green, methylene blue, acid fuchsin and so on. The golden era of cytology in the 1880s and 1890s, in which the details of the chromosomes, and the nature of cell division and fertilization were revealed, rested upon the growing range of stains and techniques described in the book.

The history of the relationship between histochemistry and cytology is a fascinating one which goes back to the very early days. In his chapter on protein and nucleic acid histochemistry, Professor Kasten takes us back to François-Vincent Raspail's use of the starch–iodine reaction in 1825. He stresses the importance of Miescher's and Zacharius's study of the relation between nuclear staining and the chemistry of the nucleus. But it was Feulgen's "nucleal reaction" which Kasten sees as the decisive influence in establishing the localization of proteins and nucleic acids in the cell, paralleling the distinctive staining reactions of nucleus and cytoplasm, and overturning the old belief in the restriction of DNA to animal tissues and RNA to those of plants.

There are obvious difficulties in writing about a collection of techniques and leading technicians for anyone but specialists. No doubt this text will be much appreciated by them. The general reader, however, will surely require a very different sort of book, one organized not around specific stains and famous figures but concentrating on general issues, such as the much-debated question of the existence of organized structures in the cell, their persistence and their replication. Intimately connected with this issue were fundamental questions over the nature of fixing and staining themselves. Did the process of fixing create structures which then adsorbed the stains to produce a spurious architecture within the cell? Was staining simply physical adsorption as Albert Fischer maintained? Were the cytoplasmic organelles discovered in the nineteenth century mere artefacts as W. B. Hardy held? This debate is mentioned on p.199 but not developed. From the book, one would not have guessed that the tenets of cytology were seriously undermined by these fundamental criticisms, and that the emerging tradition of experimental cytology, its practitioners having become sceptical of the claims of classical cytology, concentrated upon the biophysical properties of protoplasm and the presence of surface phenomena in the cytoplasm.

Needham and Peters in the 1930s envisaged a "cytoskeleton" in the cytoplasm which was a colloidal formation and not an organelle in the sense of classical cytology. This rejection of the tradition of classical cytology was not reversed until the techniques of freeze-drying, electron microscopy and ultracentrifugation had been introduced in the 1930s and successfully developed in the 1940s. Kasten points out how important was Feulgen's "nucleal reaction" in this change of opinion. It should also be stressed that histochemists were in the van of the movement beginning in 1950 to establish nucleic acids rather than proteins as the hereditary material. This decision arose from the results of the quantitative study by histochemists of the content of DNA and protein in the chromosome cycle of dividing cells and from the discovery of the halving of DNA content during reduction division. That the contribution of the histochemists is often ignored suggests that this tradition has yet to receive a just evaluation from historians. The present book offers an introduction and valuable bibliography for any such attempt. □

Robert Olby is Reader in the History of Science at the University of Leeds.

Scottish geology for the 1980s

W.S. McKerrow

Geology of Scotland, 2nd Edn.
Edited by G.Y. Craig.
Scottish Academic Press/Halsted: 1983. Pp.472. Hbk £35, $54.95; pbk £17.50.

Scotland's Environment During the Last 30,000 Years.
By Robert J. Price.
Scottish Academic Press/Columbia University Press: 1983. Pp.224. Hbk £27.50, $41.50; pbk £15.

GEOLOGICALLY, Scotland is a small but significant part of the world. Its standing is due to a combination of factors: diverse geology, good exposures and attractive landscape for the field geologist, and especially its wealth of geological literature. James Nicol was very wrong when he stated (in his 1844 *Guide to the Geology of Scotland*) that "...in a country which has been so frequently investigated by eminent geologists, it is evident that little absolutely new can be expected". The reverse is true: the better documented the geology, the easier it is to make further advances. Scottish studies in deformation, metamorphism, igneous petrogenesis and Palaeozoic stratigraphy have all led the world at different times during the past two centuries. The names of Hutton, Judd, Geikie, Lapworth, Peach and Horne, Barrow, Harker, Bailey, Richey, Read and Kennedy help to remind us of some of the advances which came from Scotland. What is Scotland doing for geology today?

Since 1965, when Craig produced the first edition of *Geology of Scotland*, our understanding of geological processes has progressed greatly. We now know more about the rates of crustal processes, structural lineaments, the deep crust, palaeolatitudes, heat flow and the continental shelf around Scotland. We also have plate tectonic models to assist us in finding analogues (from other times and other continents) to some of the major tectonic events in the geological history of the area. Most of these advances are recorded in the new edition, but the different contributors each have their own personal approach to the new facts and the new ideas. We now have much clearer views on the significance of many rocks and structures which previously were baffling or just interpreted wrongly.

Of the 12 authors, seven contributed to the first edition and have attained somewhat variable success in bringing their essays up to date. All five new contributors have maintained the high standards set in the first edition. In his introductory chapter, Harris gives a clear outline of Scottish geological development, much enhanced by his table of events (Fig. 1.2). It is unfortunate that the sections on Tertiary growth and on geomorphic evolution (present in the first edition) have disappeared; these are relatively neglected fields that should not be ignored.

The presentation and standard of editing is, in general, so good that it is perhaps unnecessary to stress the few failures. A stronger hand might have removed some hangovers from the first edition, such as the multiple discussions of the Highland Border Group (presented separately on pp. 84, 98 and 109–110) and of the Ballantrae Volcanic Group (pp. 110, 152 and 174–175). The editor might also have established some standardization of such a crucial term as "Caledonian Orogeny", where Scottish geologists should give a clear lead. Now we know that many orogenies extend over long periods of time (few are short-lived as Stille thought), we badly need to get our definitions sorted out.

One achievement of the book is that diverse parts of geology have been brought together to form a coherent unity, although this does break down in a few places. The new contributions by Brown and Mykura respectively provide excellent summaries of Devonian magmatism and stratigraphy, as do the chapters by Francis on the Carboniferous and by Lovell on the Permian and Triassic, but it is a pity that these accounts of the Late Palaeozoic igneous rocks and sediments have not been more closely integrated.

The contribution by Emeleus on Tertiary igneous rocks contains clear reviews of the latest work in geochemistry relevant to petrogenesis. This chapter takes cognizance of recent geophysical studies, though in general geophysics is not well discussed throughout the book — such a failing is to be regretted, especially in view of much recent work on the deep geology of Scotland.

Sissons gives a brief summary of the complex developments in Scotland during the Quaternary. His ability to condense a large amount of data becomes evident by study of another new contribution to Scottish geology, *Scotland's Environment During the Last 30,000 Years.* The author, R.J. Price, states (p.23) that:

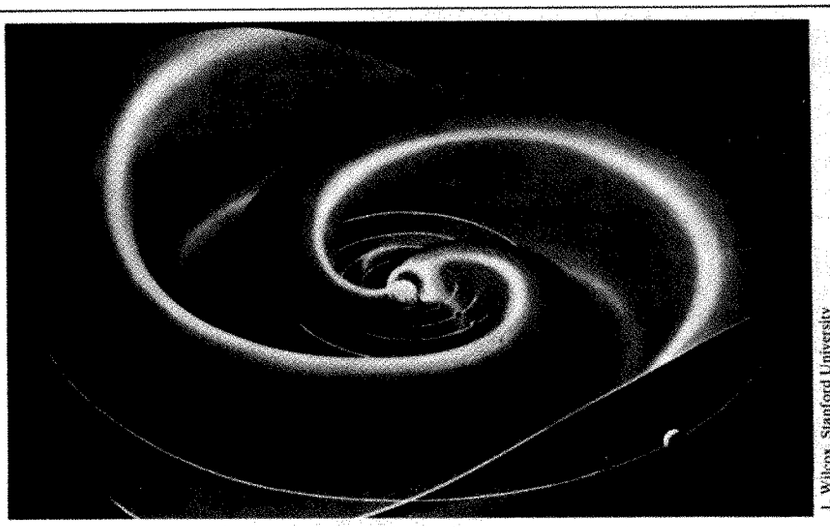
> This book is concerned not only with the geomorphological and marine sedimentary record but with the broad environmental changes which can be identified as a result of detailed investigations not only in these fields but in all aspects of the geomorphological, stratigraphical, palaeontological and archaeological record.

Price lives up to his claim in discussions of geomorphology and chronology, but some geologists will find him weak on sedimentology.

Price emphasizes a most important geological truth (pp. 8–11): the record is best in the oceans, where sedimentation has been more or less continuous; it is next best on the shelf, then on coasts and low ground, but it is almost non-existent in mountains. As a result, the key to many recent advances in Quaternary stratigraphy lies on the sea floor. Will river terraces be the next sediments to repay renewed investigation? They have produced many good results in the English Midlands; could they not also do the same in Scotland?

Both books reviewed here are clearly presented and well illustrated, with full lists of references. They should prove useful to a wide readership: the scientist, the student and the scientifically orientated tourist. Scotland has still further significant contributions to make to geology, and these two books will help to provide a sound basis for future work. □

W.S. McKerrow is a Lecturer in the Department of Geology and Mineralogy at the University of Oxford, and a Fellow of Wolfson College.



J. Wilcox, Stanford University

An artist's conception of the interplanetary medium out to the orbit of Jupiter, showing the spiral structure of the interplanetary magnetic field and the wavy shape of the interplanetary neutral sheet. (The neutral sheet, which is invisible, divides magnetic field lines pointing towards the Sun from those pointing away.) The illustration is taken from *Astronomy from Space: Sputnik to Space Telescope*, edited by James Cornell and Paul Gorenstein, and recently published by MIT Press. Price is $17.50, £15.75.

New from Princeton

Catastrophes and Earth History
The New Uniformitarianism
John A. Van Couvering
Princeton Series in Geology and Paleontology
Afred G. Fischer, Editor
C: $65.00. P: $19.50

Earth's Earliest Biosphere
Its Origin and Evolution
Edited by J. William Schopf
C: $95.00. P: $42.50

The History of the Earth's Crust
A Symposium
Edited by Robert A. Phinney
C: $33.00. P: $11.50

Quantum Theory and Measurement
Edited by John Archibald Wheeler and Wojciech Hubert Zulek
Princeton Series in Physics
P.W. Anderson and A.S. Wightman, Editors
C: $75.00. P: $19.50



Random Walks in Biology
Howard C. Berg
$16.50



Molecular Approaches to Evolution
Jacques Ninio
C: $25.00. P: $12.50

Mate Choice in Plants
Mary F. Wilson and Nancy Burley
Monographs in Population Biology, 19
Robert N. May, Editor
C: $35.00. P: $12.50

Kinetics of Heterogeneous Catalytic Reactions
Michel Boudart and G. Djéga-Mariadassou
Physical Chemistry: Science and Engineering
Edited by John M. Prausnitz and Leo Brewer
C: $35.00. P: $13.50

Princeton University Press 41 William Street Princeton, NJ 08540

Circle No.02 on Reader Service Card.

Women Scientists
The Road to Liberation

Edited by **Derek Richter**
Institute of Neurology, University of London

April 1982 £10.00 313pp ISBN 0 333 32468 4

Anyone should find these glimpses of lives in other cultures intriguing."

"Enlightening for any aspiring scientist who thinks a scientific career is a logical progression from research post to lectureship to chair." **Georgina Ferry, New Scientist**

This book draws together the personal and intellectual biographies of eleven women biomedical scientists. The individual stories are informative, fascinating, and sometimes moving, and cover cultures as diverse as those of India, Iran, USA and the USSR.

Please send orders or requests for further information to Felicity Parker, Product Manager, Scientific and Medical Division, Macmillan Press Ltd., Houndmills, Basingstoke, Hampshire RG21 2XS, UK.

SCIENTIFIC & MEDICAL | **M** | **MACMILLAN PRESS LTD**

Circle No.68 on Reader Service Card.

Announcing the new current-awareness journal from Cambridge

New for 1984

BIO/ESSAYS

Editor-in-chief: **Dr W.J. Whelan** *University of Miami*

Cambridge University Press is pleased to announce this important new monthly which will be launched in July 1984 and published for the ICSU Press, the publishing house of the International Council of Scientific Unions.

BIO/ESSAYS meets the demand for a current-awareness journal devoted to reviews, comment, debate and progress in the frontier areas of molecular, cellular, and developmental biology and their impact on science and society.

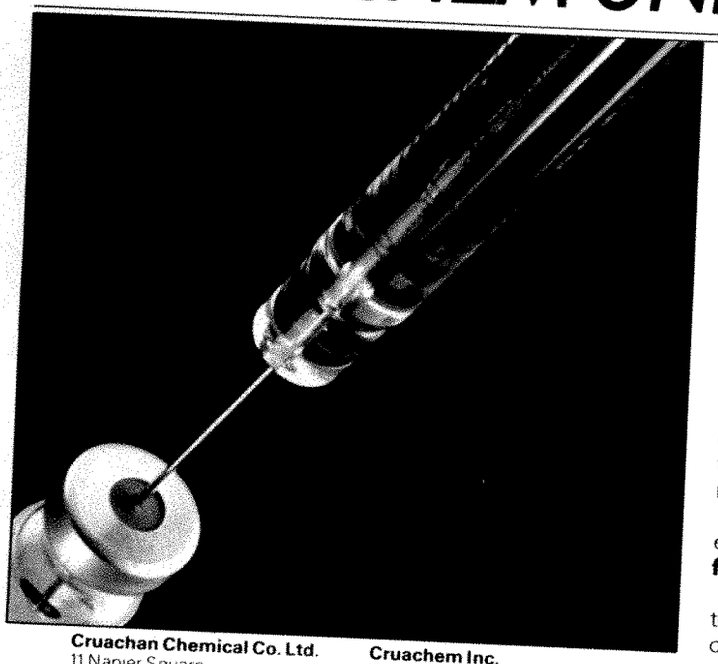
Whilst BIO/ESSAYS will concentrate primarily on advances in knowledge from basic research, it will also pay significant attention to actual or potential applications in medicine, industry and agriculture and is of particular interest to those working, or planning a career, in this field. It will debate speculative or controversial aspects of biological research, and disseminate authoritative views and exchanges of views.

Please contact Aileen Askwith, Journals Publicity Controller, for further information.

CAMBRIDGE UNIVERSITY PRESS
The Edinburgh Building, Shaftesbury Road, Cambridge CB2 2RU
32 East 57th Street, New York, NY 10022

Circle No.29 on Reader Service Card.

CRUACHEM ONE-SHOT PACKS



The Cruachem SPS-grade range of intermediates s new standards of purity and proven performance in DNA synthesis.

Now, these materials are available in pre-weighed, pre-dried ONE-SHOT PACKS.

Each septum-capped vial is packed under Argon an contains the appropriate quantity of material for a single nucleotide addition cycle in a synthesis on a 5 micromolar scal

Now there's no need to waste time weighing out an drying intermediates before your synthesis gets under way.

Keep a stock of these packs in your freezer and you synthesis start-up time is reduced to a few minutes. Couplin efficiency is unimpaired after six months storage at -20°C.

SPS GRADE SOLVENTS are also available packed under Argon in septum-capped bottles for direct connectior to your synthesis system using syringe-needles, eliminating the need to pre-dry and distill solvents and top-up solvent bottles.

The Cruachem SPS-GRADE range has been expanded to include deoxy-Uridine intermediates and **functionalised Controlled-Pore Glass supports.**

Like all SPS Grade materials, these are individually tested in an actual oligonucleotide synthesis before passing our Quality Control.

Cruachan Chemical Co. Ltd.
11 Napier Square,
Livingston, EH54 5DG,
Scotland
Tel (0506) 32146
Telex 72165 CRUACHEM

Cruachem Inc.
P.O. Box 5787,
Bend, Oregon 97708
(503) 382-5860

CRUACHEM

DNA SYNTHESIS MADE EASY

Circle No.51 on Reader Service Card.



serotec

MONOCLONAL ANTIBODIES, ANTI-HUMAN, ANTI-ANIMAL ANTISERA.

SEROTEC has emerged within a very short time as one of the leading suppliers of quality monoclonal antibodies and polyclonal antisera in the world.

We are therefore pleased to announce the introduction of a hundred and fifty new products to the already comprehensive Serotec range.

If you would like further information on the expanding range of Serotec products, and you are not already on our mailing list, apply now for our new 1984 catalogue.

SEROTEC LTD
Station Road, Blackthorn, Bicester, Oxon OX6 0TP.
Tel: (0869) 243241.

Circle No.41 on Reader Service Card.

Equipment for microbiology

This week's selection of new products covers all those that would be used in a microbiology laboratory — from reagents to immunoassay kits.

Biologicals

● Endothelial cell growth supplement (ECGS) is now being supplied by Stratech Scientific. ECGS has been shown to replace feeder cell layers in clonal growth of hybridoma and other cell types. It shows consistency in growth-promoting activity, simplifies culture maintenance and is free from detectable bacteria, fungi and mycoplasma. It is supplied in a lyophilized form which is stable for 2 yr at 4°C. One vial, containing 15 mg, will supplement up to 300 ml of medium.
Circle No. 100 on Reader Service Card.

● Advanced Laboratory Techniques has now added a number of fluorescein (FTC) labelled compounds to its reagent range. These compounds are immunoreactive and intended for use in immunoassay systems. Included in the FTC-labelled range are: amikacin, carbamazepine, digoxin, human placental lactogen, phenobarbital, phenytoin, L-thyroxine and tobramycin. Most of these compounds may be conjugated to various alternative fluorescent labels.
Circle No. 101 on Reader Service Card.

● Amersham has added six new restriction endonucleases and one new modifying enzyme to its range of restriction and modifying enzymes. The additions are: *Acc* II (isolated from *Acinetobacter calcoaceticus*); *Ava* I (isolated from the blue green alga *Anabaena variabilis*); *Ava* II (isolated from the blue green alga *Anabaena variabilis*); *Dra* I (isolated from *Deinococcus radiophilus*); *Eco* RV (isolated from *Escherichia coli* J62 pLG74) and *Sac* I (isolated from *Streptomyces achromogenes*). The modifying enzyme is DNA polymerase I from *E. coli*.
Circle No. 102 on Reader Service Card.

Anglian Biotechnology has asked us to make an addition to our report on avian myeloblastosis virus (AMV) reverse transcriptase (see *Nature* **307**, 196). They would like us to point out that the product is manufactured using a technique pioneered by the president of Molecular Genetic Resources, Dr G.E. Houts, and that any queries involving AMV reverse transcriptase should be directed to Molecular Genetic Resources Inc., 6201 Johns Road, Suite 8, Tampa, Florida 33614, USA.

● A range of specific protein rich media is now available from Advanced Laboratory Techniques. The media are suitable for the preparation of serum standards for immunoassay or as a source material for the preparation of protein. The following are immediately available: C-reactive protein (CRP), liver ferritin, placental lactogen (PL) and thyroxine-binding globulin (TBG). All the media are supplied freeze-dried and sold as weight of specific protein. Small samples are available for evaluation.
Circle No. 103 on Reader Service Card.

● Two new mounting media, hydramount and dimethyl hydantoin formaldehyde resin, have been added to the BDH Gurr range of stains and microscopy materials. Hydramount is a fast-drying aqueous mounting medium which has adhesive properties when set so that the problems of specimen damage resulting from movements of the coverslip are minimized. Hydramount is suitable for botanical and zoological applications. It has a refractive index of 1.4668 and a viscosity of 4.71 cP at 20°C. It is designed for use in micro-entomology or in the preparation of aquatic micro-invertebrates. Dimethyl hydantoin formaldehyde resin (DMHF) is a water-soluble resin for mounting whole or dissected entomological material. It is designed for use in preparing taxonomic specimens for reference collections.
Circle No. 104 on Reader Service Card.

● An improved version of the microbore valve has been introduced by Rheodyne. The new model features 0.2, 0 5 and 1 μl chambers, a 0.13 mm diameter outlet passage and a built-in syringe needle part with 0.3 μl hold-up volume. The previous microbore valves (7413, 7410) are still available, but the 7520 is designed to replace them in areas of microbore HPLC where sample size is critical.
Circle No. 105 on Reader Service Card.

● Colony stimulating factor and human interleukin 2 are now being produced by Genzyme Koch-Light. Colony stimulating factor (mouse), colony stimulating factor (human) and interleukin 2 (human) can all be supplied. The new lymphokines add to Genzyme's existing range of research biologicals — interleukin 1 (mouse), nerve growth factor (human) and collagen II (chicken).
Circle No. 106 on Reader Service Card.

● The new hand-held UVX Radiometer from UVP has three interchangeable UV sensors. Each sensor has a 36-inch electrically-shielded cable enabling accurate readings from inaccessible locations. Designed for general use within UV-A, UV-B and UV-C wave bands, the sensors come equipped with filters to reduce the solarization characteristically associated with 254 nm UV sensors. The UVX Radiometer features direct digital readout, automatic test circuitry and a read–hold switch.
Circle No. 107 on Reader Service Card.

● RNasin is now available from Anglian Biotechnology. RNasin is purified by Promega Biotec and can be used in *in vitro* translation systems using rabbit reticulocyte lysate and in cDNA work using avian myeloblastosis virus reverse transcriptase.
Circle No. 108 on Reader Service Card.

● Two new endoglycosidases are available from Boehringer Mannheim Biochemicals for use in structural studies of glycopeptides and in the removal of sugars from glycoproteins. These glycosidases show very different specificities although both should be used in studies where specific cleavage of glycopeptides or glycoproteins is desired. Endoglycosidase D is specific for the digestion of complex sugars, while endoglycosidase H is useful for the digestion of high-mannose chains.
Circle No. 109 on Reader Service Card.

● Beckman has announced an addition to its range of Ready-Solv scintillation counting cocktails. The new CP extended clear-phase cocktails solubilize aqueous samples for counting. The cocktails hold up to 33% of most aqueous samples as a continuous clear liquid emulsion from neat to maximum capacity with no two-phase breaks. It will count the standard sample size of 1 ml in miniature counting vials. It can also be used for the elution of samples in filter counting techniques and for high protein content samples. Ready-Solv CP can be used in any liquid scintillation counter.
Circle No. 110 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

General laboratory equipment

●The MSJ-T series of fermentors from Bioengineering Associates automates the production of large batches of plant or animal cell cultures. The fermentors are designed for use with cell suspensions or microcarrier beads. Models are available in vessel sizes of 10, 20, 30, 40, 50, 70 and 90 litres. The system is controlled by the microprocessor-based Bioprocessor which automates control of *p*H, dissolved oxygen and temperature. It also runs steam sterilization of the vessel and the medium. Twelve analog and eight digital input ports are available. A computer can be added via the RS232 serial port or the optional IEE-488 interface bus. In addition to the MSJ series, Bioengineering Associates can supply the MD series of bench top fermentors which are offered in five vessel sizes from 1.2 to 10 litres.
Circle No. 111 on Reader Service Card.

● Four new thermostatic water baths are available from PolyScience. The units feature a durable polypropylene tray equipped with a heater, circulating pump and a temperature control circuit. The operating range is from ambient to 50°C with a control accuracy of ±0.1°C. These baths are useful in chemistry, biology and photographic laboratories as well as blood bank applications.
Circle No. 112 on Reader Service Card.

● AMS (Analytical Measuring Systems) has announced the release of a new image analyser to augment its existing system III and VIDS ranges. Called 40-10, the instrument is specifically designed for routine laboratory use. Applications include fluorescent bacteria counting, colony counting, area fraction measurements, diffusion zone area measurements, porosity and cell counting. The system is microprocessor-controlled and includes a built-in printer. The detector is digital, giving 256 grey level settings, and there is an automatic detection system.
Circle No. 113 on Reader Service Card.

● Hybritech has released the Gamma-Photon system for isotopic immunoassays. The solid state 24-well counter allows sample throughput of 500 tubes per hour, and provides for automated data reduction using a Hewlett-Packard model 87XM computer. The instrument offers data reduction software which gives data reduction of Hybritech's immission rate measuring apparatus format solid-phase monoclonal antibody-based Tandem-R immunometric assays. Gamma-Photon also includes data reduction routines for conventional competitive-binding radio-immunoassays, and for screening assays including hepatitis tests and pregnancy tests.
Circle No. 114 on Reader Service Card.

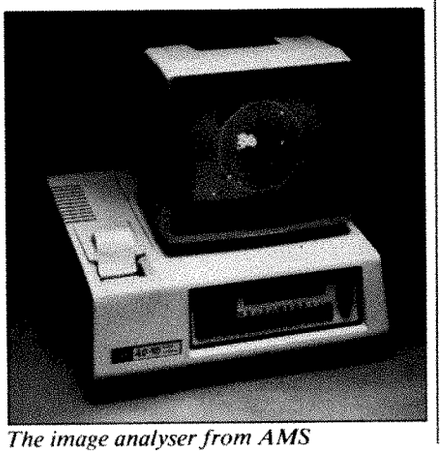
● The Titer-Pak from Robbins Scientific consists of a plastic box with telescoping cover. This contains a removable 96-hole rack which is pre-loaded with pipette tips. It is designed for use with multi-nozzle and standard pipettors. The complete Titer-Pak is disposable and is made of autoclavable polypropylene. It is also available sterilized by radiation.
Circle No. 115 on Reader Service Card.

● A three-dimensional hydraulic microdrive has been introduced by Medical Systems. The MO-103 is small and features a driving actuator which is remotely located from the headstage. Axes *x*, *y* and *z* can be driven smoothly through a cubic centimeter without zigzagging or limping. Control knobs are graduated in steps of 2 μm.
Circle No. 116 on Reader Service Card.

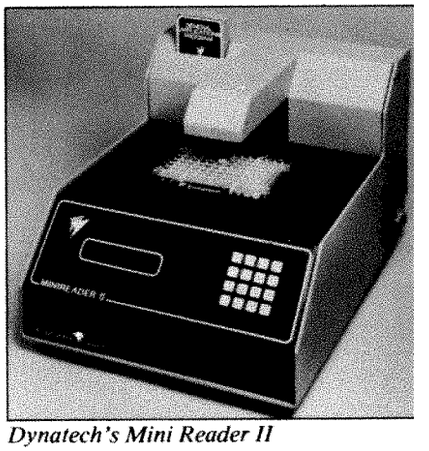
● The new Harris Lo Temp 16-V upright freezer has a capacity of 16 cubic feet divided into five chambers. Each chamber is fitted with an insulated, magnetically-sealed sub-door. The freezer is designed for life sciences, pharmaceutical and blood applications and comes in three models: −40, −70, or −85°C low temperature point. Standard on all models are casters and a safety handle and options include air or auxiliary water cooling and a standby CO_2 system.
Circle No. 117 on Reader Service Card.

● Heraeus has introduced three new types of tissue culture dish to the British market. Flexiperm is a reusable culture vessel made of silicon rubber. Its lower surface adheres to all smooth surfaces to form containers for cell culture. Two containers are available: Flexiperm slide which is designed for use with 76 x 26 mm microscope slides and Flexiperm disk which is normally used in conjunction with a Petriperm culture dish. The Quadriperm is a rectangular cell culture dish with four subdivisions in which cells may be cultivated on standard 76 x 25 mm microscope slides.
Circle No. 118 on Reader Service Card.

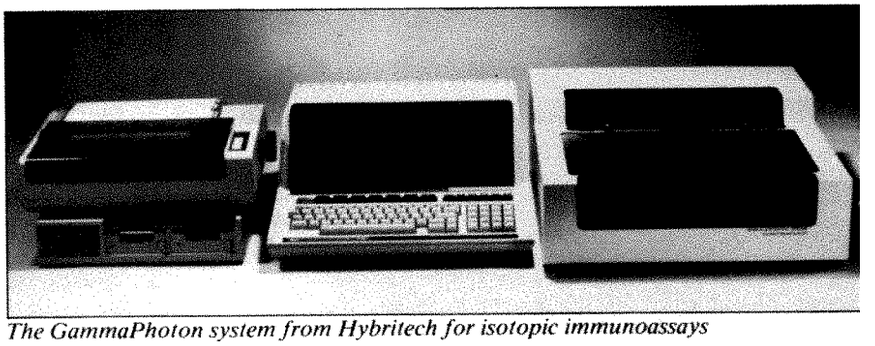
● The Mini Reader II is a manual ELISA reader produced by Dynatech which is coupled to a printer. It incorporates a plug-in cartridge programming system. A 96-well micro-ELISA plate can be read by the instrument and the results printed in approximately 2 min. The machine can store a series of readings and will print these readings when required in either a tabular format, a matrix showing positive and negative values or in optical density readings. In addition, the Mini Reader II provides a visual display of readings, well-by-well, with the results shown on the LED display. The machine has an integral keyboard for data entry which permits the user to select blanking on any well of the plate or number of wells in addition to permitting other data entry requirements such as stored information on print or demand used in conjunction with the program cartridges.
Circle No. 119 on Reader Service Card.



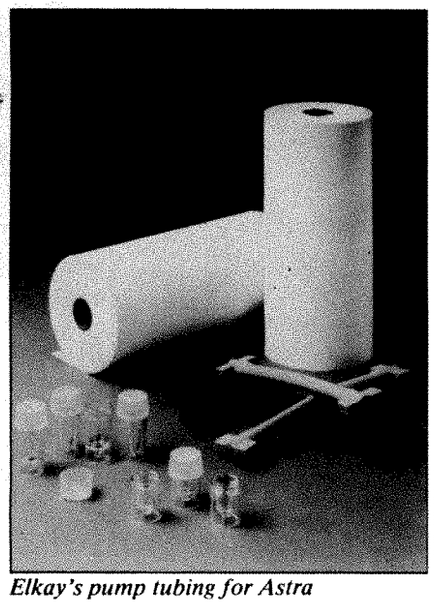
The image analyser from AMS



Dynatech's Mini Reader II



The GammaPhoton system from Hybritech for isotopic immunoassays



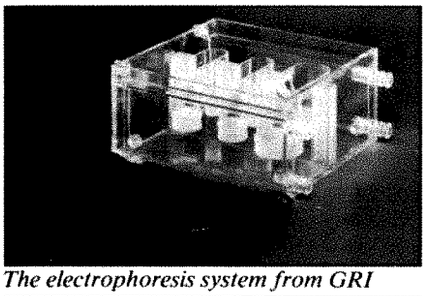
Elkay's pump tubing for Astra

● With the introduction of new Elkay pump tubing for use with Beckman Astra analysers, there is now an alternative single source for plastic consumables for Astra instruments. The silicone tubing is packaged in a press snap lidded polystyrene box.
Circle No. 120 on Reader Service Card.

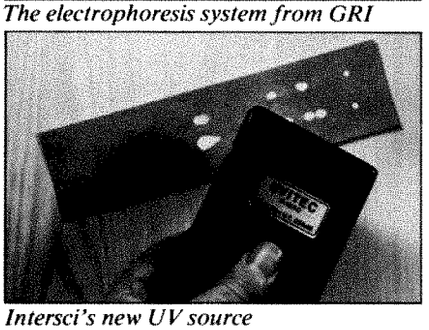
● LDC/Milton Roy has released two new versions of the spectroMonitor, model 1204A, variable wavelength detector. One version includes a maxN series fluid cell and a digital absorbance display. The standard version of the spectroMonitor III detector includes a 1,000 p.s.i. high pressure fluid cell and an analog absorbance display. The spectroMonitor III baseline drift is 5×10^{-4} A h^{-1} after suitable warm-up. The new versions of the spectroMonitor III detector feature a linear sensitivity range of 0.001–2.0 A full scale.
Circle No. 121 on Reader Service Card.

● A new range of pharmaceutical and laboratory autoclaves has been introduced by Edwards High Vacuum. The Edwards Fedegari series FOF autoclaves use the dynamic steam principle of sterilization and have a microcomputer control system dedicated to this sterilization process. There are 24 types of cycle available. Each cycle type must be further programmed by the user with the parameter values required to produce the final programmed cycle.
Circle No. 122 on Reader Service Card.

● A new range of UV/visible spectrophotometers, has been introduced by Pye Unicam. The PU8600 series of microprocessor/keypad-operated instruments is headed by two main versions–the PU8600, designed for industrial and teaching applications, and the PU8610 intended for use in the life sciences. A range of new accessories has also been produced.
Circle No. 123 on Reader Service Card.



The electrophoresis system from GRI



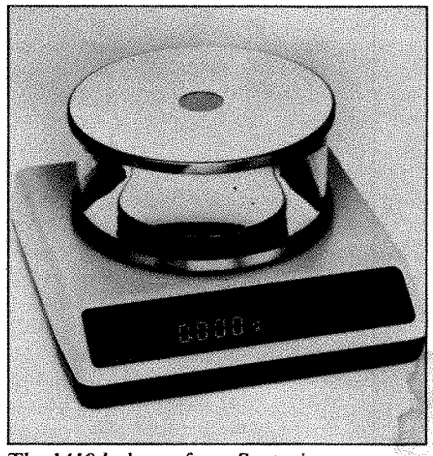
Intersci's new UV source

● Genetic Research Instrumentation has developed an electrophoresis system that is designed for the elution of protein samples from gels and the concentration of macromolecules in solution. The concentrated protein may be harvested either as a concentrate solution or bound to specific activity membranes. The system is based on its sample elution wells. These are two cylindrical wells which are bound to each other by a short bridge. The bottom of each well is covered by a membrane. Proteins migrate from one cup to the other under a constant electrical field of 5W within 1 h, either as a solution or bound to the membrane. Up to three samples may be processed simultaneously and two sizes of sample wells are available. The chamber is water-cooled and supplied complete with three sample wells, blank spacers, safety lid and input leads.
Circle No. 124 on Reader Service Card.

● Intersci has introduced a small but powerful UV source that is suitable for examining thin layer chromatography plates. The Uvitec Micro is available in short wave (254 nm) and long wave (365 nm) versions and comes complete with batteries and viewing box.
Circle No. 125 on Reader Service Card.

● Two new Tissue-Tek vacuum infiltration processors are available from Miles Laboratories. These are microprocessor-controlled flow-through tissue processors that automatically perform the tasks of fixation, dehydration, clearing and paraffin-impregnation of tissue. The specimens are safely inside a completely enclosed retort during processing. The processors are available in new 100- and 200-specimen capacity models as well as the original 300-specimen size. They incorporate a control pad which enables the operator to prepare and store up to 10 different processing programs.
Circle No. 126 on Reader Service Card.

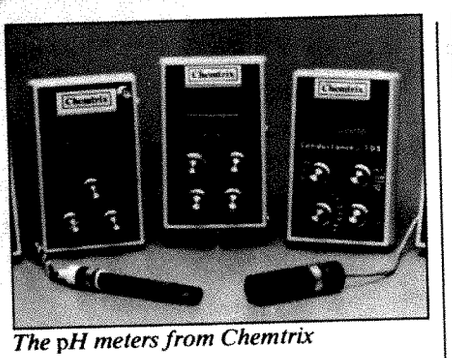
● Coulter Electronics has introduced a new multi-parameter discrete chemistry analyser, Dacos. It can analyse any sample, at any time, with a throughput of 450 tests per hour, carrying out single tests, profiles and statistics. It houses a multi-wavelength optical bench, which rotates around a ring of reaction cuvettes to provide continuous absorbance readings at up to eight wavelengths every 8 s. Dacos uses low sample and reagent volumes with a minimum reaction volume of 120 μl. It uses reusable reaction cuvettes to keep consumable costs down. One or two reagents may be added and both sample and reagent blanks are taken during the analysis.
Circle No. 127 on Reader Service Card.



The 1419 balance from Sartorius

● Brinkman Instruments has introduced the Sartorius series 1400 milligram balances. The series includes the model 1405 MP8 (220-g capacity, 0.001-g read ability) and the dual-range models 1412 MP8 and 1419 MP8 (30 to 300-g and 60 to 600-g capacities, respectively, and 0.001 and 0.01-g readability). The quad-range model 1475 MP8 (420-g capacity) features a fine reading of 0.001 g which can be activated anywhere within the weighing range by pushing the tare button. Additional readabilities of 0.002, 0.005 and 0.01 g are automatically displayed at predetermined levels. Additional features include display control of the last digit (always on, always off, or automatically controlled), auto-adjustable display update speed, adjustable digital filter, seven-segment digital display with stabilized readout indicator, automatic electronic calibration and full-range electronic tare. All models come with a stainless steel and glass draft shield.
Circle No. 128 on Reader Service Card.

● The new Elkay XLDG macropipettor with digital capacity selection dispenses volumes from 1 to 5 ml with adjustable 500 μl increments to within ± 0.4% reproducibility. It also has a hexagonal body, feather touch plunger action, positive click-stop, digital capacity adjustment and colour-coding to ensure correct tip usage.
Circle No. 129 on Reader Service Card.



The pH meters from Chemtrix



Wheaton's Unispense/Omni-Pette

● Wheaton Instruments has introduced the Wheaton Unispense/Omni-Pette, an automatic liquid delivery system for aliquoting as well as aspirating. A feature of the system is the Omni-Pette remote control dispensing head which allows fluids to be delivered at some distance from the pump. The Wheaton Unispense/Omni-Pette will feed, wash and innoculate cells in 90 and 24-well microtest plates when the appropriate microtest manifold is attached to the Omni-Pette. The Wheaton Unispense /Omni-Pette is suited to delivering small volumes of fluid in the 0.1 to 20 ml range. The system is controlled manually by a push button, foot switch or by the flush-mount switch. When programmed in the automatic mode, the Unispense/Omni-Pette will cycle at regular intervals which can also be programmed for 0.5–5 s delay intervals between cycles. A five-digit LED display records the number of doses dispensed, while a cycle alert signals the end of each dispense cycle.
Circle No. 130 on Reader Service Card.

● Cytogard is the newest addition to Gelman Sciences' laminar flow units. It has been designed to allow the safe handling of cytotoxic drugs. The cabinet, manufactured in compliance with Australian standard AS 2567, the only legislative standard in the world for the handling of cytotoxic drugs, is also designed to satisfy British standard 5726 class II for containment. The construction cabinet has a stainless steel working area. It also includes a pre-filter and final high efficiency particulate air (HEPA) filter for maximum protection and a carbon absorption cell for handling volatile liquids.
Circle No. 131 on Reader Service Card.

● A new family of multi-function *p*H meters has been produced by Chemtrix. The model 410 *p*H and mV meter is accurate to ±0.1 *p*H and ±0.1% mV units. When temperature is crucial, the model 420 *p*H/mV meter automatically compensates for sample temperature variations over the 0–100°C range. In addition to this the model 430 *p*H/mV/temperature meter measures temperature over the 50 to 200°C range with ±0.5°C accuracy. All units have large LCD displays. They are battery powered and operate over the 0–14 *p*H and ±1,999 mV ranges.
Circle No. 132 on Reader Service Card.

● Brinkmann Instruments has released the Epson HX-20 system for Sartorius electronic balances. The system includes an Epson HX-20 computer; an interface for connection to Sartorius MP6, MP7 or MP8 electronic balances and standard programs for statistics, net weight or counting. The HX-20 computer is small enough to fit in a briefcase and requires no power cord.
Circle No. 133 on Reader Service Card.

● Tissue-Tek III uni-cassettes, Tissue II cassettes and Tissue-Tek embed rings are now available from Miles S tific in a selection of colours. Uni-cas are available in five new colours: tan, orange, gold and aqua, in addition t original five colours: white, pink, g blue and yellow. The Uni-cassette s also includes a biopsy cassette for specimens, which is grey. Mega-cass available for larger tissue speci Tissue-Tek II cassettes are also availa the same 10 basic colours as Uni-cas but there is also a clear, polypro cassette that allows embedded tissue seen. Tissue-Tek embedding ring available in colours for the first time being white, pink, blue, green and y The rings allow larger specimens embedded than does Mega-Cassette
Circle No. 134 on Reader Service Ca

● Futura II star-series refillable cc ation electrodes from Beckma designed to measure *p*H in di situations. These include those in ionic strength is low, such as in ac and drinking water, those where t ature varies substantially or in s which are sensitive to silver. Sta electrodes operate in the range of *p* 14 and are accurate to ±0.01 *p*H. Re time in the *p*H 4 to 7 range at c temperature is stable to ±0.02 *p*H For temperature changes from 2 75°C in *p*H 4 buffer, response is st ±0.02 pH in 30 s. Slope is 97 to temperature range is 5 to 100°C a thermal point is 7 *p*H.
Circle No. 135 on Reader Service C

Literature and assay kits

● A new test for serum creatinine is now available from J.T. Baker Chemicals. In cooperation with Dutch clinical laboratories, J.T. Baker has developed a creatinine test based on the Jaffé method but free from bilirubin interference. Bilirubin, by use of a special reagent, is oxidized before the reaction rate is measured. The test has been designed for the kinetic measurement of creatinine. Application sheets currently available include Cobas Bio, RA-1000, PA-800 and manual techniques.
Circle No. 136 on Reader Service Card.

● Boehringer Mannheim Biochemicals now offers an *in vitro* immuno-turbidimetric test kit for the determination of fibronectin levels in human plasma. The turbidity, which results from the antigen–antibody reaction, is measured with a spectrophotometer. The assay uses a standard curve obtained from the set of standards included in the kit. Sample values are compared to this and the plasma fibronectin levels are then determined. The shelf life has been extended and the kit may now be stored at 4°C.
Circle No. 137 on Reader Service Card.

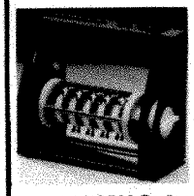
● A new ^{3}H RIA kit is now availat Seragen for specific measuren prostaglandin E_1 (PGE_1). This 100- contains all the necessary reagents test, including antisera specific fc which show 9% cross-reactic PGE_2. Seragen's new PGE_1 a sensitive to 20 pg per 0.1ml.
Circle No. 138 on Reader Service C

● An instructional brochure available from Tago that descr structure and function of plasi proteins in the transport of cholest triglycerides. Other topics covere brochure include the clinical sig of apolipoprotein measurements immunoassay systems and po studies using Tago's Diffu-Gen k
Circle No. 139 on Reader Service

● A new brochure is availat Boehringer Mannheim Biochemi brochure contains a complete list molecular biology products, modifying enzymes, restriction linkers and primers, inhibitors, nu and nucleosides.
Circle No. 140 on Reader Service

ADVERTISEMENT

DIANORM -GD-1 new!
5-Cell Equilibrium Dialyzer
for (ligand) binding and distribution studies under standardized conditions.
5 different DIANORM® – rotating-cell-types (PTFE) are available: minimal volumes 0.125, 0.6, 0.7, 1.5, 3.0 ml. Equilibrium reached in 15-180 minutes. Voltage of the drive is transformed down to 14 V thereby avoiding any risk when using a temperature controlled water bath.



DIANORM-Geräte, P.O. Box 126, D-8000 München 65, FRG

Circle No.60 on Reader Service Card.

NATURE CLASSIFIED

LONDON OFFICE Keith Walesby 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024)
AMERICAN OFFICES NEW YORK Miss Gene Fein 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900
SAN FRANCISCO JJHS (415) 392 6794 ●**PASADENA** JJHS (213) 796 9200
CHICAGO Didier & Broderick (312) 498 4520 ● **BOSTON** CEL Assoc (617) 848 9306
CANADIAN OFFICE TORONTO Peter Drake 17 Pine Crescent, Toronto, Ontario M4E 1L1 (416) 690 2423

RATES UK — Rest of world — £11.00 per centimetre. Minimum 3 cm. £2 is charged for the re-direction of replies to advertisements with a box number.

CONDITIONS. The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.

DURHAM UNIVERSITY

SENIOR RESEARCH ASSISTANT IN SYNCHROTRON RADIATION RESEARCH

plications are invited for the post Senior Research Assistant tenable he Physics Department for two rs from early 1984. The successful licant will work, under the super- on of Dr.B.K.Tanner, on an .R.C. supported project to elop X-ray diffraction techniques he characterization of strains in V semiconductors. Part of the k will be carried out on the chrotron Radiation Source at esbury Laboratory.

nitial salary in the range £7630 — 30 on National Range 1A plus rannuation.

pplications (3 copies) naming e referees should be sent by 2 ch 1984 to the Registrar, Science oratories, South Road, Durham 1 3LE, from whom further iculars may be obtained. (1255)A

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT SAN ANTONIO DENTAL SCHOOL

ASSOCIATE DEAN FOR RESEARCH AND ADVANCED EDUCATION

Academician and scientist to fill position in an innovative and expanding dental school. Responsibilities include organization and supervision of an active school wide research program and coordination and administration of dental school post-doctoral programs. The position also offers an opportunity to develop independent research activities. D.D.S. or D.M.D. and advanced degree preferred.

Send curriculum vitae to Dr. Richard Carr, Dental Dean's Office, 7703 Floyd Curl Drive, San Antonio, Texas 78284. The University of Texas Health Science Center is an equal opportunity/affirmative action employer. (NW317)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (University of London)

RESEARCH ASSISTANT IN HOMINID PALAEONTOLOGY

Applications are invited from graduates to assist in the analysis of hominid dental remains. Previous experience with computing and multivariate statistical techniques would be an advantage. The appointment is funded by the NERC for two years. Salary within the scale £6,311 — £8,531 plus £1,186 London Allowance.

Further particulars should be obtained by applying in writing to Professor Bernard Wood, Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB (01-636 8333 ext 7179). Applications, with the names and addresses of two referees, should reach the above by 16 March 1984. (1272)A

UNIVERSITY OF NOTTINGHAM FACULTY OF AGRICULTURAL SCIENCE

Applications are invited for the position of Research Assistant/Fellow to join a group interested in the growth of animals to study the control of muscle protein deposition in ruminants. Suitable applicants will have a higher degree in Biochemistry, Physiology or Agricultural Science. Experience in animal cell tissue culture would be an added asset. Funds are being provided by the Agricultural Research Council, initially for a period of one year.

Applications, naming two academic referees, should be addressed to Dr P J Buttery, Department of Applied Biochemistry and Food Science, University of Nottingham School of Agriculture, Sutton Bonington, Nr Loughborough, Leics LE12 5RD, to arrive as soon as possible. Informal enquiries on Kegworth (05097) 2386 would be most welcome. (1275)A

COLD SPRING HARBOR LABORATORY

Post-Doctoral Research Positions in Molecular Biology, Cellular Biology, and Neurobiology

Applications are invited for post-doctoral positions with the following Cold Spring Harbor Laboratory Staff:

- **David Beach** — Cell cycle control genes in yeast.
- **Susan Bonitz** — Regulation of yeast mitochondrial genes by nuclear gene products.
- **Ed Harlow** — Molecular analysis of viral and cellular tumor antigens.
- **Susan Hockfield** — Studies of the nervous system using recombinant DNA and hybridoma technology.
- **H. Earl Ruley** — Molecular analysis of oncogene function.
- **Pablo Scolnik** — Insertional mutagenesis in prokaryotes. Primary structure of promoters in phototrophic bacteria.
- **Bruce Stillman** — Eukaryotic DNA replication and adenovirus molecular biology.
- **Douglas Youvan** — Molecular genetics and biophysics of the bacterial photosynthetic apparatus.
- **Mark Zoller** — The study of DNA/protein interactions in yeast using *in vitro* mutagenesis.

Applications and inquiries should be directed to the specific staff member of interest. **Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 11724.** Cold Spring Harbor Laboratory is an Affirmative Action/Equal Opportunity Employer.

(NW310)A

GROUP LEADER MOLECULAR BIOLOGY

Unigene Laboratories, Inc., a multidisciplinary biotechnology research corporation, is seeking a distinguished scientist for the position of Group Leader in Molecular Biology. The successful candidate will have 5-10 years of post-Ph.D. research experience and an outstanding publication record demonstrating a broad background in all aspects of molecular biology. Scientific creativity and superior professional credentials are prerequisites, as the individual will be responsible for supervising and troubleshooting a team of senior and junior staff. Previous experience as a Group Leader, Project Leader or Principal Investigator in Molecular Biology is essential, while industrial experience is desirable but not required.

Unigene is a sharply-focused, financially secure institution utilizing Molecular Biology, Immunology, Protein Chemistry and Organic Chemistry to conduct state-of-the-art research in the field of human health care. Our ultra-modern research facility is located in Fairfield, New Jersey. The company offers an excellent salary and comprehensive benefits package, including equity participation, along with the opportunity to work in a stimulating research environment which fosters continuing professional development.

For a challenging and rewarding opportunity to participate in the rapidly expanding health care field with a young and aggressive company, please send a CV and three references in confidence to:

Dr. Warren P. Levy
Unigene Laboratories
110 Little Falls Road
Fairfield, New Jersey 07006

(NW330)A

unigene
An equal opportunity employer

UNIVERSITY OF ILLINOIS PROFESSOR OF CROP MOLECULAR GENETICS

An outstanding scientist is needed for a key position in the newly established CENTER OF EXCELLENCE IN CROP MOLECULAR GENETICS AND GENETIC ENGINEERING, funded in part by a grant from the Standard Oil Company (Ohio). We are seeking a molecular biologist experienced in using recombinant DNA techniques, to introduce useful genes into economically important plants such as maize and soybeans. Applicants should provide documentation of proven research experience and capability in genetic engineering and plant molecular biology. Appointment will be in the Department of Agronomy of the University of Illinois, with possible joint appointments in affiliated departments. A person hired at the associate or full professor level will be granted tenure. For the duration of SOHIO funding, the person will be designated the SOHIO Professor of Crop Molecular Genetics. Rank will be commensurate with qualifications and salary will be highly competitive.

The genetic engineering group in the University of Illinois Agronomy Department presently comprises five senior scientists in well-established research laboratories. Fifteen other University of Illinois scientists, working in molecular genetics and genetic engineering of higher plants, are identified as collaborators of the SOHIO-UI Center of Excellence.

In addition to establishing a vigorous research program, the successful applicant will be expected to cooperate with and provide expertise in support of existing research programs of the group.

Applications will be accepted until 15 April 1984 or until a suitable applicant is found. Submit curriculum vitae, a statement of research goals and teaching interests, a list of publications, and names, addresses and telephone numbers of five references to: Dr. T. Hymowitz, Department of Agronomy, University of Illinois, 1102 South Goodwin Avenue, Urbana, Illinois 61801 USA.

The University of Illinois is an Affirmative Action Equal Opportunity Employer.

(NW333)A

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF BIOCHEMISTRY

LECTURESHIP

Applications are invited for the above position now vacant due to the appointment of Dr C R Lowe as the Director of Biotechnology in the University of Cambridge. The post will involve the teaching of Biochemistry and Biotechnology to science and medical students and the successful applicant will be expected to establish a research group for investigating fundamental biochemical problems and their applications. The applicant should have 2-4 years post-doctoral experience in addition to a postgraduate qualification in Biochemistry, Molecular Biology or Chemistry.

Salary scale: £7,190 — £14,125 per annum.

Further information about the above post and about the Biochemistry Department may be obtained from Professor K Akhtar, F.R.S. Department of Biochemistry, University of Southampton SO9 3TU.

Further particulars may be obtained from A J Small, Staffing Secretary, The University, Highfield, Southampton SO9 5NH, to whom applications (7 copies from applicants in United Kingdom and one from others) giving a brief curriculum vitae and names and addresses of three referees should be sent before 23 March 1984. Please quote Ref: 84/3/AJS/N (1262)A

Southan
THE
UNIVER

CHAIR OF CHEMIS

Applications are invited fo
of Chemistry made vacar
retirement of Profes
Fleischmann. The Dep
wishes to make an appoin
an area of electro-che
Candidates with interests
applications of materials
solid state chemistry or
chemistry are also welcom

Further details may be o
from the Secretary and Re
The University, Southa
SO9 5NH, to whom appl
(10 copies from persons in
should be sent before 30 Ap
Interested persons may als
informally to the Chairman
Department of Chem
Professor G. R. Luckhurst.

(12

MICROBIAL PHYSIOL

Tenure-track position in mi
physiology at the ass
professor level and ava
September 1984. PhD re
with postdoctoral expe
desirable. Applicant must h
strong commitment to exce
in teaching and research and
expected to develop a gra
course in appropriate are
expertise. Undergraduate tea
expected.

Send curriculum vitae, transc
reprints, a brief statemer
research interests, and three l
of recommendation by 15 M
1984 to: Dr. Lewis Co
Chairman, Search Commi
Department of Biology, Mem
State University, Memphis, T
38152. Memphis State is an E
Opportunity University.

(NW327

UNIVERSITY OF MINNESOTA

AUSTIN, Minn.

POSTDOCTORAL ASSOCIATE(S)

One or two positions (resea
fellow or research associa
depending on experience)
work on the regulation
phospholipid metabolism
biological membranes. Resear
interests may include recep
mediated lipid turnover, enzyr
purification (phospholipases)
aspects of cell biology (myocyte
platelets).

Please sent inquiries to: Dr. H.
O. Schmid, The Hormel Institu
University of Minnesota, Aust
MN 55912. Phone: (50
433-8804.

*The University of Minnesota is a
Equal Opportunity Educator an
Employer and specifically invit
and encourages women an
minority applicants.* (NW321)A

BIOLOGY & ENVIRONMENT GROUP EIR, SWITZERLAND
POST-DOCTORAL RESEARCH POSITION RADIATION BIOLOGY/ MOLECULAR BIOLOGY

pen from April 1st, 84, for up to hree years to study DNA repair in a rimary tissue culture system. ackground either in biochemistry r molecular biology of DNA eeded. Starting Salary 50 to 5,000 sFr.

lease send résumé to: Dr. Verner Burkart, Biology & nvironment Group, Swiss ederal Institute for Reactor esearch (EIR) CH-5303 Vürenlingen (Switzerland). nclude two letters of support nd publications with applica-ion. (W763)A

THE UNIVERSITY OF PAPUA NEW GUINEA
Port Moresby
LECTURER IN BIOCHEMISTRY
P821004/84

pplications are invited for the ove position which will become cant in June, 1984, in the epartment of Human Biology in the aculty of Medicine. This epartment undertakes the pre-inical training of medical and ntal students in Biochemistry, natomy, Pharmacology and ysiology, and is responsible for st-graduate course in basic medical iences. Departmental and terdepartmental research is couraged. The successful applicant ll have had teaching experience in ochemistry to medical students; it is e aim of this Department to present e pre-clinical medical subjects in a nctional and clinically relevant ntext, and therefore a medical alification would be an advantage, would teaching/research perience in a developing country.

Salary: Lecturer II K17,870 per num plus gratuity; Lecturer I 6,020 per annum plus gratuity.

Applications including a full rriculum vitae, a recent small otograph and the names and dresses of three referees should be rwarded to the Deputy Registrar taffing), University of Papua New uinea, PO Box 320, University pua New Guinea, to reach him no ter than 30th March 1984. In order expedite the appointment ocedure, applicants are advised to k their referees to send confidential ports directly to the University thout waiting to be contacted. ndidates resident in the UK should nd an additional copy of their plication to the Association of ommonwealth Universities ppts), 36 Gordon Square, London C1H 0PF, from whom formation about conditions of rvice is available.
(W776)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following positions:

RESEARCH SCHOOL OF PHYSICAL SCIENCES
DEPARTMENT OF THEORETICAL PHYSICS
RESEARCH FELLOWS/ SENIOR RESEARCH FELLOWS

The Department (Head: Professor K J Le Couteur, FAA) conducts studies on nuclear structure and reactions, particles and fields, statistical mechanics, solid state physics and plasma physics. Preference in this instance will be given to applicants in theoretical nuclear physics or particle physics and field theory. Excellent computing facilities are available in the School, and interdisciplinary interaction with other groups in the School and in the University is encouraged.

Two posts are available and appointments will date from late 1984.

Closing date: 15 March 1984.

Conditions of Appointment: Salary will be in accordance with qualifications and experience within the ranges: Senior Research Fellow $A32,679 — $A38,980 pa; Research Fellow $A23,394 — $A30,734 pa. Current exchange rate is $A1 = UK 0.65p.

Appointment, unless otherwise stated, will be: Senior Research Fellow/Research Fellow normally up to three years with the possibility of extension to maximum of five years.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australian, or to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PE, for further particulars before submitting applications. (W775)A

UNIVERSITY OF ALBERTA

RESEARCH ASSOCIATE IN NEUROPHYSIOLOGY

DIVISION OF NEUROSURGERY

Applications are invited for the above position. Candidates must have a Ph.D. in Neurophysiology, state of the art skills and a good publications record. Should be enthusiastic about working with clinicians to apply basic science methods to medical problems such as epilepsy and pain. Send curriculum to Dr. Bryce Weir, 11-102 Clinical Sciences Building, University of Alberta, Edmonton, Canada T6G 2G3. (NW315)A

Leukaemia Research Fund Centre at the Institute of Cancer Research

A Leukaemia Research Fund Centre is being established at the Chester Beatty Laboratories of the Institute of Cancer Research in the Fulham Road, London. The Centre (Director: Dr. M. F. Greaves) will have a broad based programme of investigations on the cellular and molecular basis of human leukaemia.

Research Positions Available from 1st April, 1984

A. Post-Doctoral Research Fellowship:

A three-year post-doctoral research fellowship is available for studies on the biochemistry of membrane associated proteins of normal and malignant haemopoietic cells. Experience in immunochemistry and membrane protein analysis will be required. Salary on the MRC non-clinical scientific grades as appropriate depending on experience. **(Ref. 300/G/39)**

B. Research Assistants:

(1) Molecular Biology: A research assistant is required to help establish a molecular biology laboratory and to actively participate in its research programme. The latter will primarily concern studies of the regulation of proto-oncogene expression during haemopoietic growth and differentiation. **(Ref. 301/B/43)**

(2) Monoclonal Antibodies: A research assistant is required to be responsible for monoclonal antibody (hybridoma) production. Priorities in this area will be antibodies to cell surface associated receptors and proto-oncogene products. **(Ref. 301/B/44)**

(3) Flow Cytometry: A research assistant is required to be responsible for a flow cytometry section of the laboratory which will include a Becton Dickinson FACS IV (440), analyzer and computing facilities. Projects will include multi-parameter analysis and isolation of haemopoietic progenitor cells and new approaches to the immunodiagnosis of leukaemia. **(Ref. 301/B/45)**

Salary for these posts on the MRC research officer or technician scales as appropriate depending on experience and responsibilities to date.

Applications for any of the above posts should be sent with a full CV in duplicate **to the Personnel Officer, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting the appropriate reference number.** (1265)A

BIOGEN

A leading biotechnology company with laboratories in the US and Switzerland, is looking for its Geneva laboratories for

an IMMUNOLOGIST

with several years experience in the area of cell mediated immunity, T-cell hybridomas and lymphokines,

2 PROTEIN CHEMISTS

with experience in large scale protein purification and/or strong background in conformational studies of proteins,

a MICROBIOLOGIST

with extensive experience in fermentation research and development.

We offer a challenging scientific career with the opportunity to interact with talented colleagues in a stimulating environment. If this offer appeals to you, then reply to:

Personnel Manager, BIOGEN S.A., Route des Acacias 46, 1227 *Geneva, Switzerland.*

Please send your résumé and a list of three individuals who may be contacted for references on your behalf. All replies will be evaluated in confidence. (W767)A

Important Laboratoire Pharmaceutique Filiale d'un Groupe International
recherche

IMMUNOLOGISTE
NIVEAU DOCTORAT

30/35 ans,
possédant une bonne connaissance en biochimie et alergologie,
désireux de s'insérer dans une équipe de recherche et bénéficiant si possible d'une expérience industrielle analogue.

Merci d'adresser CV et références sous réf. 87881 à CONTESSE PUBLICITE
20, avenue de l'Opéra 75040 PARIS CEDEX 01
qui transmettra (W771)A

UNIVERSITY OF WAIKATO
New Zealand
MICROBIOLOGIST/ BIOCHEMIST/SOIL SCIENTIST
for work on
DENITRIFICATION BY RHIZOBIA
(Vacancy A 84/3)

A Research Scientist is required to continue work on a contract from the Ministry of Agriculture and Fisheries to investigate the role of rhizobial denitrification in soils. A postdoctoral worker is preferred, but other experienced researchers will be considered.

The work will involve the field and laboratory measurement of microbial denitrification, and experience in the use of gas chromotography as well as in soil microbiology or biochemistry, would be an advantage. The position may be taken up immediately and is for one year, with the possibility of a short extension. The salary for a postdoctoral candidate would probably be the equivalent of the top of the junior lecturer scale (currently NZ$18,732 pa) or the first or second step of the lecturer scale (currently NZ$21,660 pa and NZ$22,421 pa respectively).

Enquiries concerning the research should be made directly to Dr R M Daniel, School of Science, at the University of Waikato, Hamilton, New Zealand.

Applications should be made in a prescribed format which is available from the Registrar, University of Waikato, Hamilton, New Zealand, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Each applicant is asked to request his/her referees to write directly to the Registrar with a confidential report on the applicant's suitability for the position.

Applications close with the Registrar on 2 April 1984.
(W777)A

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF ZOOLOGY
LECTURESHIP IN ZOOLOGY

Applications are invited for a lectureship in the field of Animal Behaviour, especially from those with research experience is causal and/or developmental aspects of behaviour, to complement existing interests in behavioural ecology and evolution.

This appointment will be in the salary range £7,190 to £14,125 per annum and will start in September 1984.

Further particulars and application forms, returnable not later than 13 March 1984, may be obtained from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham, NG7 2RD. Ref No 923. (1268)A

THE UNIVERSITY OF TENNESSEE MEMORIAL RESEARCH CENTER (UTMRC)

invites applications for two tenu track positions at the assistant associate professor level. T UTMRC, a fully equipped biome cal research institute with excell small and large animal facilities, staffed by basic and clinical scienti with established programs in molec lar and cellular immunology, onc ogy, experimental hermatology, a pathology, including neuroscien Preference will be given to M.D. Ph.D. candidates who are capable developing independent resear programs that interface with those other UTMRC senior investigat and complement those of t academic clinical departments of University of Tennessee Memor Hospital. The successful applica will be provided core supp including salary and necessa laboratory start-up funds; it expected that additional resea support will be derived from grants other funding mechanisms. Invol ment in educational activities possible through participation in p and postdoctoral programs of University of Tennessee, the C Ridge National Laboratory, and the undergrduate and gradu medical education programs of University of Tennessee. Rank a salary are negotiable.

Interested parties are invited furnish their curriculum vitae and names and addresses of three re ences to: Emilio A. Machado, M. Chairman, Search Committ University of Tennessee Memo Research Center, 1924 Alcoa Hi way, Knoxville, Tenn. 37920. Affirmative Action/Title IX/Sect 504 Employer. (NW313)/

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF MEDICAL MICROBIOLOGY
RESEARCH ASSISTANT

A graduate is required to join a gr investigating the molecular basis Gonococcal infections. The work involve fractionation of biologic active macromolecules, immu chemical analysis with monocl antibodies and growth of cells tissue culture. Applicants sho have an honours degree in Bio mistry, Microbiology or a rel subject. The post is funded by MRC for three years commenci March 1984.

Salary will be on Grade 1B £6, — £8,530 per annum for Rese and Analogous Staff.

Further particulars may obtained by contacting Dr J Heckels, Southampton (0703) 777 ext 3918 or from Mr A J Sn Staffing Secretary, Univers Southampton SO9 5NH to wh applications (2 copies) should be giving date of birth, a full curricu vitae and the names and addresse two referees as soon as possi Please quote reference MA1 1284/AJS/N. (1267)

Head of Microbial Biochemistry Department

Greenford, Middlesex

An experienced research scientist is required to manage a multi-disciplinary department of about 40 scientists and technical staff.

The major disciplines in the department are biochemistry and microbiology. Research is at present concentrated on the search for selective techniques, often involving target enzymes, aimed at discovering novel biological activity of natural or synthetic origin. Current projects include work with bacteria, fungi and viruses and the design of automated assays to identify pharmacologically active agents. The department also includes ecology and fermentation groups which examine a diversity of habitats, for micro-organisms of possible biological or medicinal application and carry out studies to optimise the production of secondary metabolites.

The successful candidate is likely to be under 40 years and should have a proven innovative research record, together with administrative and communication skills, and the ability to motivate staff to a high standard of scientific achievement.

Major Company benefits include a Company car, profit sharing bonus scheme, non-contributory pension scheme and relocation assistance if appropriate.

Please write or telephone for an application form, or initially forward a full C.V. to: The Personnel Manager, Glaxo Group Research Limited, Greenford Road, Greenford,Middlesex. Tel: 01-422 3434 ext 2568, quoting ref: U/515.

Glaxo *Group Research Ltd.*

(1252)A

IMMUNEX CORPORATION is a multi-disciplinary biotechnology company pursuing research and development of important immunoregulatory diagnostic and therapeutic products. An opening currently exists for a:

MOLECULAR BIOLOGIST

experienced in the expression of eukaryotic genes in microorganisms (bacteria and/or fungi.) A Ph.D. and post-doctoral experience is required. Knowledge of immunology is not necessary.

Please send a resume and the names of three references to:

Personnel Director
Immunex Corporation
51 University Street
Seattle, WA 98101

(NW324)A

immunex

Reproductive Biologist

Experienced embryologist with Ph.D. required to manage busy laboratory and devoted to both service and research of human infertility.

Facilities include a successful IVF programme (16 delivered infants to date) gamete and embryo cryopreservation, techniques for the study and treatment of oligospermia, the induction and intensive monitoring of ovulation and active research into the causes and treatment of early pregnancy wastage.

The laboratory has a nucleus staff of 3 biologists and 2 technologists and is linked to a large private Infertility Clinic with research undertaken in affiliation with the University of Western Australia. The successful applicant is expected to manage the laboratory, supervise research projects and liaise with the clinical staff to optimise patient management.

Salary negotiable in keeping with applicant's experience and expectations.

Apply by 31st March 1984 with detailed curriculum vitae to:
Dr. J.L. Yovich, Director, PIVET Laboratory, 442 Barker Road, Subiaco, Western Australia. Tel: 09-382-1677.

(W761)A

Maag

Dr. R. MAAG Ltd., A Swiss agrochemical company with research center in Deilsdorf/ Switzerland, has an opening for a

PLANT PHYSIOLOGIST

Applications are invited from individuals with a PhD degree in plant physiology and, preferably, with several years of experience in mode of action studies of herbicides. The successful candidate should be well acquainted with the plant physiological and biochemical methods to study the following pathways: Photosynthesis (photosystem I+II inhibition, electron transport, phosphorylation, CO_2 assimilation); respiration (O_2 consumption, ATPase inhibition); chlorophyl, carotinoid, lipid, DNA, RNA and protein synthesis. Experience in auxine physiology is an asset. Modern laboratories and equipment are available.

Applicants (Swiss citizens) should submit a Curriculum Vitae, a list of their publications, and the names of three references to Dr. R. MAAG Ltd., personnel department, 8157 Dielsdorf, Switzerland.

(W772)A

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
PO BOX 30772, NAIROBI, KENYA

DEPUTY DIRECTOR

(REF: SCI/83/7): Readvertisement

The ICIPE is an international institution headquartered in Nairobi, Kenya. It undertakes advanced research in insect science and post management, and offers high-level training to insect scientists and technologists from Africa and other developing countries. It has 40 senior scientists; and is funded by a consortium of donors comprising United Nations and international bodies, governmental aid agencies, and private foundations.

Applications are invited from highly qualified scientists and widely experienced research managers for the senior research management position of DEPUTY DIRECTOR at the ICIPE.

The Deputy Director reports directly to the Director of the Centre, and in his absence, acts for him. He will, in addition, have overall responsibility for the Research Division of the ICIPE, which includes the research programmes, research support units, and technical support services (including field station facilities); and maintain close liaison with supportive Divisions within the ICIPE (on Administration, Finance, and Communication and Training). The successful candidate will be a person with a solid research background in insect science or pest management or plant protection, he will have had considerable experience in science policy matters and research management; and he will be a successful internationalist able to work in an African environment. A more detailed job description will be sent to qualified candidates.

The renumeration package will be competitive, and will reflect the vital importance of this position within the ICIPE.

Applications, giving updated curriculum vitae and addresses of three referees should be addressed (in confidence) to: The Director, ICIPE, PO Box 30772, NAIROBI, Kenya.

Closing Date: 31st March 1984.

(W769)A

---

nature

the weekly international journal of science

seeks an unusual sub-editor

to help 500,000 readers keep up with science

Essential qualifications:

Literacy — Zeal — Meticulousness — Tact

Also desirable, **two or more** of the following:

Two years experience as a sub-editor
Wide general knowledge of science
Scientific or technical degree
Work experience in a professional environment
First-hand experience of research

Duties include negotiation with scientist authors of acceptable and intelligible forms of words and preparation of manuscripts for the press. Opportunities for other journalistic work not excluded. Salary by negotiation. Age, Sex, etc. immaterial. Applications from exceptional part-timers considered.

Replies by 8 March with full *curriculum vitae* to the Editor, *Nature*, Macmillan Journals Limited, 4 Little Essex Street, London WC2A 3LF.

(1233)A

Postdoctoral position in field hydrology
Centre d'études nordiques
Université Laval, Québec

This position is available immediately (June 1st, 1984) for a researcher in field hydrology in the periglacial environment. Research topics concern snow, surface hyrdrology and permafrost thermal regime at study sites in northern Québec. The selected candidate will join an already existing group composed of a geophysicist, a geomorphologist and a botanist, which is involved in landforms and permafrost mapping along with the study of permafrost related ecological conditions. The post-doctoral researcher will have autonomy in the planning of his work and field instrumentation set-up as he will be a full member of the research group. Logistical support is provided within this context.

Although all members of the present group can communicate in English, the working and living environment is francophone.

This is a two year position. **Send curriculum vitae with summary of research interest and the name of three respondants. Reprints would be appreciated. Send to: Dr. Michel Allard, Centre d'études nordiques, Université Laval, Sainte-Foy, Québec, Canada, G1K 7P4.**

Applications are invited from all; *in accordance with Canadian immigration regulations applications from Canadian citizens and landed immigrants will be considered first.*

(NW316)A

UNIVERSITY OF DUNDEE

Molecular Genetics Laboratory

DEPARTMENT OF BIOCHEMISTRY

TEMPORARY LECTURESHIP IN PROKARYOTIC GENETICS/ MOLECULAR BIOLOGY

A temporary lectureship is available in the Biochemistry Department for a period of up to five years. Applicants should show a strong research interest in any aspect of prokaryotic genetics/molecular biology. The successful applicant will be expected to be actively involved in the research activities of the Department, either independently or as part of the research group of Dr. C.F. Higgins, currently investigating various aspects of prokaryotic gene expression and the molecular biology /genetics of bacterial membrane proteins.

Further details can be obtained from Dr. Higgins (Department of Biochemistry; telephone 0382-23181 Ext. 500). Applications containing full career details and the names of 2 referees, should be sent as soon as possible to The Personnel Office, University of Dundee, Dundee DD1 4HN. Please quote reference. EST/1/84J

(1251)A

SYRACUSE UNIVERSITY

DEPARTMENT OF BIOLOGY

ASSISTANT PROFESSOR, GENETICIST

Applications for a tenure tra position are invited from individu with doctoral training in genetics a post-doctoral training in an academ setting. Applicants in an area eukaryotic genetics who are studyi problems using molecul approaches are preferred, b applicants with outstanding potent in other areas of genetics will also considered. Successful candidat will be expected to pursue a vigoro independent research program, a to teach undergraduates in genet and graduate students in an area their specialization.

Applications should inclu curriculum vitae, recent publicatio and grant support, a statement proposed research plans, and t names of three scientists willing write letters of recommendation. T starting date for this position will between Sept. 1, 1984 and Sept. 1985. Applications should be sent April 15, 1984 to Dr. David Sulliv Department of Biology, Syrac University, Syracuse, New Yo 13210. Syracuse University is Affirmative Action/Equ Opportunity Employer.(NW319)A

Allelix INC.

IMMUNOCHEMISTS

llelix Inc., Canada's first major otechnology company, has a acancy for a postdoctoral fellow in s Molecular Immunology Group. e are seeking candidates with perience in immunoassay techques and methods of protein urification, characterization and odification, particularly as plied to immunoglobulins of both olyclonal and monoclonal origin. pplicants should have a strong ublication record and be able to ork as a member of a team on ojects of both a basic and applied ture.

lelix has a broad base in all major otechnology disciplines and is ell financed by three large nadian organizations. Research conducted in a modern, well-uipped laboratory near Toronto. alaries and benefits are at fully mpetitive levels.

erested candidates are invited to nd résumés, including three ferences and quoting file number 64 to:

Dr. D. C. Burke
Scientific Director
Allelix Inc.
6850 Goreway Drive
Mississauga, Ontario
L4V 1P1.

???

AFRICAN PROFESSIONALS
FRICAN MICROBIOLOGISTS

arasitologists Immunologists, stopathologists, and experts in otomicroscopy, are needed by ernational laboratory in Kenya. rican nationals with MS or PhD erested in these positions are ited to submit résumés for acement and relocation sistance.

HEAD LIBRARIAN AND COMMUNICATIONS SYSTEMS MANAGER

eded by international oratories in Kenya. Kenyan izens are invited to submit sumés for placement and ancial assistance.

INTERGOVERNMENTAL OMMITTEE FOR MIGRATION
ROT-Africa
1346 Conn. Ave., NW
Washington, DC 20036.

ICM/ISS
Cranmer House
39 Brixton Rd.
London, SW9 6DD.

(NW309)A

Bureau of Mineral Resources, Geology & Geophysics Australia

RESEARCH SCIENTIST/SENIOR RESEARCH SCIENTISTS

Marine Geophysicists

The Division of Marine Geosciences and Petroleum Geology in the Australian Bureau of Mineral Resources, Canberra, has vacancies for Marine geophysicists (3 positions).

The principal research aims of the Marine Division are to define the regional geological framework, petroleum prospectivity and evolution of the sedimentary basins of Australia's extensive continental shelf and margins; further to develop new scientific concepts of basin structures and evolution of passive margins and also the active margins of the South Pacific. The main objective is the stimulation of ongoing programs of offshore petroleum exploration. The Division is presently in the process of acquiring a research vessel and seismic processing centre.

The successful candidates should be experienced in multichannel seismic operations, in seismic structural and stratigraphic interpretation and in at least some aspects of geohistory analysis. Associated experience in other fields of marine geophysics and petroleum would be valuable but not essential.

Classification will be at Research Scientist or Senior Research Scientist level depending on the successful candidate's qualifications and experience.

Qualifications:
A PhD (or equivalent) in marine geophysics together with demonstrated research ability.

Salary:
Research Scientist (RS) $A24344 – $A30,038
Senior Research Scientist (SRS) $A31,092 – $A35,806

Conditions:
Conditions of service include superannuation, long service leave, four weeks annual leave and removal expenses to Canberra. Permanent appointment is available to persons who are Australian citizens. A term engagement will be considered for persons not meeting this criterion.

Canberra, the national capital, is located approximately 280 km southwest of Sydney and has excellent educational, recreational and sporting facilities.

Applications together with full personal and professional details and the names of at least three referees should be sent to:
The Director
Bureau of Mineral Resources
GPO Box 378
Canberra ACT 2601
AUSTRALIA

Applications close 16 March 1984.

(W780)A

AUSTRALIA

UNIVERSITÄT FREIBURG
anticipates the appointment of a
FULL PROFESSOR
at the Lehrstuhl für Makromolekulare Chemie.

Applicant should have interest in synthetic polymer or biopolymer esearch. The candidate should have a strong commitment to graduate nd undergraduate teaching. Scientific cooperation within DFG joint esearch groups, especially "Funktion durch Organisation in makro-nolekularen Systemen", is offered.

Applicants should supply curriculum vitae and lists of ublications and teaching experiences. They should be sent to he Dekan der Fakultät Chemie – Pharmazie, Universität reiburg, Rheinstraße 10, D-7800 Freiburg, West-Germany, by March 15, 1984.

(W774)A

PANLAB GENETICS, INC.

Director and Staff Scientists for Fungal Molecular Biology Research

Panlab Genetics Inc. an internationally known service research organization having nearly 200 clients in 15 countries, is seeking to assemble a team in its Seattle laboratory to develop recombinant DNA and vector technology in filamentous fungi. Applications are invited from post doctoral molecular biologists for the above positions. Experience with filamentous fungi is desirable but not essential. An attractive salary plus relocation expenses are offered.

Please send curriculum vitae including 3 referees to: Dr. Christopher Ball, Panlab Genetics, Inc., 110 – 110th Ave. N.E., Suite 704, Bellevue, WA 98004.

An equal opportunity employer.

(NW318)A

TRANSGENE

As a consequence of continued growth and expansion, and of a recent move into new, larger premises, TRANSGENE, a leading genetic engineering company based in STRASBOURG, FRANCE, has immediate openings in the following positions.

— SENIOR MOLECULAR BIOLOGIST

with extensive experience in eukaryotic cell vectors and in the expression of foreign proteins in cultured cells.

— BACTERIOLOGIST

with research experience in the physiology and genetic manipulation of microorganisms of industrial importance (the streptococci, lactobacilli, streptomyces . . .).

— MOLECULAR BIOLOGIST

with 2-3 years practical experience of cDNA cloning techniques.

— PROTEIN CHEMIST

with practical experience in the synthesis of peptides.

TRANSGENE is an independent recombinant DNA research and development company, backed by a group of major French industrial and financial organizations.

Interested candidates are invited to submit, in confidence, a full curriculum vitae, a list of publications, and the names and addresses of three referees to:

Dr. J. P. LECOCQ
Scientific Director
11 rue de Molsheim
67000 STRASBOURG
FRANCE
Tel: (88) 22.24.90.

(W756)A

UNIVERSITY OF GRONINGEN
Kapteyn Astronomical Institute

RESEARCH ASTRONOMERS

Two positions are available, each for 2 years. Commencing date flexible, preferably September 1984. The institute's research interests lie in extragalactic and interstellar research, both observational and theoretical, and advanced facilities for image processing and model calculations are available. Observing possibilities exist with first rank instruments both nationally and abroad. The infrared sky survey data obtained with the IRAS satellite are available for comparative work.

Candidates must be in the possession of a PhD or equivalent degree in order to begin these appointments. One of the positions will be given to a candidate with a demonstrated interest in observational astronomy and will involve half of the 2-years at the UK-Dutch La Palma Observatory as a resident astronomer.

Applicants are requested to send a curriculum vitae, list of publications and a summary of current research interests, and to arrange to have 3 letters of reference sent directly to: Prof. R. J. Allen, chairman Astronomy Department, Postbus 800, 9700 AV Groningen, The Netherlands, phone 50116695, telex 53572 stars nl. Closing date: 31 March 1984. (W770)A

IMMUNOLOGY R & D

IMREG, INC., a publicly held biotechnology company engaged in development of immunoregulators for human application is expanding its scientific staff, and has openings for scientists with the following qualifications:

(1) PhD level scientists with postdoctoral training and experience in cellular immunology, peptide biochemistry, and recombinant DNA technology.

(2) Research Associates with M.S. degrees or B.S. degrees in all of the above areas.

(3) M.D. clinical scientist with interest and experience in laboratory or clinical immunology to assist in design and coordination of clinical trials of the Company's immunomodulators.

These are intellectually challenging positions at the advancing edge of the rapidly developing area of human lymphokines.

We offer competitive salaries appropriately scaled for training, experience and record fo productivity in research, and an attractive fringe benefit package including an incentive stock option plan. We are located in an attractive, fully-equipped setting in one of America's most interesting cities. The Company maintains linkages to the local and national academic immunologic community. Send curriculum vitae and names of three references in confidence to John L. Carlton, Vice-President of Operations and Finance, IMREG, INC., P.O. Box 56643, New Orleans, 70156. (NW308)A

UWIST

University of Wales

APPLIED BIOLOGY LECTURERS

Microbiology and Molecular Biology Position

RESEARCH ASSOCIATE

supported by a SERC Biotechnology Directorate Grant to characterise the dehalogenase transposons of Pseudomonas putida. Applicants must have a wide experience in molecular biology techniques.

Salary: Within Range 1A Research and Analogous Staff £7,190 – £11,615 per annum.

Requests (quoting Ref. B31) for details and application form to Staffing Office, UWIST, PO Box 68, Cardiff CF1 3XA.

Further information can be obtained from Professor J. H. Slater, on Cardiff (0222) 34130.

Closing Date: 9 March 1984.

(1269)A

UNIVERSITY OF STRATHCLYDE
Department of Bioscience and Biotechnology
(Applied Microbiology Division)
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a Post-doctoral Research Fellowship (tenable for three years) to work with Dr D R Berry and Dr J Johnston on the genetic manipulation of yeast strains. Candidates should have experience of nucleic acid biochemistry or molecular biology.

Salary on Range IA (£7,190 – £11,615 pa) of the national salary scale for Research Staff. USS benefit.

Applications (two copies) with curriculum vitae and the names and addresses of three referees (quote ref: R5/84) should be sent to Dr R S Henderson, Department of Bioscience and Biotechnology, University of Strathclyde, 204 George Street, Glasgow G1 1XW by 23 March 1984. (1257)A

CONNECTIVE TISSUE RESEARCH
FACULTY OF DENTISTRY
THE UNIVERSITY OF ALBERTA

Applications are invited for a full-time position as a Research Scholar for fundamental studies on connective tissue involving periodontal disease and wound healing. Present research includes studies on the structures and functions of collagens and proteoglycans and mechanisms of connective tissue degradation. Expansion of these studies or sound independent research in another area by Research Scholars with the requisite experience will be considered. Facilities include automatic amino acid sequencing, tissue culture and electron microscopy with associated clinical areas.

These positions are dependent upon successful application for competitive awards by an external granting agency. Appointment will be on a long-term basis and will enjoy the previleges of the University academic rank at which the appointment is made (Assistant Professor, Associate Professor or Professor level presently $29,720 – $41,820; $37,420 – $53,658 and from $48,010). University of Alberta is an equal opportunity employer.

Interested applicants should forward their curriculum vitae, outlines of their research interests and names of three referees to Dr. J. A. hargreaves, Director of Graduate Studies and Research, Faculty of Dentistry, The University of Alberta, Edmonton, Alberta T6G 2N8.

The closing date for application is May 1, 1984. (NW314)A

University College of Swansea

Senior Research Assistant

plications are invited for an **RC-funded Senior Research sistant in the Department of ysics,** to join an existing High ltage Group based at the above llege and UWIST at Cardiff. plicants should have a good ial degree in Physics, Electrical gineering or a related subject, gether with some relevant search experience. The research, hich is closely integrated with that a number of European groups, l involve a study of the growth of ace charge in transient electrical charges in gases at voltages up 250 kV. The experimental work l be carried out mainly in the high ltage laboratory at Swansea ng an existing experimental angement for which micro-ocessor-based multi-channel ta acquisition systems are being veloped.

e appointment, which will be for o years from the soonest date at can be arranged, will be at a mmencing salary of £8,080 per num plus USS/USDPS benefits.

ormal enquiries may be made Professor J. Dutton, partment of Physics at vansea or Professor R. T. ters, Department of Physics, ctronics and Electrical gineering, UWIST, Cardiff, t further particulars and plication forms (2 copies) st be obtained from the rsonnel Office, University llege of Swansea, Singleton rk, Swansea SA2 8PP, to hich office they should be urned by Thursday, March 1, 4.

(1256)A

Public Service Commission of Canada / Commission de la Fonction publique du Canada

Research Scientist, Marine Organic Geochemistry

Fisheries and Oceans
Champlain Centre for Marine Science and Surveys
Quebec City, Quebec

We require a senior scientist to be responsible for conducting a research program on behaviour pathways and fluxes of organic substances, particularly contaminants in the coastal marine environment. We will rely on you to carry on research in physical, chemical, and biological oceanography on processes in the Estuary and Gulf of St. Lawrence and in coastal waters in Northern Quebec. These activities will be transferred to a new research institute near Mont-Joli sometime in 1986-87.

You require a doctorate degree in oceanography with specialization in organic geochemistry from a recognized university, or a lesser degree with research experience and scientific productivity equivalent to a doctorate. In addition to extensive field and successful research experience, it is essential that you have published original articles. Willingness to work in the field in all seasons for long periods is mandatory. Knowledge of English and French is essential. Please note that in some cases language training may be offered.

We offer a salary ranging from $26,834 to $49,913 commensurate with your qualifications and experience.

Send your application and/or résumé and list of publications, quoting reference number 84-NCRSO-DFO-RES-5, to:
Joan Girling (613) 593-5331
Public Service Commission of Canada
Ottawa, Ontario

Closing date: 30 March 1984

Tout renseignement est disponible en français en s'adressant à la personne susmentionnée.

(W778)A

The Public Service of Canada is an equal opportunity employer

Canada

UNIVERSITY OF EDINBURGH

DEPARTMENT OF BOTANY

CTURER IN MYCOLOGY

lications are invited for the ve post available from 1st ober, 1984.

andidates should hold a PhD ee, or the equivalent, and have at three years postdoctoral rience in any aspect of fungal arch although candidates with an est in experimental aspects are y to be preferred. The successful idate will be expected to develop her personal research ramme and assist in the training oth general and more specialised cts of mycology.

lary on the academic scale for urers (£7,190 — £14,125) rding to qualifications and rience.

urther particulars may be ained from the Secretary, versity of Edinburgh, Old lege, Edinburgh, quoting ence number 1105. Closing date pplications 1st May, 1984.

(1289)A

MOLECULAR BIOLOGISTS

The Department of Biology of Memphis State University invites applications for a tenure track faculty position in the area of molecular biology at the level of assistant or associate professor. The applicant must have postdoctoral experience with demonstrated excellence in molecular biology including recombinant DNA methodology. This position includes ample space and funding for research. It is expected that the successful candidate will develop a research program that will qualify for extra mural funding. The individual will also be expected to participate in appropriate departmental teaching.

Interested individuals should submit a résumé, a statement of research plans, reprints of publications and have 3 letters of recommendation to: Dr Lewis B Coons, Chairman, Search Committee, Department of Biology, Memphis State University, Memphis TN 38152. The Memphis State University is an equal opportunity university. The closing date is April 30, 1984. (NW328)A

NATURE CONSERVANCY COUNCIL

DIRECTOR SCOTLAND

£19,243 — £23,159

Applications are invited for the post of Director Scotland which will be vacant in January 1985 on the retirement of the present Director Dr. J. Morton Boyd, BSC PhD DSc FRSE.

The person appointed will be responsible for NCC's activities in Scotland including a staff of 115 and 61 National Nature Reserves and over 800 Sites of Special Scientific Interest and will work closely with the Chairman of Council's Advisory Committee for Scotland. As a member of the Board of Directors the Director contributes to the formulation and implementation of Council's policies and to the wider management of NCC throughout Great Britain.

Candidates must be capable of working at the highest levels with Central and Local Government and Agencies, with land managers and users, and with scientific, professional and voluntary bodies.

A proven capacity for leadership and management is required as is an appropriate background of experiece. The post provides outstanding opportunities for developing nature conservation.

The post is graded at Deputy Chief Scientific Officer and is based in Edinburgh.

Interviews will be held in London (19/20 Belgrave Square) during May 1984.

For application forms and further details contact Mrs J. Whitting, Nature Conservancy Council, Godwin House, George Street, Huntingdon, Cambs. Tel: Huntingdon 56191 ext 278.

Closing date for receipt of completed application forms is 30 March 1984.

(1282)A

esa

EUROPEAN SPACE AGENCY

The European Space Agency seeks for its European Space Research and Technology Centre, Noordwijk, an

ASTRONOMER MILLIMETER AND SUB-MILLIMETER WAVE ASTRONOMY

Applications are invited from astronomers with experience in millimetre wave heterodyne observations of molecular clouds. Applicants should have demonstrated research ability and a sound knowledge of heterodyne receivers. The post requires an ability to lead a small group in collaborative research with other European astronomers, to direct the development of advanced instrumentation for submillimetre wave astronomy from space, and to lead a study group examining the feasibility of an 8 metre diameter space telescope for submillimetre wave astronomy.

An excellent knowledge of English or French is essential with a working knowledge of the other language.

We offer international working conditions, expatriation and family allowances, good social security and pension.

Interested candidates are requested to apply, sending a detailed curriculum vitae to the Head of Personnel, ESTEC, P.O. Box 299, 2200 AG Noordwijk, the Netherlands.

(W764)A

PLANT EVOLUTIONARY BIOLOGIST — FACULTY POSITION

The Department of Plant Biology, University of Illinois at Urbana-Champaign, invites applications for a tenure-track position at the assistant professor level for appointment in August 1984. Applicants must have a PhD and have research experience in evolutionary studies of higher plants and in plant systematics utilizing approaches of molecular biology. The appointee will be expected to conduct research in evolutionary relationships of plants and contribute to both graduate and undergraduate teaching programs. It is anticipated that this person will complement and interact with present faculty in evolutionary and systematic biology. Salary is open, depending on the applicant's qualifications.

Applications must include a curriculum vitae, a summary of research and teaching interests and at least three letters of reference which are to be sent to: Dr. David S. Seigler, Search Committee Chairman, Department of Plant Biology, University of Illinois, 505 South Goodwin Street, Urbana, Illinois 61801; telephone (217) 333-7577 or 333-3261. In order to ensure full consideration, applications must be received by March 19, 1984. The University of Illinois is an Affirmative Action/Equal Opportunity Employer.

(NW334)A

McGill University
DEPARTMENT OF PLANT SCIENCE

Applications are invited for two Assistant Professor positions

1. **Plant Pathologist/Molecular Biologist.** Teach undergraduate plant physiology and an additional botany or plant pathology course, plus graduate teaching and research in area of expertise. PhD, background in molecular biology of plant pathogens/host pathogen relations required.
2. **Plant Pathologist** with strong background in quantitative biology. Teach undergraduate crop diseases and nematology; develop graduate course and research in epidemiology and crop disease modelling. PhD required.

Send application, C.V., and three references to Dr J.F. Peterson for position 1, and to Dr H.R. Klinck for position 2 at Search Committee, Department of Plant Science, Macdonald College, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, Canada H9X 1CO.

Final selection will not be made before April 15. Starting date, 1 September 1984. Starting salary $29,500. All qualified applicants are encouraged to apply, but in accordance with Canadian Immigration regulations, this advertisement is directed to Canadian citizens and permanent residents of Canada. (NW323)A

M R C CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW
MIDDLESEX HA1 3UJ

PAEDIATRIC RESEARCH GROUP — MASS SPECTROSCOPIST

Applications are invited from graduates for this scientific post to participate with biochemists and clinicians in a research programme in paediatric metabolism. Areas of particular interest are inborn errors of metabolism, and metabolic homeostasis and its control. The post holder will be responsible for the co-ordination and conduct of the mass spectrometric work of the group (Head, Dr. T.E. Stacey) which will include use of stable isotopes.

Previous practical experience in mass spectrometry is essential, preferably in organic chemistry or biochemistry. The appointment is for 3 years in the first instance. Salary will be in the range £8,376 – £11,426 p.a. inclusive, depending on qualifications and experience.

Candidates who wish to apply should send a curriculum vitae and the names of two professional referees to Jean Cottam, Personnel Section, quoting ref. no. 123/1/3912. Closing date for application 16th March 1984. (1283)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF EARTH SCIENCES

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post for work on radiogenic isotope geology. The appointment is available immediately for a fixed term of up to 18 months. Applicants should have experience in radiogenic isotope geology and preferably interests in igneous geochemistry. The successful candidate will be expected to initiate research projects, collaborate on current research and assist in the training of research students in the isotope geology laboratories.

Salary within the range £7190 £10250 on the IA Range for Resea and Analogous Staff (£7190 £11615) according to age, qualifi tions and experience.

Informal enquiries may be made Professor Briden, Department Earth Sciences, The University Leeds.

Application forms and furt particulars may be obtained from Registrar, The University, Le LS2 9JT quoting reference num 49/27/D. Closing date for appli tions is 31 March 1984.

Applicants from overseas m apply by telegram or telex (556 UNILDS G) in the first instance. (1264)A

UNIVERSITY OF CALIFORNIA
SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY

HEAD, DIVISION OF IMMUNOLOGY

The School of Medicine of the University of California, San Francisco is seeking a Head for the Division of Immunology within the Department of Microbiology and Immunology. The Head will occupy a tenured faculty position with joint appointments in the Department of Microbiology and Immunology, and the Cancer Research Institute. Qualified individuals will have training and experience in molecular biology and immunogenetics, and a demonstrated capacity for independent, creative activity of distinction. In addition to responsibilities in research and teaching immunology to medical students, other health sciences students and graduate students, the Head will lead a graduate degree program in immunology utilizing the resources for immunology and related disciplines on the University of California, San Francisco campus.

Applications with curricula vitae should be sent to Dr. John D. Stobo, HSW-473, University of California, San Francisco, California 94143. An Affirmative Action/Equal Opportunity Employer. (NW311)A

INSTITUTE OF CANCE RESEARCH AND ROYA MARSDEN HOSPITAL
Sutton, Surrey

JOINT DEPARTMENT OF PHYSI

MEDICAL ULTRASONI PHYSICIST
at University Lecturer Lev

A vacancy has arisen for a physi with appropriate experience applications of ultrasonics medicine, to take responsibility f major part of our research Hospital service work in this a Evidence will particularly be so of a candidate's ability both initiate and carry out innova research and to collabor constructively in its execution clinical and other colleag Appointment will be made at appropriate point on the M Scientist (Non-Clinical) scale, G II/I (£7,190 — £14,125 pa) London Allowance of £1,186 pa

Applications in duplicate sh give full details of qualifications experience with the names of persons familiar with the candida work. These should be sent to Personnel Officer, Institute Cancer Research, 34 Sumner Pl London SW7 3NU, quoting 300/G/42. Further details can obtained by telephoning Mrs Sh Dunstan, Tel: 01-642 6011 ext Applications must be received later than 16th March, 1984. (1273)A

KING'S COLLEGE LONDON
UNIVERSITY OF LONDON

APPOINTMENT OF PRINCIPAL

The appointment of the Principal of King's College, Lord Cameron, comes to an end of 31st July 1985 and it is intended that a successor should be appointed from 1st August 1985.

The Principal is the chief academic and administrative officer of the College.

The salary is negotiable and living accommodation is provided.

King's College is due to merge in 1985 with Queen Elizabeth College and Chelsea College to form a single School of the University of London under the name of King's College London, and the person appointed to the post of Principal will be head of the combined College.

Persons interested in being considered for this appointment, or wishing to suggest the names of others, are invited to write in confidence not later than 31st March 1984 to: The Rt. Hon. Earl Jellicoe, DSO, MC, Chairman of the Council, King's College London, Strand, London WC2R 2LS, from whom further particulars are available. (1288)A

SSISTANT, ASSOCIATE OR FULL PROFESSOR OF PLANT PATHOLOGY PLANT VIROLOGY

e Department of Plant Pathology, versity fo California, Berkeley, ounces a tenure track position in area of plant virology. Level of ointment will depend upon erience. A PhD is required. plicants should have training and earch experience in plant virology. ference will be given to those plicants with demonstrated ertise in the biochemical and lecular biological techniques. A nmitment to applying such hods toward a solution of virus ease development and virus-host eraction, and etiology and lemiology of plant virus diseases. teaching responsibilities will ude participation in a team taught dergraduate Comparative ology course and major involve- t in a graduate course in Plant ology.

pplicants should submit a riculum vitae, transcripts, lications and a brief description esearch goals, and arrange for e letters of reference to be sent to: Morris, Chair, Search Commit- Department of Plant Pathology, versity of California, Berkeley 94720, by April 30, 1984.

he University of California is an al Opportunity, Affirmative ion Employer. (NW331)A

IVERSITY OF LEICESTER

PARTMENT OF BIOCHEMISTRY

ESEARCH ASSISTANT 1B

ent graduates from relevant fields invited to apply for the above t, to work on applications of in o mutagenesis to cloned genes olved in oncogenesis.

he post is funded by the Cancer earch Campaign and will be for years, starting as soon as sible. The salary will be on the 1B e, starting at £6,310.

pplications with curriculum vitae the names of two referees should ent to Dr. I.C. Eperon, University Leicester, Department of chemistry, Adrian Building, versity Road, Leicester LE1 7RH 0533 551234, ext. 391) from m further information can be ained. (1253)A

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF CHEMISTRY
(SERC Daresbury)

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from chemists and physicists for the above post tenable for up to two and a half years. Synchrotron radiation and fast particles will be used to study primary processes resulting from the absorption of high energy radiation; the dependence of luminescence decays on photon energy and magnetic field will be measured. The main experiments will be carried out at the SERC's Daresbury Laboratory; the research assistant could be based in Sheffield or Daresbury. Research experience with synchrotron radiation, vacuum ultraviolet, single-photon counting or luminescence and an interest in computing would be useful.

Salary £7190 to £7630 a year on Range IA: possibility of higher salary at reduced tenure.

Details from Dr. B. Brocklehurst, Department of Chemistry, The University, Sheffield S10 2TN to whom applications, naming two referees, should be sent as soon as possible. Quote ref: R40/G. (1284)A

GODDARD INSTITUTE FOR SPACE STUDIES POSTDOCTORAL POSITION IN PHYSICS AND ASTROPHYSICS

Two postdoctoral positions will be available at the Goddard Institute for Space Studies in the summer of 1984. One is for a radio or infrared astronomer to work in the general area of molecular clouds and galactic structure. The other is for a laboratory astrophysicist to work on the millimeter-wave and far IR spectroscopy of molecular ions and radicals that might be detected in space.

Candidates should send their curriculum vitae to Dr Patrick Thaddeus, and should arrange to have three letters of recommendation sent to Goddard Institute for Space Studies, New York, New York 10025, at the same address. (NW325)A

CSIRO
TAXONOMIST
$A24,344 — $A35,806
Division of Entomology
Canberra Act Australia

Field: Taxonomy of Mites (Acari).

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Entomology is a member of the Institute of Biological Resources.

General: The Division carries out basic and applied research on many entomological problems of economic importance, and is the main centre in Australia for research on insects and related arthropods. A wide range of ecological studies is in progress and there are numerous other projects covering diverse fields of entomology. The professional staff numbers over 120, including 8 full-time and several part-time taxonomists, who are responsible for research on the Australian insect fauna and the curation of the Australian National Insect Collection (ANIC). The Division has excellent research and library facilities.

Duties: To undertake taxonomic research on mites and be responsible for the development and curation of the ANIC collection of Australian Acari. Substantial holdings of some families are already on hand. Opportunities will be available to participate in faunal survey and other relevant field work. Some identification responsibilities are involved.

Qualifications: A PhD degree or equivalent qualifications, with considerable research experience and a special interest in the taxonomy of Acari.

Tenure: Indefinite with Australian Government superannuation benefits available.

Note: Applicants with an exceptional level of research achievement may be considered for appointment at a higher salary.

Applications: Stating full personal and professional details, the names of at least two referees and quoting reference No A2188, should be directed to:

The Chief,
CSIRO Division of Entomology,
GPO Box 1700,
CANBERRA ACT 2601.
AUSTRALIA.
By March 23, 1984. (W765)A

UNIVERSITY OF READING

DEPARTMENT OF AGRICULTURE & HORTICULTURE

RESEARCH FELLOW

required immediately in the Sub-Department of Horticulture to work with Dr P Hadley on the action of chemical plant growth regulators on the growth and physiology of crop plants. The post is for a fixed term of three years.

Candidates should hold a PhD degree and have research experience in plant physiology and/or plant biochemistry. Interests in carbon assimilation and carbon economy would be an advantage. Starting salary £7,190 pa. Apply, quoting Ref R7A, for application form to Personnel Office, University of Reading, Whiteknights, Reading RG6 2AH. (1271)A

Athrofa Gogledd Ddwyrain Cymru
The North East Wales Institute
of higher education

DEESIDE, CLWYD — RESEARCH DIVISION

TECHNICIAN BIOTECHNOLOGY

Scale 1 — 3 (£2,976 — £6,135)

Applications are invited for a Technician to assist an industrial/SERC supported team working on immunoassays for novel purposes. The post is initially available for 23 months. Applicants, should have biological and biochemical experience.

Further details and application form available from the Registrar, The North East Wales Institute, Deeside, Clwyd. Tel: Deeside 817531 ext 254. Closing date for receipt of applications 9th March 1984. (1278)A

Electro neuropharmacologist

PhD degree neurophysiology

An important pharmaceutical Research Centre in the West Paris suburbs is seeking a Neurophysiologist to direct its Electrophysiology Laboratory for the investigation of the mechanisms of action of psychotropic and neurotropic agents.

Candidates in their early thirties should have a PhD degree or equivalent in Neurophysiology with knowledge of neuropharmacology, biological statistics and the use of microcomputers. Adequete spoken French is a requirement.

Please send hand-written letter, C.V., photo, salary requirements with ref. 2822/N on the enveloppe to **MEDIA P.A.** - 9, boulevard des Italiens - 75002 PARIS. (W766)A

MEDIA PA

Max-Planck-Institut für Biologie, Tübingen
POST-DOCTORAL POSITION

Position for research in Molecular Biology/Immunology of Parasitic Protozoa. Applications are invited from scientists interested in studying the interaction of parasite surface receptors with host cells, and the control of their expression during parasite differentiation. Preference will be given to applicants with a background in molecular biology/immunology. Experience of parasitic protozoa is not necessary. Appointments from April or later, salary according to stipend regulations (DM 2,200-2,400/month, tax-free).

Applications with curriculum vitae, publications list and names of referees should be sent to: Prof. Dr. P. Overath, Max-Planck-Institut für Biologie, Correnssstrasse 38, 74 Tübingen, W. Germany. (W768)A

Pathologist

In the development of any new drug, evaluating its safety for man is an essential element. As part of a continued expansion of such activity Beecham Pharmaceuticals has acquired a purpose built site at Stock, near Chelmsford, Essex.

We are now seeking to recruit a laboratory animal pathologist to carry out the histopathological assessment of tissues from animals used in safety studies.

Applicants should have a degree in veterinary science or a good honours degree (at least 2.1 standard) in a related subject. Relevant practical experience would be a distinct advantage.

We can offer an attractive salary, pleasant working conditions together with the usual fringe benefits of a large and successful company.

For an application form, please write to the Personnel Department, Beecham Pharmaceuticals Research Division, Honeypot Lane, Stock, Essex quoting ref. 3/84.

(1292)A

Beecham Pharmaceuticals

RESEARCH Assistant, MSc or PhD in biochemistry. Experience and publications in membrane immunochemistry of human leukemia cells. Salary at technical level, commensurate with experience. Hospital appointment in Toronto, renewable annually, to begin July 1984. Curriculum vitae and references to Dr M Baker, 399 Bathurst Street, Toronto, Ontario, M5T 2S8 Canada. (NW312)A

UNIVERSITY OF UTAH
DEPARTMENT OF BIOENGINEERING

Applications from candidates with an earned doctorate are invited for one/two tenure track faculty positions, assistant to full professor level. The appointee(s) will teach courses in bioengineering and will be expected to conduct an outstanding independent program of research. Joint appointments with other departments possible.

Send curriculum vitae and three recent publications to: Prof Richard A Normann, Department of Bioengineering, University of Utah, Salt Lake City, Utah, 84112. Application deadline April 15, 1984. An affirmative action/equal opportunity employer. (NW294)A

IMMUNOLOGY FACULTY POSITION

Tenure Track, at either Assistant/Associate or Full Professor, Department of Microbiology, Southwestern Medical School, Dallas.

Areas of particular interest include molecular genetics, cellular immunology and cell cloning. Rank and salary open. Current members of faculty are: Drs Capra, Forman, Kettman, Lipsky, Sullivan, Tucker, Uhr, Vitetta, and Wasserman.

Applicants should submit curriculum vitae, description of research interests and names of 4 references to Dr J W Uhr, Department of Microbiology, Southwestern Medical School, 5323 Harry Hines Blvd., Dallas, Texas 75235.

The University of Texas Health Science Center is an equal opportunity/affirmative action employer. (NW326)A

HERIOT-WATT UNIVERSITY
DEPARTMENT OF CHEMISTRY
LECTURESHIP

Applications are invited for Lectureship in Inorganic/Analytic Chemistry from 1 October 1984. It essential that the candidate shou have a high research potential a strong interest in teaching. T Department of Chemistry is locat on the Riccarton Campus of t University. An appointment will made at an appropriate point on t Lecturer salary scale £7190 — £141 — plus USS benefits.

Applications forms and furth particulars may be obtained from t Staff Officer, Chambers Stree Edinburgh EH1 1HX quoting re 12/84 to whom completed applic tion forms should be returned by March 1984. (1258)A

CHAIRMAN
DEPARTMENT OF ANATOMY

Applications are invited for t position of Professor and Chairma Department of Anatomy, Schools Medicine and Dentistry, Universi of Alabama in Birmingham. We a seeking candidates with research a teaching experience, leadersh qualities, and administrative skil This department has teachi responsibilities for graduate stude in anatomy, medical, dental a other students.

Please submit applications anc curriculum vitae to: Antonietta Hyche, Administrative Offic University of Alabama School Medicine, Dean's Office — M 306, Birmingham, Alabama 352 An Equal Opportunity/Affirmat Action Employer. (NW263)A

PUBLIC HEALTH LABORATORY SERVICE BOARD
VIRUS REFERENCE LABORATORY
CENTRAL PUBLIC HEALT LABORATORY
VIROLOGIST

A graduate with 1st or 2nd cl honours degree and with 2 or m years experience in virology required to assist with a proj concerned with the transmission hepatitis A virus to man by shellf and procedures of inactivation.

The project is funded by DHSS a period of 2 years.

Salary on Whitley Coun scientific scale £6,759 — £8,349 p £623 London Weighting. The sc quoted is dependant upon experie relevant to the Terms and Conditic of service.

Application forms may obtained from the Person Department, Central Public Hea Laboratory, Colindale Avenu London NW9 5HT. (1270)A

Nominations invited for the

1984 BROOKDALE AWARDS FOR DISTINGUISHED CONTRIBUTIONS TO GERONTOLOGY

The Gerontological Society of America announces three Brookdale Awards for Distinguished Contributions to Gerontology:

I. To a **citizen of the United States** for contributions to Gerontology through **research in the Social or Behavioral Sciences**

II. To a **citizen of the United States** for contributions to Gerontology through **leadership in Biology or Clinical Medicine**

III. To a **citizen of another nation** for contributions to Gerontology through **research and/or leadership**

Each awardee will receive a $25,000 cash prize and will be honored at a special ceremony during the 1984 Annual Scientific Meeting of The Gerontological Society of America in San Antonio, Texas.

Nominations

Nominations can be made by any individual or organization and should be submitted in the form of a letter setting out in detail the significance of the individual's contributions. A curriculum vitae and two other letters of endorsement must accompany the nomination. Eight copies of these materials should be sent to The Gerontological Society.

Nominees must be gerontologists who have been recognized both nationally and internationally for their distinguished contributions to the field.

Deadline for Nominations

Nominations should be addressed to the

Brookdale Awards
% The Gerontological Society of America
1411 K Street N.W., Suite 300
Washington, D.C. 20005

and must be received no later than May 2, 1983.

THE GERONTOLOGICAL SOCIETY OF AMERICA

(NW322)N

SEMINARS and SYMPOSIA

INSTITUTE OF BASIC MEDICAL SCIENCES
ROYAL COLLEGE OF SURGEONS OF ENGLAND
First Announcement of Three-Day Interdisciplinary Symposium
"INTERACTIONS OF CELLS WITH NATURAL AND FOREIGN SURFACES"
28TH, 29TH and 30TH NOVEMBER, 1984
Co-Organisers:
Professors N. Crawford and D. E. M. Taylor
— at the
ROYAL COLLEGE OF SURGEONS OF ENGLAND
LINCOLN'S INN FIELDS, LONDON WC2A 3PN

This Symposium will provide an overview of an important and rapidly developing interdisciplinary field and is intended to improve understanding and generate dialogue between life scientists, clinicians and materials scientists. In particular it will provide a link between the more basic biology of cell interactions with surfaces and its application and relevance to the use of biomaterials in clinical practice.

The Symposium will include eight sessions of invited papers by experts in their fields, three oral communication sessions for free papers on basic and applied aspects and a number of poster sessions. Topics to be covered will include:

MEMBRANE STRUCTURE, ORGANISATION AND ELECTROKINETIC PROPERTIES
BIOTOLERANCE AND HAEMOCOMPATIBILITY
ASSESSMENT OF BIOCOMPATIBILITY OF MATERIALS
ANTITHROMBOTIC SURFACES: DESIRABLE PROPERTIES
CELL BIOLOGY OF WOUND HEALING AND WOUND MANAGEMENT
ORTHOPAEDIC IMPLANTS, BEARING SURFACES, FIXATIVES
CARDIOVASCULAR IMPLANTS AND EXTRACORPOREAL CIRCUITRY
TUMOUR METASTATIC SPREAD, CARCINOGENICITY OF MATERIALS
DENTAL MATERIALS, FILLERS, CEMENTS, CELL/POLYMER INTERFACES

For further information and registration forms etc, write to:
I.B.M.S. Symposium 1984
Royal College of Surgeons of England,
Lincoln's Inn Fields, London, WC2A 3PN.
(1281)M

ALCOHOL

The 15th international symposium on food subjects, organised by the National College of Food Technology, University of Reading, will be on Alcoholic Beverages. The symposium will be held at the new centre of food studies, University of Reading, 27-29 March 1984.

Details from Dr G. G. Birch, National College of Food Technology, Department of Food Technology, Food Studies Building, University of Reading, Whiteknights Reading RG6 2AP. (1279)M

ANNOUNCEMENTS

THE ROYAL SOCIETY
GUEST RESEARCH FELLOWS

The Council of the Royal Society invites applications for the support of Guest Research Fellows. The object of this scheme, established in 1980, is to assist outstanding leaders in research in the United Kingdom to invite as guests scientists of proven ability, normally resident overseas, to come and work with them for periods of at least four months and not exceeding two years. The aim is to provide for research needs which can best be met by such international collaboration. Emphasis in selection will be on the abilities and field of research of applicants and on special contributions to the applicant's research which would be provided by the visitor and are not available in the United Kingdom; applicants must be working in the United Kingdom.

Applications should be made on forms to be obtained from the Executive Secretary (GRF/UMAM), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and returned by 30 April 1984. (1261)G

ASSISTANTSHIPS

THE UNIVERSITY OF SHEFFIELD
WOLFSON INSTITUTE FOR BIOTECHNOLOGY
RESEARCH POSTS IN PLANT CELL BIOTECHNOLOGY
POST-DOCTORAL RESEARCH ASSISTANTSHIPS

Two Postdoctoral Research Assistants are required to work on natural product synethesis in plant cell culture in the food and flavour area. The projects are supported by grants from industry and are available for a period of one year in the first instance, commencing as soon as possible. Applicants should have a first degree in Microbiology, Microbial Biochemistry or Natural Product Chemistry. Salary in the range of £7,190 — £8,080 a year on Range IA for Research and Analogous Staff.

Further details from Professor M.W. Fowler or Dr. A.H. Scragg, Wolfson Institute of Biotechnology, the University, Sheffield S10 2TN (0742 — 78555 Extn. 4245) to whom applications, including the names of three references, should be sent as soon as possible. Quote ref: R38/G. (1285)P

CONFERENCES and COURSES

1984/85

INTER-UNIVERSITY MSc COURSE ON THE PHYSICS AND TECHNOLOGY OF AMORPHOUS MATERIALS

Universities of Dundee
Edinburgh — Glasgow

The one year advanced course, in this rapidly developing field, is offered jointly by the above named Universities. Course work will be given at the University of Dundee. For the project work, students will join a research group at one of the participating institutions.

Applications are invited from students with an honours degree (or its equivalent) in either Physics, Electrical Engineering, Chemistry, or a Materials Science. The course has been approved by the Science Research Council and Advanced Course Studentships are available for suitably qualified applicants.

Further information may be obtained from: Professor W E Spear, FRS, Carnegie Laboratory of Physics, The University, Dundee DD1 4HN. (1290)C

CHELSEA COLLEGE
University of London
STUDY HISTORY AND PHILOSOPHY OF SCIENCE AT CHELSEA

One year MSc courses begin Octob 1984. Facilities for MPhil and Ph by research. Applicants must posse a first degree in one of the natur sciences or mathematics. All cours approved for DES grand award Early application is advised since t closing date for applying for a DE award is 1 May.

Send for details to Dr M L Redhead, Department of History a Philosophy of Science, Chels College, University of Londo Manresa Road, London SW3 6L Tel. 01-351 2488. (1250)C

continued on page 16

FELLOWSHIPS

A POSTDOCTORAL Fellowship available in protein crystallograp related to active sites and regulato mechanisms, including enzym modified at the DNA level by reco binant techniques. Applicants fro the area of small molecule cryst lography will also be considere Applicants may write to Pr William N Lipscomb, Gib Chemical Laboratory, Harvard U versity, Cambridge, MA 0213 USA. Harvard University is Affirmative Action/Equal Opp tunity Employer. (NW301)E

UNIVERSITY OF ABERDEE
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH FELLOWSHI

Applications are invited to join research team whose objective is establish a scientific basis for physical and chemical immobili tion of certain radioactive wastes cement-based systems. In order compliment the skills of ot members of the team, applica should preferably be familiary w radiochemical methods a procedures.

The post is available immediate An initial appointment will be m until 31 March 1985 but it envisaged that the work, which supported by the Department Environment and the Commissio the European Communities, will continued for a further period.

Salary, depending on qualifi tions and experience is in the ra 1A scale for Research and Analog Staff (£7,190 — £11,615) with app priate placing.

Further particulars and appli tion forms from The Secretary, University, Aberdeen, with wh applications (2 copies) should lodged by 16 March 1984.

Informal enquiries may be mad Professor F P Glasser (T 0224-40241 Ext: 5640). (1254)

UNIVERSITY OF CAPE TOWN

Postdoctoral Research Fellowships 1985

The University of Cape Town annually invites applications for Post-doctoral Research Fellowships to be held at the University.

The Fellowships are tenable for a maximum of twelve months and the stipend attached to the current Fellowships is R1 500 p.m. (R18 000 p.a.). Successful candidates from abroad will receive an additional Travel grant up to a maximum of R2 000. (Some increase in these values to keep pace with inflation is possible for 1985). There is no restriction to any particular field of research.

Preference will be given to recently qualified research workers but reduced awards suitable for a visit of about three months have sometimes been made to tenured staff members of universities. Preference is also given to applicants whose research interests and facilities needs mesh well with those of the appropriate host department.

Applications must include full details of the applicant's envisaged research programme in addition to a full curriculum vitae and the names of two referees whom the University may consult. The policy of the University is not to discriminate in the appointment of staff on the grounds of sex, religion, race, colour or national origin. The dates upon which the successful candidates will be expected to take up their duties are flexible and can be arranged to suit the special circumstances of each candidate.

Applications should be addressed to the Head, Research Administration, University of Cape Town, Private Bag, Rondebosch 7700, South Africa, so as to reach him by 10 June 1984. The first round of award letters will be despatched by the end of August 1984.

(W773)E

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL FELLOWSHIP

Applications are invited for a Post-doctoral Fellowship for research in the Biochemistry of vision. The appointment will be for two years. Preference will be given to candidates with experience of protein chemistry and sequence analysis.

Salary on Scale 1A (£7,190 — £11,615) for Research and Analogous Staff.

Applications should be sent to Professor M Akhtar FRS, Department of Biochemistry, University of Southampton, Southampton SO9 3TU.

Please quote Ref. No 1266 as soon as possible. (1266)E

UNIVERSITY COLLEGE DUBLIN
Department of Geology

The Department invites applications from suitably qualified individuals for Department of Education Postdoctoral Fellowships 1984-85. These are administered by the Department of Education and are tenable for a period of two years with a possible extension for a third year. Applicants should hold the degree of PhD or have equivalent research experience at the time of taking up the award. They should be under 30 and preferably under 28 years of age on March 31, 1984. Appointment will be effective from a date not earlier than October 1, 1984. Salary, dependent upon qualifications and experience, will be in the range IR£6,961 — IR£7,876 per annum.

The Department of Geology is particularly interested in applications from persons with interests in isotope geochemistry, fluid inclusion studies and mineralisation, micropalaeontology or Irish Caledonian geology, but applicants with other research interests will also be considered. Interested persons are requested to contact Prof. M. J. Kennedy, Department of Geology, University College, Belfield, Dublin 4 for further particulars of the facilities and research opportunities in the Geology Department.

Applications should be made directly to the Secretary, Department of Education, (Headquarters Section 3), Marlborough Street, Dublin 1, from whom application forms and full particulars of the fellowship scheme can be obtained. The deadline for receipt of applications by the Department of Education is March 31, 1984. (1260)E

POSTDOCTORAL FELLOWSHIPS
at
The Wistar Institute

Postdoctoral positions for 2 to 3 years are continuously available to exceptionally good recent MD and PhD graduates in the field of oncogenesis, cellular differentiation, chemical carcinogenesis, membrane biochemistry, somatic cell genetics, tumor cell biology and molecular biology.

Applicants should send their curriculum vitae and names of three references to:

Coordinator of Research Training,
The Wistar Institute
of Anatomy and Biology,
36th Street at Spruce,
Philadelphia, PA 19104. (NW329)E

Organon International bv

postdoctoral research fellowship in CNS-Pharmacology

The fellowship tenable for up to 3 years is available in the Scientific Development Group of Organon International bv, The Netherlands.
The successful candidate will work in the Behaviour Group of the CNS-Pharmacology Department.
The post will appeal to pharmacologists experienced in the field of animal models for aging, Alzheimers's disease and related pharmacology.
The company's extensive and well equipped laboratories are situated at Oss, in the southern part of The Netherlands.

Written applications, including curriculum vitae, a full statement of research experience and a list of publications should be sent to: Organon International bv, Personnel Department, P.O. Box 20, 5340 BH Oss, The Netherlands, under number HK 83-16. Requests for further information should be directed to Dr. C. Broekkamp, CNS Research & Development Laboratories, phonenumber (31)-4120-62801.

Organon

Organon International bv
The Netherlands

(W779)E

CONFERENCES and COURSES continued

ANNOUNCEMENT/CALL FOR PAPERS, POSTERS, AND REGISTRATION
CONFERENCE ON THE MOLECULAR BASIS OF CANCER: AN INTERDISPLINARY ON BASIC AND APPLIED ASPECTS OF CANCER
MAY 30 — JUNE 2, 1984
ROSWELL PARK MEMORIAL INSTITUTE, BUFFALO, NEW YORK

Invited speakers:

A. Block	Buffalo	G. Loew	Menlo Park
S. Broyde	New York	K. Miller	Troy
C. Cantor	New York	S. Neidle	London
E. Clementi	Poughkeepsie	T. O'Connor	Buffalo
D. Crothers	New Haven	W. Olson	New Brunswick
C. DeLisi	Bethesda	G. Pack	Rockford
T. Dougherty	Buffalo	M. Pincus	New York
W. Duax	Buffalo	P. Politzer	New Orleans
M. Feldman	Rehovot	A. Pullman	Paris
R. Feldmann	Bethesda	B. Pullman	Paris
I. Hertman	Ness-Ziona	R. Rein	Buffalo
A. Hopfinger	Skokie	R. Sarma	Albany
T. Hopp	Seattle	H. Scheraga	Ithaca
J. Kaufman	Baltimore	R. Schwartz	Bethesda
S. Kim	Berkeley	D. Stollar	Boston
P. Kollman	San Francisco	A. Wada	Tokyo
J. Ladik	Erlangen	R. Wells	Birmingham
M. Liebman	New York	C. Wenner	Buffalo

Organizing Committee: R. Rein (Chairman), C. DeLisi, P. Lowdin, T. O'Connor, B. Pullman, H. Weinstein

This conference will provide a forum for a dialogue on topics which are in the forefront of cancer research by investigators from various areas of natural sciences. The conference will bring together experimental biologists, theoreticians, and structural chemists for a discussion centered around gene function and expression, drug design, immunological probes, and membrane properties underlying the cancerous process.

The invited and contributed papers will be promptly published. Papers for the proceedings should be submitted no later than the finish of the conference. Abstracts are due by April 1 and should include the name of the author(s), institution and a summary of the work to be presented. Send to R. Rein, Roswell Park Memorial Institute, Cancer Cell Center, Buffalo, New York 14263.

The number of participants will be limited to 400. Inquiries about details of the program and registration should be addressed to Kevin A. Craig, Medical & Scientific Communications, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14263. (NW320)C

The Scientific Affairs Division of NATO is sponsoring an Advanced Study Institute on:

CHROMOSOMAL PROTEINS AND GENE EXPRESSION

to be held at Sitges, Spain from 17 to 26 September, 1984.

The course is intended largely for post-doctoral researchers and advanced graduate students, but a limited number of independent investigators may also be admitted. Through lectures and tutorials, we will systematically discuss and analyze recently accumulated knowledge on the roles of chromosomal proteins in gene expression. Topics to be covered include: nucleosome and higher order chromatin structures, active gene chromatin structure, hypersensitive sites, histones, protamines, HMG proteins, scaffold proteins, Z-DNA binding proteins, promoter and enhancer elements, in vitro and in vivo transcription, DNA methylation, and DNA-protein interactions.

Lecturers: F. Azorin (Cambridge, Mass.), M. Beato (Marburg), C. Crane-Robinson (Portsmouth), G. Dixon (Calgary), S. Elgin (St. louis), G. Goodwin (London), C. Hélène (Paris), U. Laemmli (Geneva), P. Puigdomènech (Barcelona), G. Reeck (Kansas), D. Schumperli (Zurich), J. Subirana (Barcelona), J. Thomas (Cambridge), B. Wasylyk (Strasbourg), S. Wright (London).

Organizing committee: G. Reeck, G. Goodwin, and P. Puigdomènech.

Limited funds are available to subsidize some participants' expenses. Applications to attend the course and for financial support should be sent to Dr. G. Reeck (Kansas State University, Dept. of Biochemistry, Manhattan, Kansas 66506 USA) and should include a résumé, list of publications, description of research interest, rationale for wanting to participate in the course, and statement of financial need (including availability of other funds to support your participation). (1259)C

EMBO — Advanced Lecture Course Molecular Genetics of Mammalian Development

PAVIA — 3-15 September 1984

General topics: recombinant DNA techniques in development, germ cells, early development, dedifferentiation and its relationship to embryogenesis, developmental and temporal regulation of genes, tissue specific expression, somatic cell genetics as a tool, hormonal regulation of expression, promotors, enhancers and other regulatory elements, injection of foreign DNA into embryos and its expression, approaches in other animals.

Specific systems include: H-2, HLA and disease, antibody genes, muscle differentiation, nervous system, lens cell, X-inactivation, sex determination, the T/t locus, oncogenes and the cancer problem.

Lecturers include: C. Morandi, V. Sgaramella (Pavia, Italy), A. Carbonara (Torino, Italy), G. Tocchini-Valentini (Roma, Italy), G. Bulfield, R. Clayton, N. Hastie (Edinburgh, UK), A. McLaren, P. Goodfellow (London, UK), M. Lyon (Harwell, UK), P. Démant (Amsterdam, Netherlands), V. Pirrotta (Heidelberg, FRG), V. Chapman (Buffalo USA), K. Paigen (Berkeley, USA), P. Brûlet (Paris, France).

The Course will consist of lectures, seminars and journal clubs and its organised for EMBO by G. Astaldi Ricotti, C. Morandi, G. Della Valle (Pavia) and G. Bulfield (Edinburgh). The Course fee is $280 which includes all meals and accommodation. For further details and applications with brief CV and research interests by *1st June* (limit 60 people) to:

Dr Grahame Bulfield (EMBO Course 1984/9)
AFRC Poultry Research Centre
Roslin, Midlothian, EH25 9PS, UK.

(1280)C

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

MSc COURSES IN BIOCHEMISTRY

The University offers four full-time one-year M.Sc. courses in Biochemistry: General Biochemistry, Biochemical Endocrinology, Genetic Manipulation and Molecular Biology, Plant Biochemistry and Bioenergetics and places on these courses are available for the 1984/85 session. In some cases a full-time 2-year course may be possible.

Research projects are included in each course, related to research currently underway in the Biochemistry Group, much of which is of relevance to developing countries.

Applicants should normally possess an Honours degree or its equivalent in Biochemistry or in a Biological, Chemical or Medical Science.

Enquiries and applications about these and other postgraduate courses in Biochemistry should be sent to the Subject Chairman of Biochemistry, School of Biological Sciences, University of Sussex, Falmer, Brighton, East Sussex, BN1 9QG. (1287)C

ADVANCED COURSE OF FIS AND SHELLFISH DISEASES I EUROPE

Sponsored by the Council Europe and UNDP/FA (Mediterranean Region) to be he at the Institute of Aquacultur University of Stirling, from Ju 17-29, 1984.

Lecturers and participants will from various parts of Europ Infectious and non-infectio diseases, therapy, control a economics will be included.

Further details from Dr Ja Polglase, Institute of Aquacultur University of Stirling, Stirlin FK9 4LA, Scotland, UK. Closi date for applications 1st May 198 (1291)C

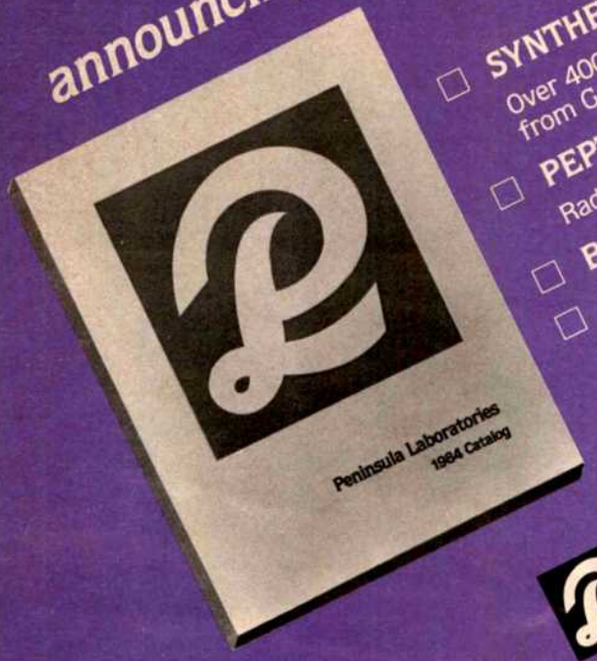
POSITIONS WANTED

APPOINTMENTS WANTE

advertise your qualities and qual cations through the most influen science weekly in the world a special reduced cost of *25p a wo* (Personal Box Numbers £1). Advertisements must be pre-paid a sent to: Nature Classified (Ap Wtd), 4 Little Essex Street, Lond WC2R 3LF. (890)E

Typesetting: J.W. Arrowsmith Ltd, Bristol and Outline Graphics Ltd, London EC4Y 0HP. Printing: Kingsdale Press Ltd, Reading, Berks, in association with Godfrey Lang Ltd, London WC1R 4EB and The William Byrd Press Inc., Richmond, Virginia, USA Published by MACMILLAN JOURNALS LTD, 4 Little Essex Street, London WC2R 3LF. — 23 February 1984.

announcing...



☐ SYNTHETIC PEPTIDES
Over 400 biologically active peptides from Gastro-Intestinal to Neuro-Peptides

☐ PEPTIDE ANTISERA
Radioimmunoassay and Immunofluorescence Kits

☐ BULK ANTISERA

☐ NUCLEOTIDE PRODUCTS
Phosphoramidites, Phosphotriesters and protected Nucleosides

☐ CUSTOM SYNTHESIS

Call or write for our 1984 catalog:

Peninsula Laboratories, Inc.

611 Taylor Way, Belmont, California 94002
(415) 592-5392 Telex 172511 (PEN LABS BLMT)
Out of State Toll-free: (800) 922-1516

Circle No.21 on Reader Service Card.

COMPUTED TOMOGRAPHY OF THE BODY

NOW IN PAPERBACK

A Radiological and Clinical Approach

Janet E. Husband MRCP, FRCR Ian Kelsey Fry DM, FRCP, FRCR

'As a general text on computed tomography this book may be thoroughly recommended.' British Medical Journal

'... A much needed book that will be of use to all those involved in CT scanning and should be read by all radiologists in training – their examiners will certainly have read it.'
British Journal of Radiology

'I would highly recommend this book to anyone interested in learning about computed body tomography.'
American Journal of Roentgenology

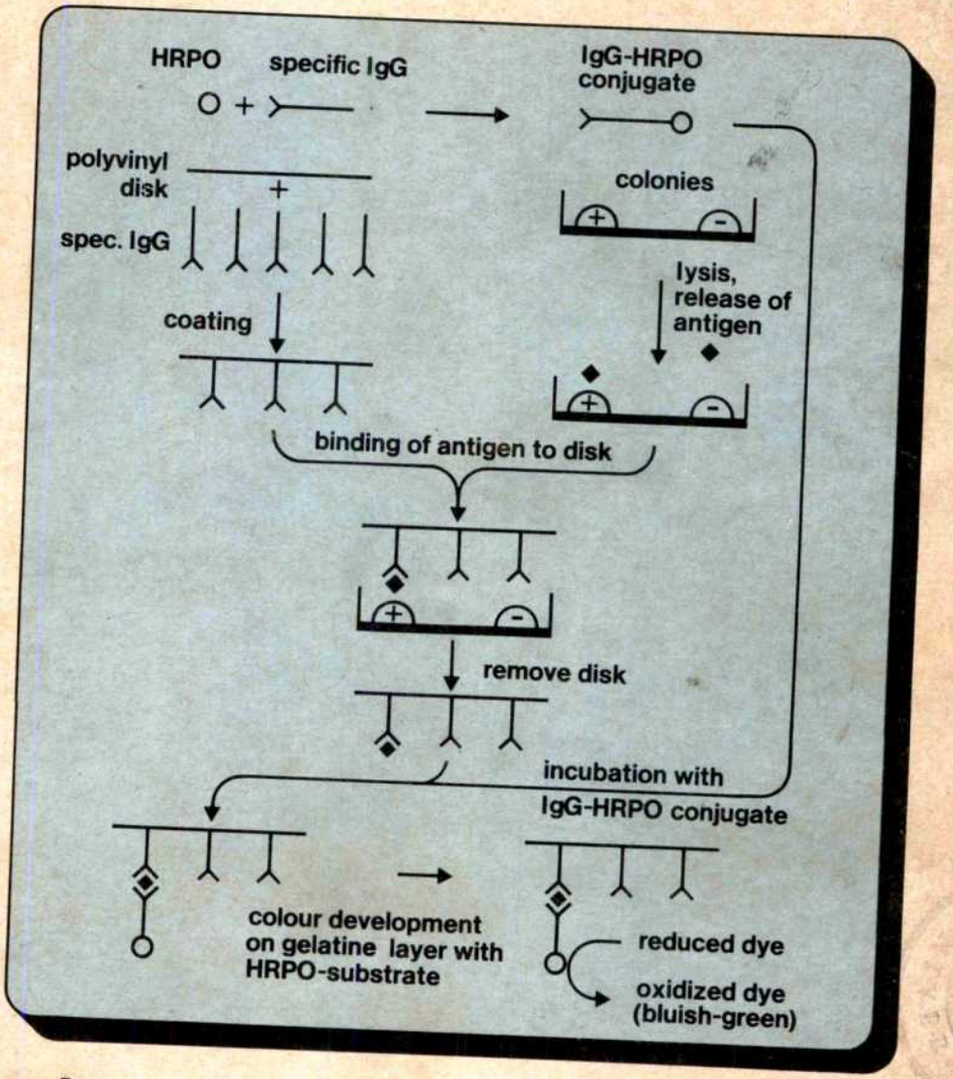
Contents
The Contributors; Preface; Basic Principles with R. Parker; General Considerations – Technical; General Considerations – Clinical; The Thorax – Mediastinum; The Thorax – Lungs, Pleura and Chest Wall; The Abdomen – Kidney; The Abdomen – Adrenals; The Abdomen – Liver and Biliary Tract; The Abdomen – Pancreas; The Abdomen – Miscellaneous Topics; Lymph Node Disease of the Abdomen and Pelvis; The Pelvis; The Musculo-Skeletal System; The Spinal Cord by P. Pullicino; The Head and Neck with C.A. Parsons; The Orbits by G.A.S. Lloyd; Computed Tomography in Oncology; Index.

October 1983	232pp (Illustrated)	SCIENTIFIC & MEDICAL / MACMILLAN PRESS LTD	£15.00	ISBN 0 333 25585 2

Please send orders and requests for information to: Carolyn Leuw
Scientific and Medical Division, The Macmillan Press Ltd, Houndmills, Basingstoke, Hants RG21 2XS, England

Circle No.67 on Reader Service Card.

Boehringer Mannheim
presents the new
Gene Expression Kit



★ simple procedure
★ fast results
★ sensitive
★ non-radioactive

For a detailed instruction please ask your local representative or

mannheim boehringer

Boehringer Mannheim GmbH
Biochemica
P.O. Box 310120
D-6800 Mannheim
W. Germany

Circle No.27 on Reader Service Card.